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Biochimica et Biophysica Acta 1458 (2000) 43–62

BIOCHIMICA ET BIOPHYSICA ACTA

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Review

Hydrogen bonds and proton transfer in general-catalytic transition-state stabilization in enzyme catalysis

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Received 1 November 1999; accepted 1 December 1999

Abstract

The question of the nature of the proton bridge involved in general acid–base catalysis in both enzymic and non-enzymic systems is considered in the light of long-known but insufficiently appreciated work of Jencks and his coworkers and of more recent results from neutron-diffraction crystallography and NMR spectroscopic studies, as well as results from isotope-effect investigations. These lines of inquiry lead toward the view that the bridging proton, when between electronegative atoms, is in a stable potential at the transition state, not participating strongly in the reaction-coordinate motion. Furthermore they suggest that bond order is well-conserved at unity for bridging protons, and give rough estimates of the degree to which the proton will respond to structural changes in its bonding partners. Thus if a center involved in general-catalytic bridging becomes more basic, the proton is expected to move toward it while maintaining a unit total bond order. For a unit increase in the pK of a bridging partner, the other partner is expected to acquire about 0.06 units of negative charge. The implications are considered for charge distribution in enzymic transition states as the basicity of catalytic residues changes in the course of molecular evolution or during progress along a catalytic pathway.

Keywords: Enzyme; Catalysis; Hydrogen bond; Transition state; Active site; Charge distribution

1. Introduction

Many enzymes employ transition-state proton bridging (general acid–base catalysis) as an element in their strategy of catalytic acceleration, a feature of direct relevance to bioenergetics [1–4]. A number of enzymes in this category are participants in bioenergetic pathways, including glycolysis, the tricarboxylic acid cycle, and the electron-transport/oxidative phosphorylation sequences. Their generally high catalytic power thus helps to determine the success of these mechanisms for the generation, conversion, storage, and retrieval of biological energy. Additionally, the magnitude of the catalytic power of any enzyme in any pathway determines the quantity of the enzyme that must be synthesized to meet the metabolic demands of the host organism: high catalytic power requires fewer molecules of enzyme to produce the same flux through the catalyzed reaction. Other things being equal, high catalytic power and a lower burden of biosynthetic demand thus reduce the bioenergetic cost of a particular step in the organismic economy [5].

Our purpose in this minireview is to examine the

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0005-2728/00/$ – see front matter
PII: S0005-2728(00)00059-1

current status of ideas about the nature of the transition-state proton bridges by which general acid–base catalysis results in net transition-state stabilization, i.e., in catalysis. Some of the issues that are raised by this question are independent of whether general acid–base catalysis occurs in simple solution or within the active site of an enzyme. We will advance tentatively a model we believe is in accordance with current information, and will suggest that the nature of the general-catalytic proton bridge is an important issue in the understanding of enzyme catalytic power and its evolution.

1.1. General acid–base catalytic proton bridging in enzyme catalysis

Transition-state stabilization by proton bridging can occur when a reaction, usually consisting of the formation or fission of a covalent bond or bonds, creates a change in the acidity or basicity of a participating center. Then the formation of a bridge with a proton donor or acceptor can lead to a reduction in the free energy of the activated complex that exceeds the reduction experienced from hydrogen bridging at the same site in the preceding reactant state. The result is net transition-state stabilization [6–8].

Illustrative examples from enzyme-catalyzed reactions are given in Fig. 1. In all of these examples, there is a *primary reaction* or bonding change, considered to be the chief reason that the overall reaction 'requires catalysis', and a proton-bridging interaction, commonly considered an ancillary process that leads to catalysis of the primary reaction. If the catalytic bridging interaction results in net proton transfer, then it is appropriate to refer to this net chemical reaction as a *secondary reaction*. It is, however, by no means necessary for catalytic proton bridging to lead to a net chemical reaction: bridging could clearly stabilize a transition state such that the proton remains in the product state with the same bonding partner as in the reactant state. In this case, it would be inappropriate to refer to a secondary reaction. A further characteristic of the cases of general catalysis to be considered in this article is that the bridging occurs between electronegative atoms such as oxygen, nitrogen, or sulfur, and not between carbon and other atoms. Toward the end of the article, we will briefly consider cases in which proton bridging to and from carbon is occurring.

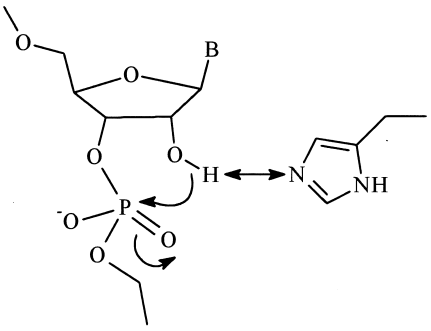
These proton bridges clearly contribute to catalytic accelerations in enzymic systems, as is well known, but the question of whether the primary and secondary processes are coupled to each other in enzyme action has been little examined, nor has the possible significance of such coupling for an understanding of enzyme catalysis and evolution. We next examine two models for the general-catalytic proton bridge, the canonical model postulating coupled processes and the alternative model postulating uncoupled processes.

1.2. The canonical model

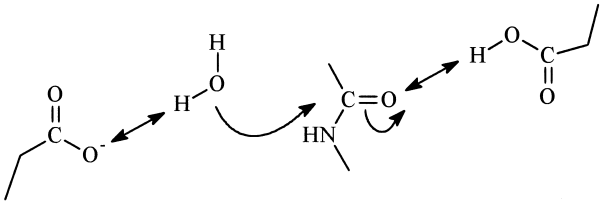
The most common mechanistic description of the nature of the proton-bridging interaction is one in which the bonding changes for the primary reaction are physically coupled to the net transfer of a proton across the catalytic bridge [6–19]. The motions together form all or part of a normal-coordinate motion of the activated complex as illustrated in Fig. 2a.

In this hypothetical transition-state reaction coordinate, the atomic motions leading to completion of the primary reaction of *carbonyl addition* (shortening of the forming C–O σ-bond, lengthening of the breaking C–O π-bond) are coupled to the motions leading to the secondary reaction of *proton transfer* (shortening of the forming O–H σ-bond, lengthening of the breaking H–O σ-bond). The primary and secondary reactions thus occur *simultaneously* in the sense that both are incomplete before the transition state is entered and complete after departure from the transition state. In this sense also, the two reactions may be said to occur 'in concert' or to be *concerted*. The coupled reactions are not necessarily *synchronous* in the sense that the degree of completion of each reaction need not be the same as the degree of completion of the other reaction at every point along the pathway through the transition state. Bernasconi [20] has treated in detail the concepts of *balance* and *imbalance* among component reactions in the transition states for complex reactions.

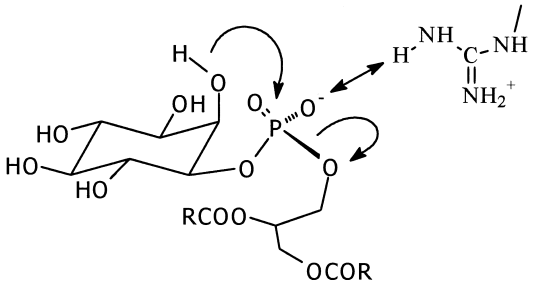
Jencks [6,7] pointed out that a prerequisite exists if the coupling between a primary reaction and a secondary proton-transfer reaction is to lead to catalysis. The prerequisite constitutes a relationship among



(a) ribonuclease action: general-base catalysis of attack by hydroxyl at a phosphoryl center



(b) HIV protease action: general-acid/base catalysis of attack by water at a carbonyl center



(c) action of bacterial phospholipase C: general-acid catalysis of phosphoryl transfer

Fig. 1. Examples of the role of proton bridging (double-headed arrows) in transition-state stabilization in enzyme catalysis. (a) General-base catalysis by a histidine residue of the attack of the 2′-OH of the substrate on phosphorus in the initial step of ribonuclease action [53]. Net proton transfer from O to N occurs as the P–O bond forms. Equilibration of the protonated histidine residue with buffer completes the catalytic cycle, restoring the residue to its unprotonated form. (b) General-acid and general-base catalysis of the attack of water on a substrate peptide bond in the action of acid proteases such as the HIV protease [54]. Net proton transfer occurs at both carboxylic-acid centers; equilibration then restores the enzyme to its initial protonation state. (c) General-acid catalysis of phosphoryl transfer in the action of a bacterial phospholipase C [55]. Net proton transfer does *not* occur. Instead the interacting residue forms a stabilizing proton bridge to a phosphoryl oxygen bearing a temporary excess of negative charge in the transition state.
←

the acidities of the species involved in the primary reaction and those involved in catalytic bridging (see Fig. 3). Intrinsic to Jencks's reasoning is the observed fact that the proton-transfer reactions involved in bridging (commonly transfers among O, N, and S centers) are extremely rapid compared to typical primary reactions like the carbonyl-hydration reaction shown in Fig. 3. Jencks's rule states that the catalytic entity must have an acidity relative to its partner in the reactant state such that proton transfer is thermodynamically unfavorable in the reactant state. In the example, the pK of the carboxylate – in its protonated state – is about 5, that of the reactant water molecule 15.7, so proton transfer in the reactant state is unfavorable by 10–11 orders of magnitude. In addition, the catalytic entity must have an acidity relative to its partner in the product state so that the proton transfer which has occurred is thermodynamically favorable in the product state. In the example, the (now protonated) carboxylic acid has a pK of 5, the HO–C group of the carbonyl adduct if protonated would have a pK around −2 so that the proton transfer shown as accomplished is indeed favorable by about 7 orders of magnitude.

Thus a proton transfer that would be endergonic in the reactant state becomes gradually more exergonic as the structure of the reacting molecules approaches the structure of the product state. If the structure of the transition state is sufficiently advanced toward the product-state structure (about 65% in this example), then partial proton transfer at the transition state will become energetically profitable. On this model, it is the progress of the primary reaction (heavy-atom reorganization) that controls the acidity of the proton donor in the secondary (proton-transfer) reaction, so that – although the component reactions are taken to be coupled – it is only the progress of the primary-reaction component

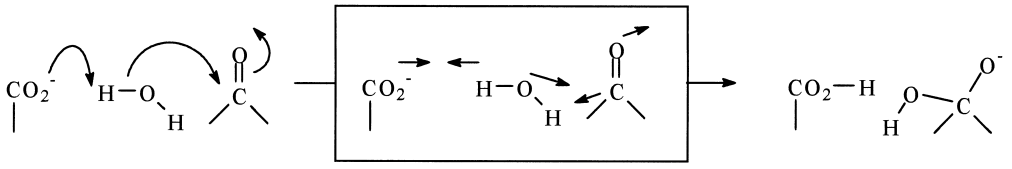
at the transition state that dictates whether and to what degree the proton-transfer component will have a catalytic effect.

1.3. The alternative model

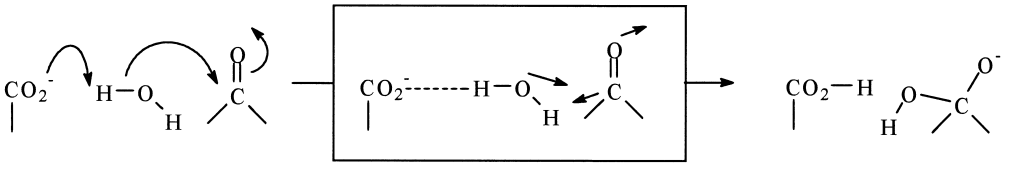
An alternative to the canonical model was suggested by Swain et al. ([8]; see also [9–19]) on the ground that isotope effects for general acid–base catalysis are generally smaller than those for a number of reactions in which proton-transfer processes are almost surely coupled to other processes in a single transition-state reaction coordinate (see Section 1.4). A much preferable form of this model was proposed by Cordes and Kreevoy [21] and later expanded and supported by critical experimental data by Eliason and Kreevoy [22]. We will present the model in essentially the terms of Eliason and Kreevoy.

The alternative model is described in part in Fig. 2B. Here it is postulated that the reaction coordinate is not formed of coupled motions of the primary component of heavy-atom (i.e., non-hydrogen) reorganization and the secondary component of proton transfer. Instead the reaction coordinate is taken to be composed solely of motions of the primary, heavy-atom reorganization component; the secondary proton-bridging component is considered to be uncoupled from the reaction-coordinate motion. This would lead to substantially smaller hydrogen isotope effects at the bridging position. This would satisfy the objection of Swain et al. [8] that the isotope effects in general catalysis were too small to reflect a coupled proton-transfer component in the reaction coordinate.

The model was required, however, to account for the sometimes considerable rate accelerations deriving from the presence of the general catalytic entity in the transition state. The canonical model attributes the catalytic effect to the exergonic proton transfer that occurs in transition states in which the pri-



(a) the general-catalysis reaction coordinate on the canonical model



(b) the general-catalysis reaction coordinate on the alternative model

Fig. 2. Distinction of the canonical and alternative models of general acid–base catalysis. (a) The canonical model. The arrows in the transition-state structure suggest the schematic amplitudes of atoms in the reaction-coordinate motion, the motion along the unstable normal coordinate of the transition state that leads to decomposition back to reactants or on to products. The transfer of the proton between substrate and catalyst is coupled with the heavy-atom reorganization of the remaining part of the transition state, i.e., the formation of the O–C σ-bond and fission of the C–O π-bond. The proton is thus a participant in this unstable mode and may be said to exist in an unstable potential at the transition state. (b) The alternative model. Here the proton bridge between catalyst and the remainder of the transition state does not participate in the unstable normal coordinate or reaction coordinate. The proton now exists in a stable potential at the transition state. The proton bridge may be considered a hydrogen bond, and the energy released by the formation of this hydrogen bond stabilizes the transition state and thus generates the catalytic effect. Net proton transfer may or may not result from catalysis on this model, depending on the relative basicities of the hydrogen-bonding partners as the molecules emerge from the transition state. To achieve the requisite energy of formation and to generate the solvent isotope effects observed in general catalysis, the hydrogen bond must be a 'low-barrier hydrogen bond' (Eliason and Kreevoy [22]).

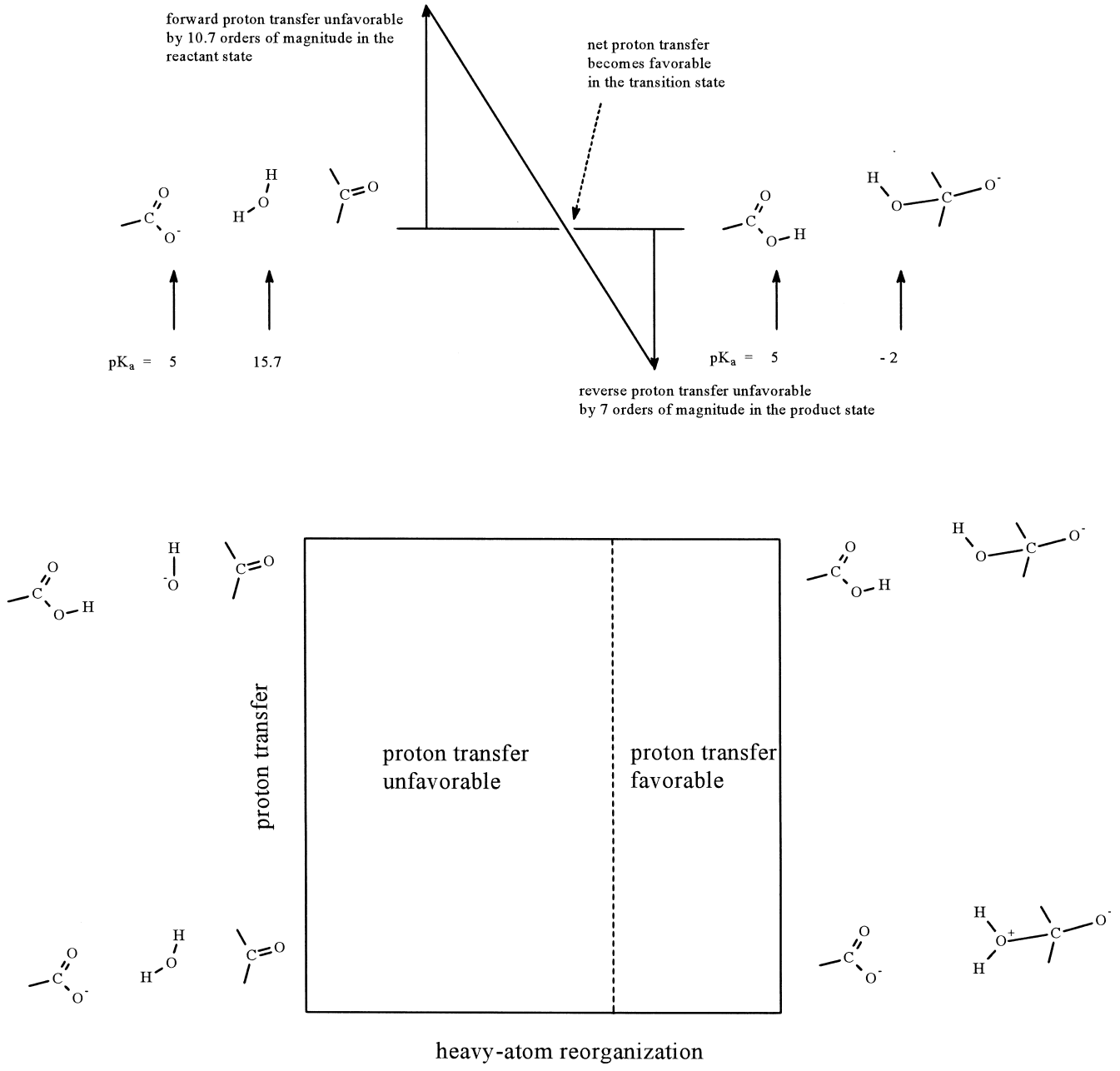


Fig. 3. Origin of catalytic acceleration in general acid–base catalysis on the canonical model in which net proton transfer accompanies heavy-atom reorganization, illustrated by the general-base catalyzed addition of water to a carbonyl group. The system obeys Jencks's rule [28]. In the reactant state, net proton transfer from substrate (water) to catalyst is unfavorable, while in the product state, net proton transfer from the catalyst to the product (conjugate base of the tetrahedral adduct but note that proton transfer *to the neutral OH group not to the negative oxygen center* is envisioned, since if the catalyst had not accepted the proton, the unstable tautomer of the carbonyl tetrahedral hydrate (often called $T^{\pm}$) would have formed) is unfavorable. Thus a range of 'early' transition-state structures does not allow for exergonic proton transfer to the catalyst at the transition state, while a range of 'late' transition-state structures does allow for exergonic proton transfer to the catalyst. Jencks's rule thus requires a cross-over of the relative pK_a values of catalyst and substrate proton donor. The release of energy by partial proton donation to the catalyst at the transition state decreases the free energy of the transition state and is the origin of the catalytic effect.

mary process has suitably progressed, but by uncoupling the two processes the alternative model renders this explanation of catalysis more difficult to apply. Instead, the alternative model holds that an unusually strong hydrogen bond is formed in general-catalytic transition states and produces the net transition-state stabilization observed.

The hydrogen bonding proposed clearly had to be unusual because of the free-energy release necessarily involved, as shown by the following argument [8]. Consider the catalytic effect of a base of pK 7 vs. the effect of a water molecule (pK −1.7) in a reaction possessing a Brønsted coefficient β of 0.5. The relative rate constants k_B for catalysis by the base B and k_W for catalysis by a water molecule are connected to their basicity constants by the Brønsted relationship as:

$$k_B/k_W = (K_B/K_W)^{\beta} = 10^{8.7\times 0.5} = 10^{4.4}\ \Delta G = -6.2\ \text{kcal/mol}\ (-26\ \text{kJ/mol}) \quad (1)$$

The rate constant ratio k_B/k_W is the equilibrium constant for the reaction

$$H_2O - H - X + B \rightarrow B - H - X + H_2O \quad (2)$$

where X represents the region of the transition state in which the primary, heavy-atom reorganization process is occurring. The calculation then indicates that the B–H–X hydrogen bond is stronger than the H_2O–H–X hydrogen bond by 6.2 kcal/mol, an unusual level of competition for any base against water in aqueous solution. Swain et al. [8] suggested the unusual strength might arise from mutual polarization of the hydrogen bridge and the (presumed) highly polarizable forming and breaking bonds within X. Eliason and Kreevoy [22], however, proposed that the strength arose from the fact that the hydrogen bridge was of the type lately referred to as a 'low-barrier hydrogen bond', and since such bridges had at that point been observed experimentally by Kreevoy and his coworkers, their model is obviously preferable.

1.4. Isotope effects: the role of proton inventories [11,12,18,19,23–25]

As explained above, a main reason for proposing the alternative model of the general-catalytic proton bridge was the fact that general catalysis is associated with smaller hydrogen isotope effects than would be anticipated for a reaction involving proton transfer coupled with heavy-atom reorganization. For example, bimolecular elimination reactions (E2 reactions) generally produce isotope effects k_H/k_D around 5–10, while those for general acid–base catalytic proton bridging are rarely larger than 3–4, are often as small as 1.5–2 and occasionally approach unity. This suggested to these authors [8,9] that proton transfer was not in fact occurring in the reaction coordinate of the transition state for general acid–base catalysis.

A complicating factor in the isotope-effect evidence is that studies of general-catalyzed reactions, including enzymic reactions, are very commonly conducted in water and occasionally in other protic solvents, but only very rarely in aprotic solvents. The protons that form general-catalytic bridges in transition states are, in the reactant states, essentially universally attached to oxygen, nitrogen, or sulfur centers with the result that their exchange with protic solvents occurs very rapidly and in fact generally at a diffusion-controlled rate. Thus the only way that the isotope effects for general-catalytic proton bridging can be measured is by isotopic labeling of the solvent so that the isotopic label in the catalytic site is introduced by exchange.

The most common experiments are deuterium solvent isotope effects, in which a rate constant measured for H_2O solution is compared with one for D_2O solution. In the case of small-molecule reactions, this fact raises the question whether the observed isotope effect does not have contributions from isotope effects on solvation changes or indeed whether the *entire* isotope effect does not arise from such isotopic medium effects. A main motivation for proposing substitution of the alternative model of general catalysis for the canonical model was that the observed (solvent) isotope effects were smaller than expected for proton transfer coupled to heavy-atom reorganization according to the canonical model. But if a medium effect in the opposite direction to the normal solvent isotope effect for the actual proton-bridging interaction were present, then the observed isotope effects might well be smaller than the expected values for canonical proton bridging, with the latter being hidden by the canceling medium-effect contributions.

The situation appeared even worse for enzymic

reactions. In addition to the possibility of medium effects arising from solvation changes in the same sense as for small-molecule reactions, there is the possibility of contributions from the very many exchangeable sites in the polypeptide structure of the enzyme. These considerations clearly dictated that the contributions from general-catalytic bridges in both small-molecule and enzymic reactions had to be isolated from the possible solvation and protein-structural isotope effects that could afflict the observations.

A reasonable solution was offered by the use of mixtures of isotopic solvents, a technique that was pioneered in the 1930s by Gross and Butler and then re-explored in the 1950s and 1960s, largely by Long, Gold, Swain and Thornton, and Kresge. Kresge [26] wrote a particularly influential review, referring to all this earlier literature, in which he provided a convenient and more general algebraic form for the Gross–Butler relationship between the observed solvent isotope effect and the individual isotope-effect contributions that make it up. Applied to the problem of medium or protein-structural effects combined with effects from general-catalytic proton bridging, the algebraic predictions reduce to three fairly simple cases:

$$k_n/k_0 = 1 + n(\varphi - 1) \text{ linear}$$

(no medium/structural effect) (3)

$$k_n/k_0 = Z^n \text{ exponential}$$

(medium/structural effect only) (4)

$$k_n/k_0 = [1 + n(\varphi - 1)]\, Z^n \text{ both medium/structural}$$

and bridging effects (5)

where n is the atom fraction of D in the solvent, k_n is the rate constant for a mixed solvent containing atom fraction n of D, k_0 is the rate constant for pure H_2O ($n=0$), $1/\varphi$ is the isotope effect for the proton-bridging site, and $1/Z$ is the medium isotope effect.

Distinctions among the models represented by these three cases are frequently possible with data of reasonably attainable precision. Fig. 4 shows the three models as they would be represented by experimental data for an observed isotope effect of 3. The

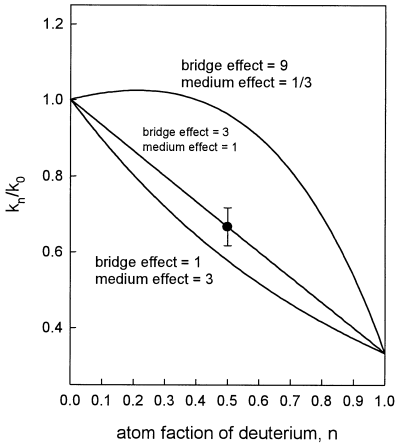


Fig. 4. Use of the proton-inventory method (rate constants as a function of deuterium content in mixtures of protium and deuterium oxides) to distinguish the component of a solvent isotope effect that is caused by a catalytic proton bridge from the component caused by small isotope effects at many points in the solvent or in the polypeptide structure of an enzyme ('medium effect'). The ratio k_n/k_0, where k_n is the rate constant in a mixed isotopic solvent containing atom fraction n of deuterium and k_0 is the rate constant in pure protium oxide ($n=0$), is plotted against the atom fraction of deuterium n. The three curves represent different models for the origin of an overall solvent isotope effect of 3. The linear curve is calculated for a model in which the entire isotope effect of 3 comes from a single site such as a catalytic proton bridge and no effect of the medium or protein structure is present; this is the model of Eq. 3 in the text. The lower, exponential curve assigns the entire effect of 3 to a medium or protein-structural effect (many small isotope effects at many different sites), as in Eq. 4 of the text. The upper curve corresponds to combined effects in which a large isotope effect of 9 at a single site is partly masked by an inverse medium/structural effect of 1/3 to give an overall effect of 3 (Eq. 5 in the text). The point shown in the center has error limits of ±10%, emphasizing that the technique can distinguish the limiting cases with reasonable experimental precision when the isotope effects are of the magnitudes shown.

situations in which this effect arises from a single site only (no medium effect), from a pure medium effect, and from a combination of the two are distinguishable with rate-constant precision of about 10%. The technique of employing rate measurements in mixtures of protium and deuterium oxides to produce an analysis of the number of exchangeable sites contributing to the isotope effect has become known, particularly among biochemists, as the *proton-inventory* method.

Generally, the project of using proton inventories to sort out the main contributions to general-cataly-

sis solvent isotope effects has been successful. In small-molecule systems, the isotope effects of 1.5–4 observed in general catalysis emerge as deriving from a single transition-state site, presumably the catalytic proton bridge. The original concerns about isotope-effect magnitudes that led to the proposal of an alternative model to the canonical model of coupled proton transfer and heavy-atom reorganization have thus been confirmed as justified. No important cases of medium effects that conceal or confuse the contributions of catalytic sites have been found.

In the case of enzymic reactions, an analogous result is in hand. When internal reactions after substrate binding are at issue (as in solvent isotope effects on parameters like k_{cat}) the general experience is an absence of medium effects. When substrate binding is kinetically included (as in solvent isotope effects on parameters like k_{cat}/K_m), medium effects are often observed but have been analytically divorced from the catalytic effects by the proton-inventory approach. The proton-bridge contributions to enzymic catalysis appear in fact to share the range of magnitudes of about 1.5–4 seen in non-enzymic reactions.

2. Approaches to distinction of the canonical and alternative models of the general-catalytic proton bridge

In this article, we would like to portray the application of two approaches to the distinction of the canonical and alternative models of general-catalytic proton bridging. One approach is venerable, having been introduced and used in the 1970s by W.P. Jencks and his coworkers, and is based on a detailed structure–reactivity formulation. Its importance for the problem of general-catalysis models seems not to have been sufficiently appreciated, and we would like now to emphasize its significance. The second approach has been very recently introduced by Limbach and his coworkers and is based on solid-state observations of hydrogen-bonded systems.

2.1. Structure–reactivity relationships [27–56]

Jencks and his coworkers introduced [27,29] a most original method of mechanistic study. One value of their technique is that it can be used for deducing the nature of the potential in which a transition-state proton is located, i.e., whether the potential is unstable (as the canonical model of general catalysis holds the potential for the catalytic bridge to be) or stable (as the alternative model of general catalysis holds the potential for the catalytic bridge to be). These studies rely on a complex of ideas about structure–reactivity relationships that developed over several decades but with particular intensity in the 1960s and 1970s. Jencks gathered the concepts into a systematic format that he called the *Bema Hapothle* [30], the letters of this invented phrase constituting an anagram of the initials of various scientists who contributed to the concept (the absence of a 'J' testifies to the well-known modesty of Jencks himself). The particular aspect that concerns us here is the use of interrelationships among the parameters of free-energy relationships that themselves describe the dependence of rate constants (thus transition-state free energies) for various reacting structures upon properties of the reactant or product states.

The approach of Jencks and his coworkers will be illustrated by one of their most thoroughgoing studies in this area [32], that of the reaction shown in Fig. 5. The reaction consists of the elimination from an adduct of the alcohol ROH, the proton of the product ROH being provided by a general-acid catalyst HA. It is feasible in the experimental system to vary ROH over a series of structures so that the pK_a of the alcohol varies from 12.4 (2,2,2-trifluoroethanol) to 16.0 (ethanol) and also to vary the general catalyst HA over a pK_a range from -1.74 (the hydronium ion) to 6.5 (dihydrogenphosphate). Gravitz and Jencks [32] measured a selection of the rate constants along both of these structural variables. The data (rate constants k) can then be treated according to two linear free-energy relationships:

$$\log k = [-\alpha(pK_{ROH})]\, pK_{HA} + \text{constant} \quad (6)$$

$$\log k = [\beta(pK_{HA})]\, pK_{ROH} + \text{constant}. \quad (7)$$

as is also shown in Fig. 5. The first of these relationships (Eq. 6) is the familiar Brønsted relationship for general acid catalysis, with its coefficient α. In the Gravitz–Jencks system, a value of α can be obtained for each choice of leaving-group ROH, so that the coefficient can be considered a function of pK_{ROH},

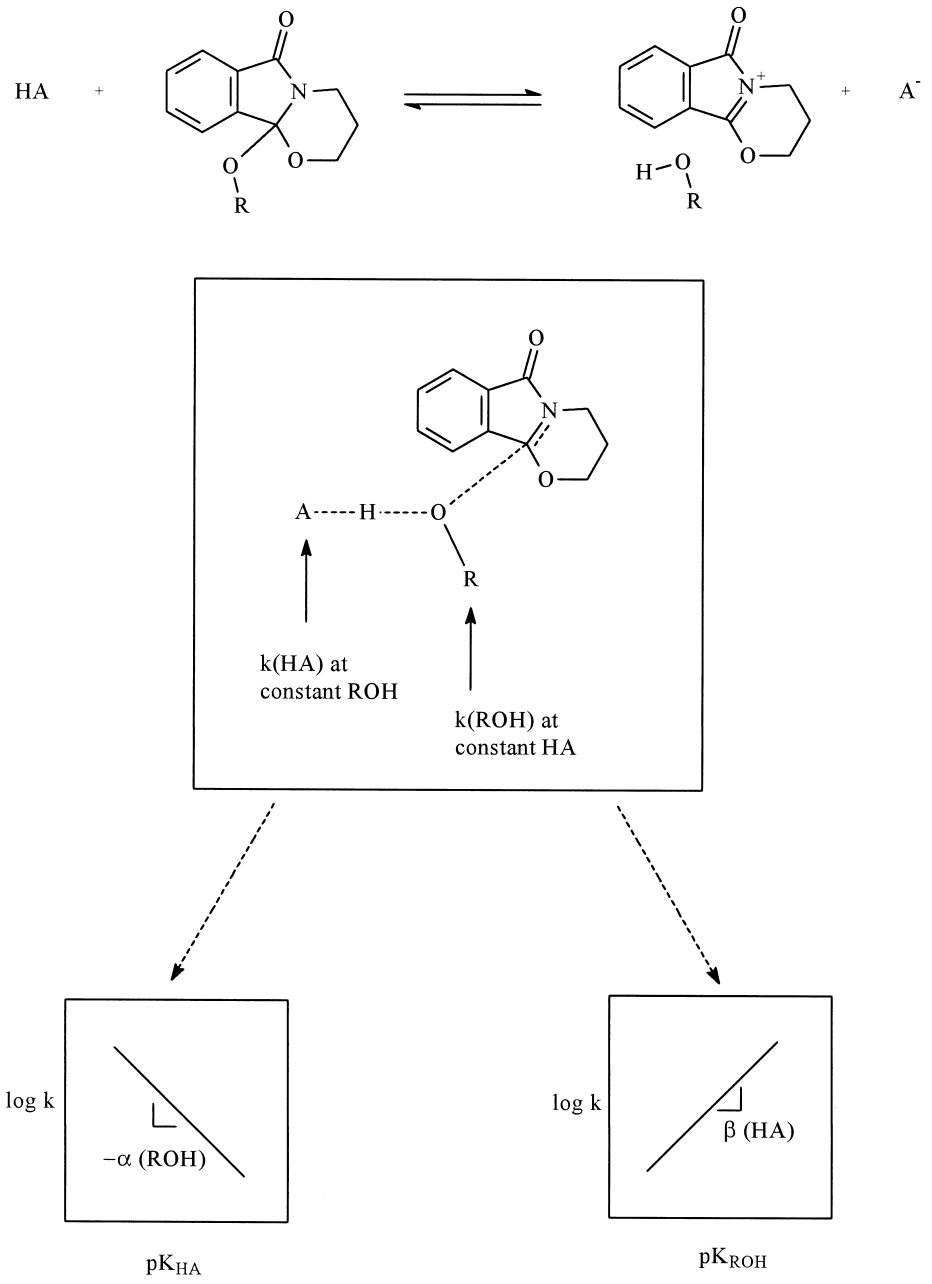


Fig. 5. The system of Gravitz and Jencks [32]. Structure–reactivity data consisting of rate constants k for the elimination of ROH with catalysis by the general acid HA can be obtained with variation of R for each choice of HA (alternatively stated, with variation of HA for each choice of R) and plotted in the two ways shown, corresponding to Eqs. 6 and 7 of the text.

and is therefore given in Eq. 6 as $\alpha(\mathrm{p}K_{\mathrm{ROH}})$. The second relationship (Eq. 7) is an extended Brønsted relationship. Here likewise the coefficient is designated $\beta(\mathrm{p}K_{\mathrm{HA}})$ because a value of the coefficient can be obtained for each choice of the general-acid catalyst HA.

The recent review of Williams [35] makes particularly clear the information to be obtained from coefficients like α and β, and Williams's viewpoint is followed here. Eq. 6 compares the activation process of Eq. 8 with the equilibrium of Eq. 9:

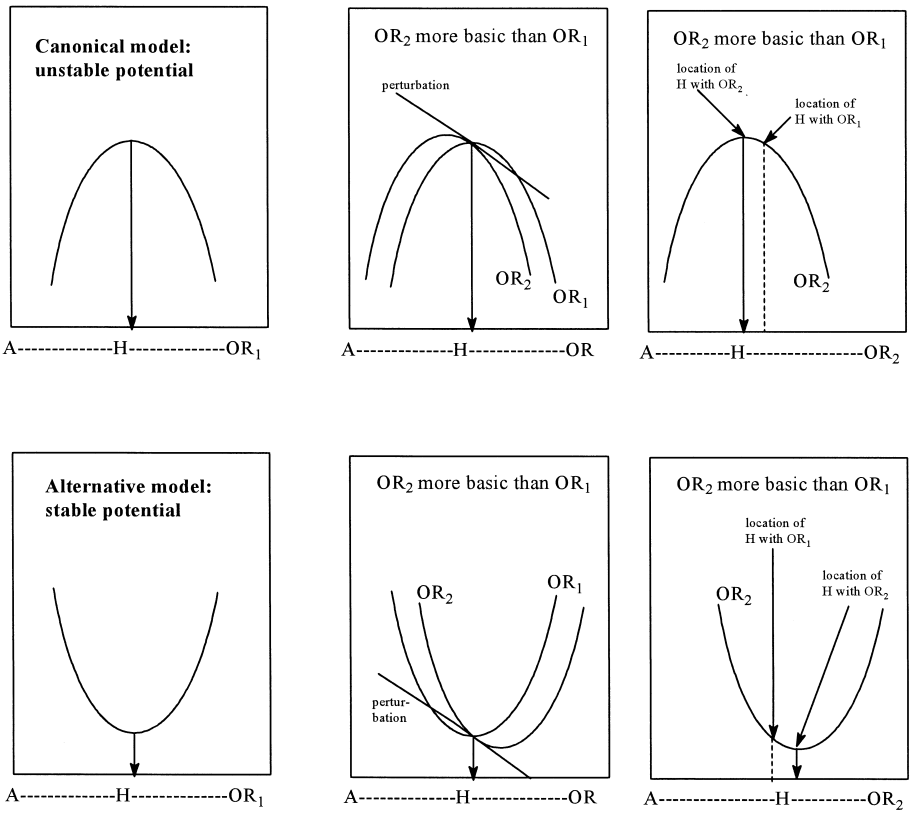


Fig. 6. Thornton's account [36] of the differing response, in hydrogen bridges with stable and unstable potentials, of the location of the bridging proton to a perturbation of the relative basicities of the binding partners of the proton. Above left: an unstable potential such as is postulated in the canonical model of general catalysis, on which the proton is a participant in the reaction-coordinate motion of the transition state. Above center: A perturbation (slanted straight line) is applied to the original (OR_1) potential that describes the conversion of OR_1 to a more basic structure OR_2. The perturbation indicates a lower energy for the proton when it is nearer the more basic partner. Addition of the perturbing potential to the original potential then produces a new one. Above right: The result is a shift in the location of the proton so that it is now closer to the relatively *less basic* partner. This is the counter-intuitive behavior typical of unstable potentials. Below left: a stable potential such as is postulated in the alternative model of general catalysis, where the transition state is stabilized by a strong hydrogen-bond proton bridge the components of which do not participate in the reaction-coordinate motion. Below center: The same perturbation as above (slanted straight line) is applied but now the proton moves closer to the relatively *more basic* partner. Below right: The new potential with the proton closer to the more basic partner. This is the intuitive result typical of stable potentials.

$$S + H - A \rightarrow S* - H - A \quad (8)$$

$$H - A \rightarrow H^+ + A^- \quad (9)$$

Here S refers to the reactant structure and S* to the part of the transition state other than that derived from the general acid HA. The slope of Eq. 6, $-\alpha(pK_{ROH}) = \partial(\log\ k)/\partial(pK_{HA})$, therefore measures the (negative) electrical charge on the moiety A in the transition state, or equivalently the degree of ionization of the H–A moiety in the transition state. Eq. 7 compares the activation process of Eq. 10 with the equilibrium of Eq. 11:

$$M - OR + HA \rightarrow M* - OR - H - A \quad (10)$$

$$H - OR \rightarrow H^+ + {}^-OR \quad (11)$$

where M refers to the remainder of the reactant state and M* to the remainder of the transition state. The slope of Eq. 7, $\beta(pK_{HA}) = \partial(\log\ k)/\partial(pK_{ROH})$, thus measures the charge, negative or positive, on the RO group in the transition state (if the degree of proton donation to RO exceeds the degree of RO–M* fission, a positive charge on RO will result, vice versa a negative charge).

Cordes and Jencks [27] first considered the phys-

ical significance of the 'cross-coefficients' obtained from plotting $\alpha(pK_{ROH})$ vs. pK_{ROH} or $\beta(pK_{HA})$ vs. pK_{HA}. The information content of the two slopes is identical:

$$\partial\alpha/\partial(pK_{ROH}) = -\partial^2(\log k)/\partial(pK_{ROH})\,\partial(pK_{HA}) \quad (12)$$

$$\partial\beta/\partial(pK_{HA}) = \partial^2(\log k)/\partial(pK_{HA})\partial(pK_{ROH}) \quad (13)$$

The magnitude of either slope answers the same question: how does the location of the proton in the catalytic proton bridge RO–H–A respond to a change in the basicity of either of the bonding partners, A or RO?

The value of this approach for our present purpose is that the direction of change in proton location is predicted by the Bema Hapothle *to be opposite on the canonical and alternative models of the catalytic bridge and thus to constitute evidence for choosing between the models.* The argument is presented in Fig. 6. As Thornton [36] was the first to show, the effect on the transition-state location of the bridging proton that is caused by changing the basicity of either bonding partner can be deduced by a perturbation approach. Fig. 6 demonstrates that a perturbation corresponding to a change from the less basic RO_1 to the more basic RO_2 (or equally from a more basic A_1 to a less basic A_2) will cause a relocation of the proton that differs depending on whether the proton bridge experiences a stable or an unstable potential. If the potential is stable (alternative model), then the proton will relocate closer to the more basic partner: an 'intuitive' result corresponding to the thermodynamically governed behavior familiar from reactant-state molecules which have universally stable potentials. If the potential is unstable (canonical model), then the proton will relocate closer to the *less basic* partner: a 'counter-intuitive' result that corresponds to the Hammond postulate in physical organic chemistry [37], a principle of which the Bema Hapothle is a generalization [30], and is characteristic of unstable potentials such as the reaction-coordinate potential of a transition state.

Now we consider the experimental findings of Gravitz and Jencks (Fig. 7). The two plots show a slope $\partial\alpha/\partial(pK_{ROH})$ (or $\partial\beta/\partial(pK_{HA})$) that indicates the proton in the general-catalysis proton bridge has moved toward the stronger base as the basicity of either the A moiety or the RO moiety is increased at the termini of the catalytic bridge. Thus the proton exhibits the intuitive behavior expected of a proton in a stable potential such as a strong hydrogen bond. This result is consistent with the alternative

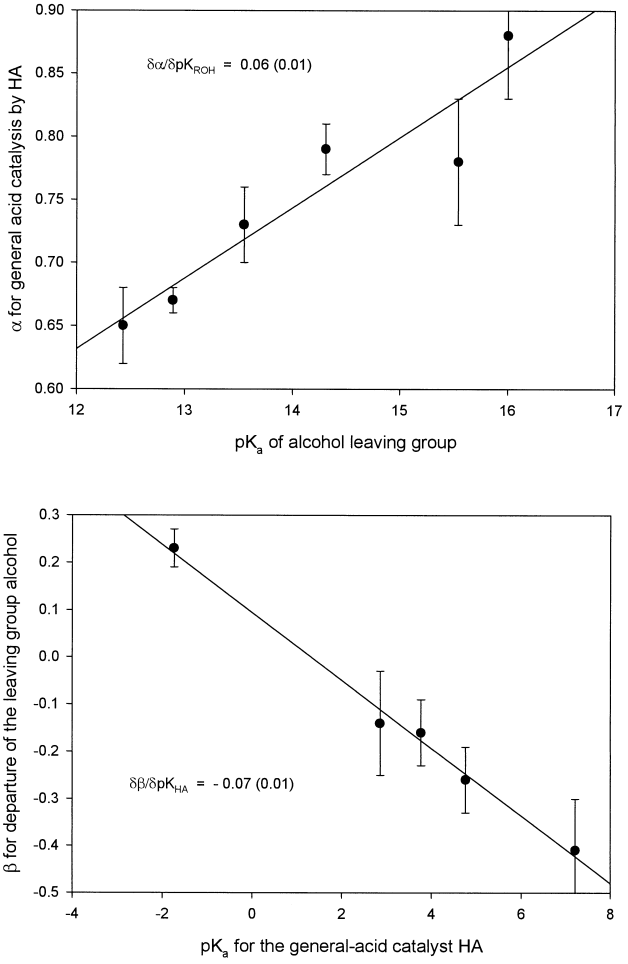


Fig. 7. Cross-correlations of linear free-energy parameters from the data of Gravitz and Jencks [32]. The parameters were recalculated with error estimates from the data of Gravitz and Jencks and do not differ importantly from theirs. Above: α for general-acid catalysis is plotted against pK_{ROH} for the leaving-group alcohol. The slope is $+0.06 \pm 0.01$, indicating that the bridging proton moves away from A and toward RO as the conjugate base of ROH becomes more basic. This is the intuitive result consistent with the alternative but not the canonical model. Below: β for variation of the leaving group ROH is plotted against pK_{HA} for the catalytic general acid. The slope is 0.07 ± 0.01, necessarily equal in absolute value to that above. As above, as A becomes a stronger base and the proton shifts toward it, the charge on RO drops from positive to increasingly negative.

model of general catalysis but not with the canonical model.

Furthermore, the slopes of the two plots in Fig. 7 suggest a valuable quantitative measure of the degree to which the location of the bridging proton responds to the basicity of the bonding partners. A unit change in the pK of one of the bonding partners may be expected to produce a shift in the proton location corresponding to a bond order change of slightly more than 0.05 units, around 0.06–0.07 units. For cases in which bond order about the proton is conserved, such a shift will result in transfer of >0.05 units of electrical charge between the termini for each unit change in the pK of a bonding partner. We shall return to this value in discussing applications of these results in enzyme catalysis.

The results obtained by Gravitz and Jencks are entirely representative of findings for general acid–base catalytic bridging between electronegative atoms. All examples cited in Lee's 1992 review [34] show the same result (the proton shifts toward an increasingly basic partner). In addition, the magnitude of the slopes is generally similar to the value of 0.06–0.07 obtained by Gravitz and Jencks. Gilbert and Jencks [56]) in 1982 provided a value of 0.05 and cited five cases in addition to Gravitz and Jencks where values "ranging up to 0.12" were observed.

2.2. Structural studies [38–43]

Within the recent past, structural studies of hydrogen-bridging systems by solid-state and low-temperature NMR methods [38,42] have been combined with results from neutron-diffraction crystallography [39–41] to provide a picture of proton bridges with stable potentials that clarify substantial features of the problem of general-catalytic proton bridging. An important example from these studies appears in Fig. 8.

The data from which Fig. 8 was constructed consist of precise values of the relative locations of proton donor, proton acceptor, and proton for hydrogen bonds between nitrogen bases, where the hydrogen donor and hydrogen acceptor are identical. When the nitrogen–nitrogen distance is large, such hydrogen bridges are 'asymmetric', i.e., the hydrogen is located closer to one partner than to the other. When the nitrogen–nitrogen distance is small, the hydrogen is located at the midpoint between the nitrogen centers and the bridge is 'symmetric'. Steiner and Saenger [39,40] made use of neutron-diffraction data to obtain values of the distances, largely for asymmetric hydrogen bonds. The data for symmetrical hydrogen bonds largely derive from NMR studies and rely on the establishment by Limbach and his coworkers of a relationship between interatomic distances and ^{15}N chemical shifts and coupling constants that permit the determination of accurate bond distances in hydrogen bridges between nitrogen bases [43].

Benedict et al. [38] showed that a plot of the sum vs. the difference of the bond distances about the bridging hydrogen produced a common curve that passed through the points for both crystallographic and NMR results. The curve indicated a decreasing sum of distances as the difference in distances became smaller (the donor atoms and acceptor atoms approached as the hydrogen bridge became more symmetrical), culminating in a minimum donor–acceptor distance when the difference in distances is zero, thus at the exactly symmetrical hydrogen bridge. In the spirit of Bürgi and Dunitz [44], the curve represents the trajectory of a hydrogen-transfer reaction, as the hydrogen donor approaches the acceptor, the system passes through a symmetrical transition state with a minimum donor–acceptor distance, and the products then depart from one another.

Fig. 8 presents the information in a way somewhat different from the presentation of Benedict et al. [38]. The distances have been converted to Pauling bond orders [45] and the order of one bond is plotted against the order of the other. Along a reaction trajectory, the two bonds could be considered the forming bond and the breaking bond. The Pauling bond order is defined by the exponential bond-distance–bond-order relationship shown in Fig. 8. The relationship has two parameters, the distance r_0 of a bond of unit order and a scaling constant b. The data in the figure were calculated by using the parameters shown, which were obtained by Benedict et al. [38] as a least-squares best fit on the assumption that the sum of the two bond orders is unity. As can be seen from Fig. 8 this relationship (represented by the diagonal straight line) holds exceedingly well, the

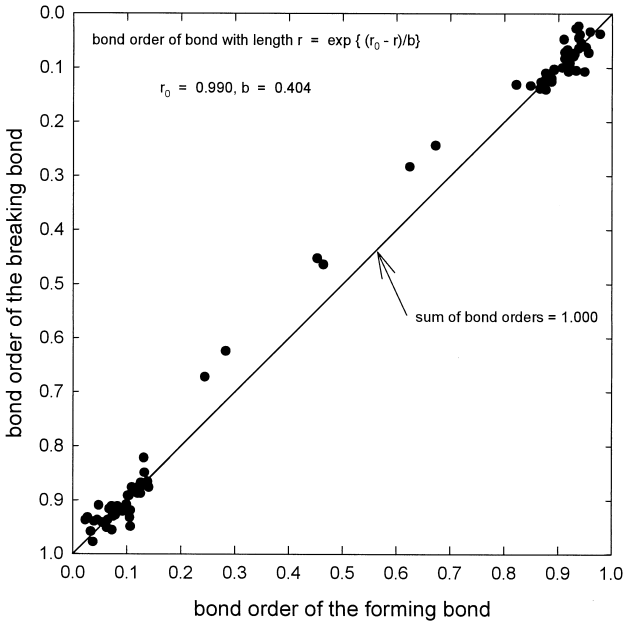


Fig. 8. Bond orders for the breaking vs. the forming bond about a bridging proton, as deduced from neutron-diffraction crystallographic and low-temperature NMR determinations of the two bond distances for hydrogen bonds between two identical nitrogen bases [38]. The bond order is defined by Pauling's formula at upper left, with the two parameters set according to the best fit of the data shown, on the assumption that the sum of the bond orders is always unity (diagonal line). Within a few percent, the assumption is met. Toward the center of the diagram, isotope fractionations corresponding to isotope effects K_H/K_D of up to 5–7 (at 100 K; around 2 at 300 K) are both observed and calculated and are relatively independent of structure within the range of bond orders of roughly 0.2–0.8.

departure from unity even for the most highly deviant points being only a few percent.

From the viewpoint we present in this article, a very important aspect of Fig. 8 is that *it provides an excellent structural model for the range of hydrogen bonds that could be involved in general acid–base catalytic proton bridging and it shows that, at least in the case of hydrogen bonds in stable potentials (as all of these, of course, necessarily are), unit bond order at hydrogen is preserved with good precision in all structures.*

A second significant aspect is the observation of substantial isotope fractionation in these stable hydrogen bridges for the bond-order region roughly 0.2–0.8 [42]. Such fractionation, corresponding to isotope effects of up to 5–7 at about 100 K and isotope effects around 2 at 300 K for formation of these hydrogen bridges, was expected on the basis of considerable work by Kreevoy and his coworkers.

A novel finding is that, within this bond-order region, the magnitude of the isotope fractionation is essentially independent of the structure (position of the proton). This result predicts that, for the formation of stable hydrogen bridges in the alternative model of general catalysis, isotope fractionation should indeed be observed as suggested by Eliason and Kreevoy [22] *and* the magnitude of the associated isotope effect should not vary much. This latter point is in accord with various observations of general-catalysis isotope effects, which are nearly always in the range 1.5–4 and commonly do not depart from the range 2–3.

Tunneling of the proton in general acid–base catalytic bridges has not been explicitly considered here. Evidence for tunneling in general-catalysis transition states not involving C–H bond formation or fission is currently lacking, although it may clearly play a role. The main conclusions reached below, however, seem not to depend much on this feature of the transition state.

2.3. *Evidence for and implications of a stable potential at the transition-state proton bridge for general acid–base catalysis*

As already described, the hypothesis that general acid–base catalytic proton bridges between electronegative atoms like oxygen, nitrogen, and sulfur (but not those involving carbon) possess a stable potential for the proton was based on the relatively small isotope effects for such bridges. Initial skepticism centered around the complexity of solvent isotope effects, but proton-inventory studies over two decades have shown the catalytic bridges themselves to yield isotope effects of only around 1.5–4, whether in enzymic or non-enzymic contexts. Later, this alternative model of general catalysis (opposed to the canonical model, in which the bridging proton occupies an unstable potential as a reaction-coordinate participant) was given substance by Eliason and Kreevoy [22] who pointed out (a) that net proton transfer could occur in favorable cases by way of a single transition state while maintaining the proton in a stable potential; (b) that isotope effects in the range 1.5–4 could well correspond to the low-barrier

hydrogen bonds then being examined experimentally and theoretically by Kreevoy and his coworkers.

The present article adds two items of evidence to the isotope-effect arguments favoring the alternative model of general catalysis. First, structure–reactivity studies of Gravitz and Jencks [32] from 1974, interpreted in terms of the Bema Hapothle [30], suggest that the proton occupies a stable potential in a general-catalytic bridge. This result holds for other cases of general acid–base catalysis as well [34,56]. This evidence was quite correctly interpreted at the time it was presented but its general implications seem not to have been adequately appreciated. Second, the crystallographic/NMR proton-bridge models developed by Limbach, Steiner, Saenger and their coworkers [38–43] show that (a) a range of stable structures that structurally simulate general-catalytic bridges, including bridges with centrally located hydrogen can be directly observed; (b) such bridges are capable of generating isotope fractionation in the range expected from isotope effects in general catalysis (as suggested by Eliason and Kreevoy [22]) and the magnitude of the fractionation is roughly independent of the location of the proton in the bridge, again in agreement with experiments on general catalysis.

These results, in our view, suggest that the alternative model for general catalysis is preferable to the canonical model. It emerges that application of the alternative model to general catalytic bridges in enzyme catalyzed reactions has implications for interpreting questions of enzyme catalytic efficiency, the substrate specificity commonly associated with enzyme action, and the evolution of both of these qualities in biological history. These implications are described in the final section of this paper.

3. Significance of a stable general-catalytic proton bridge for enzyme catalysis and evolution

The contribution of general acid–base catalysis to many enzyme-catalyzed reactions must count as perhaps the best established aspect of mechanistic enzymology. For our purposes, it is necessary to take note of two classes into which essentially all of the cases fall:

1. general acid–base catalytic proton bridging between two electronegative atoms, such as oxygen, nitrogen, or sulfur. This is the phenomenon we have treated in this article and we reached the conclusion that current evidence favors the alternative model of general catalysis, i.e., the bridging proton rests in a stable potential. We reached the further conclusion that to a good approximation, bond order is conserved at unity about the bridging proton.
2. general-base catalysis of the fission of C–H bonds, generally those activated by a neighboring carbonyl or similar functional group. There is excellent evidence that these bridging protons, in contrast to those between electronegative atoms, are in fact reaction-coordinate participants, and they exist in unstable potentials. Numerous studies have documented the large isotope effects characteristic of hydrogen transfer from carbon, and structure–reactivity studies of Gandler and Jencks [33], analogous in concept to those reviewed above, confirm from 'cross-coefficients' of structure–reactivity relationships that the proton bridge behaves as one in an unstable potential. However, evidence cited by Bernasconi [20] strongly suggested that proton transfer from carbon to general bases does *not* occur with conservation of unit bond order at the transferring proton. Table 1 of Bernasconi's review [20] gives eight examples of proton-transfer reactions from carbon in which the sum of bond orders (as calculated from structure–reactivity data) ranges from 0.96 to 0.11. It is thus clear that total bond order at the transferring proton in these cases may be substantially less than unity.

This dichotomy between proton bridges involving carbon and those not involving carbon as a bonding partner will be adhered to in the discussion below. After a very brief review of ideas about enzyme catalytic efficiency and specificity, two simple examples will be used to illustrate possible applications of the findings we have obtained above in understanding enzyme catalytic power and its evolution.

3.1. Enzyme and substrate structure, efficiency and specificity

Fig. 9 presents a schematic view of a typical en-

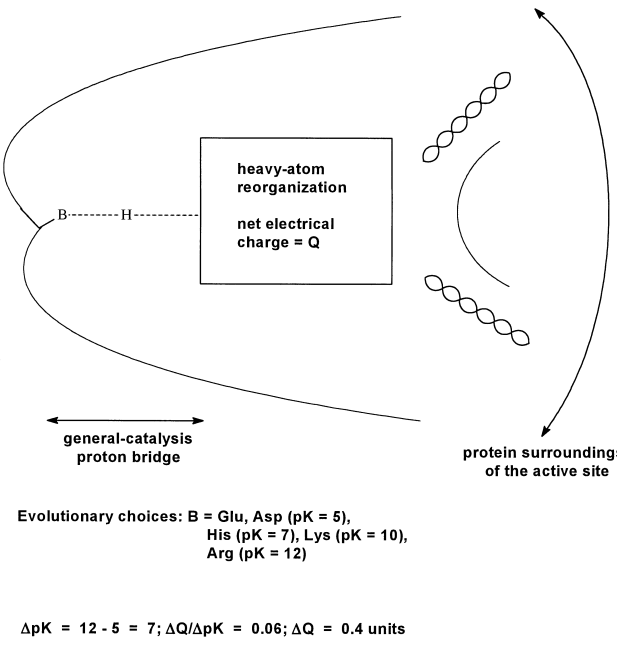


Fig. 9. Schematic account of the effect of evolutionary changes in the pK_a of a general-catalytic residue in an enzyme active site on the charge distribution within the reacting part of the transition state and thus the enzyme-transition state interaction. The basic residue B may logically have a pK_a (of its conjugate acid BH) as low as 5 for a Glu or Asp residue or as high as 12 for an Arg residue, so that the range of possible choices constitutes 7 pK_a units. If bond order at the bridging proton is conserved as argued from the results of Limbach and his coworkers [38], then changes in location of the bridging proton in response to changes in the pK_a of B will be reflected by changes in the net electrical charge within the remainder of the transition state, i.e., that part undergoing heavy-atom reorganization. From the studies of Gravitz and Jencks [32], a general-catalytic proton bridge can be expected to transmit changes in the pK_a of BH with an efficiency $\partial\beta/\partial(pK_{HB}) =$ ca. 0.06, so that the range of 7 pK_a units corresponds to a change in the net electrical charge within the heavy-atom unit by slightly over 0.4 charge units. If the proton bridge is in a stable potential (alternative model of general catalysis) then an increase in pK_a at B will result in an increase in negative charge within the heavy-atom unit. If the proton bridge is in an unstable potential (canonical model of general catalysis) then an increase in pK_a at B will result in a decrease in negative charge within the heavy-atom unit.

zyme active site, in which a general-catalytic proton bridge participates in stabilizing the transition state for a reaction that also involves heavy-atom reorganization in the remainder of the substrate-derived part of the transition state. There are a number of possible basic side chains among the 20 common amino acid residues that the enzyme may provide at the position B (whether the proton bridge represents donation from BH to the heavy-atom part of the transition state – general-acid catalysis – or proton donation from the heavy-atom part to B – general-base catalysis – makes no difference to the arguments to be considered here). What the features are of enzymic evolution or of reaction mechanisms that lead to the selection of a particular base is a question that remains currently unresolved. The range of acidity/basicity among the possible structures is quite broad: as shown in Fig. 9, a number of enzymes rely on relatively weak carboxylate bases with pK values near 5 while arginine residues at the other end of the scale have pK values near 12.

The considerations we have worked through in the present article allow us to make some predictions about what effect a change in the identity of the basic residue (e.g., in a point mutation during molecular evolution). The assumptions on which the predictions will be based are:

1. General-catalysis proton bridges between oxygen, nitrogen, and sulfur will have the proton in a stable potential and the location of the bridging proton will accordingly shift toward a residue that is changed to a more basic residue and away from a residue that is changed to a less basic residue.
2. For proton bridging between electronegative atoms, bond order will be conserved at unity about the bridging proton so that changes in the location of the proton will generate shifts in electrical charge within the bonding partners but no electrical charge will accumulate on the bridging proton itself.
3. Changes in such linear-free energy parameters as the Brønsted or extended Brønsted α or β will be taken to follow the appropriate rules of the Bema Hapothle for stable potentials (all catalytic bridges except those to carbon).
4. Following Williams [35], we will take such parameters as α or β to correspond to electric charge in the appropriate transition-state bonding partner of the bridging proton.
5. We will take the slopes of the 'cross-correlation' plots of Gravitz and Jencks [32], in agreement with the values summarized by Gilbert and Jencks [56], to give an approximate sensitivity of the

shifts to be expected (about 0.06 units of charge per unit change in pK of a bonding partner).
6. Proton transfers to and from carbon will not be treated because of the apparent unpredictability of the total bond order at the transferring proton [20].

3.2. General example

Following these concepts, consider the consequences of a point mutation that converts an Asp residue (pK 5) to an Arg residue (pK 12) at point B in an active site such as that of Fig. 9. We take the potential about the bridging proton to be stable so that the increase in basicity of residue B will result in the relocation of the proton toward the residue B (point 1). We take bond order to be conserved about the proton (point 2) so that the decrease in negative or increase in positive electrical charge at B, occasioned by relocation of the proton nearer to it, will be accompanied by an equal and opposite change in electrical charge within the entity to which the proton bridge reaches (points 3, 4). Thus the quantity Q in Fig. 9, the net electrical charge in the structure undergoing heavy-atom reorganization, will become more negative as a result of the mutation at B of Asp to Arg. The magnitude of this increase in negative charge can be estimated (points 3, 4, 5) as $\Delta Q = 0.06 \times \Delta pK = 0.06 \times 7 =$ ca. 0.4 units of charge. This is a large change in the amount of electrical charge and should be reflected in significant alterations in the interaction energetics of this moiety with its surroundings. Furthermore, because of bond-order conservation at the bridging proton, there must be a complementary increase in positive charge or decrease in negative charge at position B, which will cause an equally significant alteration in the interaction energetics of the moiety B with its surroundings.

The surroundings of the two parts of the active site are indicated schematically by curved lines and representations of helices. In any realistic case, the interactions thus represented will consist of electrostatic attractions and repulsions (see particularly Warschel [46]), hydrogen bonds of various kinds and strengths, van der Waals and hydrophobic interactions – the familiar gamut of contributions to transition-state and reactant-state stabilization or destabilization by enzymes [46,47]. When a mutation such as that we are envisioning results in the large shift in electrical charge predicted above, then the surrounding components and structures of the active site are presented with a substantially altered set of interaction partners. If the new situation leads to a more favorable total energy, then the mutation will have been a favorable one, otherwise an unfavorable one.

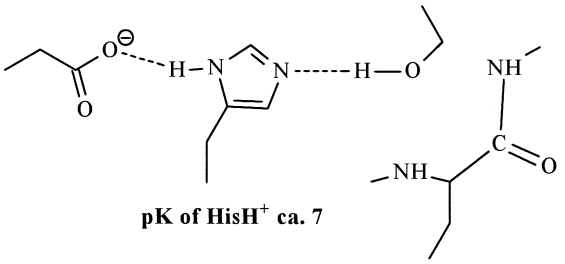
A point that emerges from our considerations here is that a change at B in the active site does not have implications only for nearby surrounding structures. Instead, because the hydrogen bridge with conserved bond order has a good efficiency for transmitting charge shifts (as indicated by the magnitudes of the 'cross-correlation' slopes), a change at B will produce possibly drastic changes in interaction of distant surrounding structures with other parts of the transition state. Of course, changes in other parts of the transition state will communicate their effects across the hydrogen bridge similarly and produce possibly large effects at the B site.

In summary, our considerations suggest that mutations in active-site structures that are realistic in terms of available protein structural components can produce large changes in electrostatic and other interactions between enzymes and bound transition-state structures, when the sites of mutation are involved in general-catalytic hydrogen bridging. The changes in enzyme-transition state interaction will not be local but may extend to considerable distances from the site of mutation through communication across catalytic hydrogen bridges. Furthermore, the conclusions reached above were based on the assumption of a stable proton bridge, for the alternative model of general catalysis.

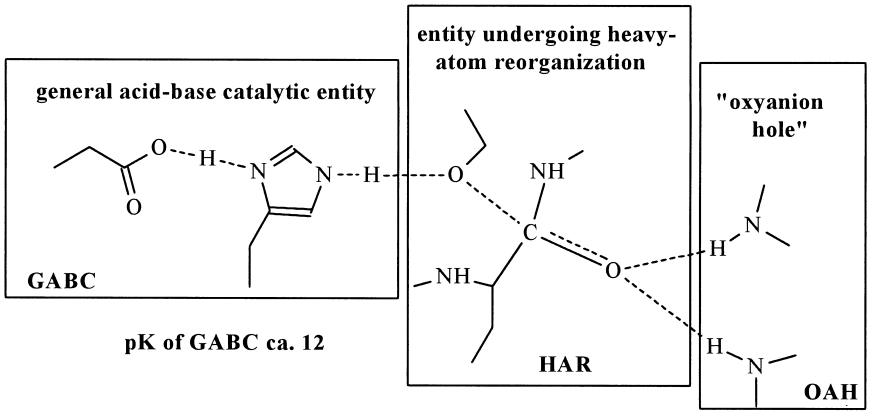
3.3. The serine-protease system [18,48–52]

A more concrete application of the concepts described above can be made to catalysis by the serine proteases, thanks to pioneering studies of Liang and Abeles [49] and Finucane and Malthouse [50], lately re-emphasized by Cassidy et al. [51]. The points involved are summarized in Fig. 10.

Fig. 10a shows the familiar structure of the active site of a serine protease with bound peptide substrate. The entity that exercises general acid–base



(a) reactant state: local charge - 1, mostly localized in Asp or Asp/His



(b) transition state: local charge - 1, now delocalized over three substructures: $Q_{GABC} + Q_{HAR} + Q_{OH} = -1$.

Fig. 10. Application of models for general-catalytic proton bridging to the question of transition-state charge distribution and origins of catalytic power in serine proteases. (a) Schematic portrayal of the reactant-state enzyme–substrate complex. Protonation of the His residue is argued on the basis of NMR observations to generate a low-barrier hydrogen bond between Asp and His [52] but this interaction seems not to perturb the p*K* of the His, which remains at the commonly observed value of about 7. A weak hydrogen bond may bind the Ser to His. The overall electrical charge in the region shown is unit negative, localized on Asp or possibly shared to some degree by Asp and His. (b) Transition state for nucleophilic attack of the Ser hydroxyl group on the substrate carbonyl function, with general-base catalysis by the Asp/His unit. The structure is divided into three substructures which are connected to each other by proton bridges. The substructure at left, the *general acid–base catalytic entity*, abbreviated GABC, consists of the Asp–His dyad. It is connected by a general-base catalytic proton bridge to the second substructure, the *entity undergoing heavy-atom reorganization*, abbreviated HAR. The reaction-coordinate motion for the transition state involves formation of the C–O σ-bond and fission of the π-bond of the carbonyl group. On the canonical model of general catalysis, the bridging proton and some atoms of the GABC also participate in the reaction coordinate. On the alternative model of general catalysis, only the atoms in the HAR participate in the reaction coordinate. The third substructure is the '*oxyanion hole*', abbreviated OAH and is formed of two backbone N–H bonds that donate proton bridges to the carbonyl-oxygen in the transition state. The transition-state overall charge is the same as in the reactant state, unit negative. The charge is now delocalized over the three substructures shown. Furthermore, the transition-state p*K* of the GABC is raised from 7 to 12 [49–51]. The charge distribution within the active site should be radically affected by this increased basicity, and the direction of the effect will depend on the nature of the proton bridges connecting the substructures, as described in the text.

catalysis in the action of serine proteases is the Asp–His dyad at left. The hydrogen bridge between Asp and His has been suggested on the basis of NMR studies to be a low-barrier hydrogen bond when the His is protonated [52], but if this is the case, the interaction seems not much to alter the overall proton affinity of the unit, the p*K* remaining in the normal range of about 7.

Fig. 10b portrays the transition-state situation as the serine hydroxyl group is nucleophilically attacking the substrate peptide–carbonyl group. The general acid–base catalytic entity (GABC), shown in the

box at the left, is connected by a hydrogen bridge (the proton at the right side of the box marked 'GABC'), which we take to be in a stable potential, to the entity undergoing heavy-atom reorganization (HAR) as the new C–O σ-bond is formed and the carbonyl π-bond is broken (center box). Further proton bridging is occurring between the carbonyl oxygen and the backbone N–H groups of the 'oxyanion hole' (box at right). *Because all hydrogen bridges shown are between electronegative atoms, we take them all to be in stable potentials and to respond appropriately to structural changes.*

A major discovery by Liang and Abeles [49] and Finucane and Malthouse [50] was that, when a transition-state analog was bound to the serine center, the p*K* of the Asp–His dyad was increased to a value of 12. We do not wish to enter into the discussion of the origin of the increased p*K* [51]. Instead, we wish to address the question: how does the fact that the transition-state p*K* of the general acid–base catalytic entity is 12 instead of 7 affect the charge distribution in the transition state? The interaction of the surroundings of the active site with the distributed charge is likely to contribute strongly to catalysis. Therefore, the effect of the p*K* shift from 7 to 12 in reorganizing the charge distribution has implications for the magnitude of catalysis through interaction with the active site surroundings.

As noted in Fig. 10 (see the caption for definitions of abbreviations used below), the local net charge in the active site is unit negative in both reactant and transition state. Taking a hypothetical transition state with a GABC p*K* of 7 as a reference state, the charge shifts induced by alteration to the real transition state with pK 12 can be estimated as above:

$$Q_{\mathrm{GABC}} + Q_{\mathrm{HAR}} + Q_{\mathrm{OH}} = -1 \tag{14}$$

$$\Delta \mathrm{p}K_{\mathrm{GABC}} = 7-12 = -5 \tag{15}$$

$$\Delta(Q_{\mathrm{HAR}} + Q_{\mathrm{OH}}) = 0.06\times\Delta \mathrm{p}K_{\mathrm{GABC}} = -0.3 \tag{16}$$

$$\Delta Q_{\mathrm{GABC}} = +0.3. \tag{17}$$

Thus the actual serine-protease transition state, where the GABC has a p*K* of 12, will experience an overall shift of 0.3 units of electrical charge out of the GABC and into the combined HAR and OH units. Indeed the effectiveness of the 'oxyanion hole' in contributing to catalysis may be critically dependent on this charge shift.

It is useful to note that a contrary result would have been obtained if the canonical, rather than the alternative, model of general catalysis had been applied. Then the direction of the charge shift would have been reversed. The increased p*K* of the GABC would have resulted in a relocation of the proton further from the more basic unit. If the efficiency of charge shift were the same as above, the result would have been an increase in *positive* charge in the HAR/OAH unit.

4. Summary

In this article, we have brought together some information from disparate sources to attempt to form a picture of how the phenomenon of general acid–base catalysis may contribute to the generation of enzyme catalytic power and how it may influence the evolution of catalytic power. Old evidence from isotope effects was reviewed, leading to the important proposal of Eliason and Kreevoy [22] that general-catalytic proton bridges are strong hydrogen bonds in stable potentials. Evidence from structure–reactivity studies of Jencks and his coworkers [27–33] was added, reinforcing the view of a stable potential and providing a quantitative measure of the effectiveness of general-catalytic proton bridges in transmitting electrical charge between bonding partners. Then recent results from Limbach and his colleagues [38–43] were considered, in which a trajectory for a stable hydrogen bridge that can lead to net proton transfer was deduced from crystallographic and NMR sources with the important observations that such bridges preserve the bond order at unity for the bridging hydrogen as well as giving isotope fractionation consistent with results from general catalysis. Finally, these results were combined to generate a scheme for estimating the magnitudes of charge shifts in enzyme transition states occasioned by realistic examples of mutations of general-catalytic residues, or of changes in their acid–base properties between reactant state and transition state. Application to the serine-protease system suggested that the increase of the p*K* of the acid–base catalytic by 5 units on

entering the transition state, as observed by Liang and Abeles [49] and Finucane and Malthouse [50], would effect a charge shift of around 0.4 units (negative) out of the acid–base catalytic dyad into the serine-substrate assembly, possibly enhancing the effectiveness of the 'oxyanion hole'. In general, the expectation of fairly large electrical-charge shifts over considerable distances emphasizes the likely non-local character of mutations and other structural effects in enzymic systems that involve hydrogen bridging.

Acknowledgements

We are pleased to thank the Stiftung Volkswagenwerk, Hannover, the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, the Fonds der Chemischen Industrie, Frankfurt, the Russian Foundation for Fundamental Research, and the Alexander-von-Humboldt-Stiftung, Bonn-Bad Godesberg.

References

[1] J.P. Richard, Biochemistry 37 (1998) 4305–4309.
[2] A.J. Kirby, Acc. Chem. Res. 30 (1997) 290–296.
[3] W.W. Cleland, P.A. Frey, J.A. Gerlt, J. Biol. Chem. 273 (1998) 25529–25532.
[4] J.P. Guthrie, R. Kluger, J. Am. Chem. Soc. 115 (1993) 11569–11572.
[5] P.C. Babbitt, J.A. Gerlt, J. Biol. Chem. 272 (1997) 30591–30594.
[6] W.P. Jencks, J. Am. Chem. Soc. 94 (1972) 4731–4732.
[7] W.P. Jencks, Chem. Rev. 72 (1972) 705–718.
[8] C.G. Swain, D.A. Kuhn, R.L. Schowen, J. Am. Chem. Soc. 87 (1965) 1553–1561.
[9] R.L. Schowen, Prog. Phys. Org. Chem. 9 (1972) 275.
[10] R.D. Gandour, G.M. Maggiora, R.L. Schowen, J. Am. Chem. Soc. 96 (1974) 6967–6979.
[11] J.P. Elrod, R.D. Gandour, J.L. Hogg, M. Kise, G.M. Maggiora, R.L. Schowen, K.S. Venkatasubban, Faraday Symp. Chem. Soc. 10 (1975) 145–153.
[12] R.L. Schowen, in: W.W. Cleland, M.H. O'Leary, D.B. Northrop (Eds.), Isotope Effects on Enzyme-Catalyzed Reactions, University Park Press, Baltimore, MD, 1977.
[13] G.M. Maggiora, R.L. Schowen, in: E.E. van Tamelen (Ed.), Bioorganic Chemistry, Vol. 1, Academic Press, New York, 1977, pp. 173–229.
[14] I.H. Williams, D. Spangler, D.A. Femec, G.M. Maggiora, R.L. Schowen, J. Am. Chem. Soc. 102 (1980) 6619–6621.
[15] I.H. Williams, G.M. Maggiora, R.L. Schowen, J. Am. Chem. Soc. 102 (1980) 7831–7839.
[16] I.H. Williams, D. Spangler, D.A. Femec, G.M. Maggiora, R.L. Schowen, J. Am. Chem. Soc. 105 (1983) 31–40.
[17] I.H. Williams, D. Spangler, G.M. Maggiora, R.L. Schowen, J. Am. Chem. Soc. 107 (1985) 7717–7723.
[18] R.L. Schowen, in: J.F. Liebman, A. Greenberg (Eds.), Mechanistic Principles of Enzyme Activity, VCH Publishers, New York, 1988, pp. 119–168.
[19] F.J. Alvarez, R.L. Schowen, Isot. Org. Chem. 7 (1987) 1–60.
[20] C.F. Bernasconi, Adv. Phys. Org. Chem. 27 (1992) 119–238.
[21] E.H. Cordes, Prog. Phys. Org. Chem. 4 (1967) 1–44.
[22] R. Eliason, M.M. Kreevoy, J. Am. Chem. Soc. 100 (1978) 7037–7041.
[23] K.B. Schowen, R.L. Schowen, Methods Enzymol. 87C (1982) 551–606.
[24] K.S. Venkatasubban, R.L. Schowen, Crit. Rev. Biochem. 17 (1984) 1.
[25] D.M. Quinn, L.D. Sutton, in: P.F. Cook (Ed.), Enzyme Mechanism from Isotope Effects, CRC Press, Boca Raton, FL, 1991, pp. 73–126.
[26] A.J. Kresge, Pure Appl. Chem. 8 (1964) 243–258.
[27] E.H. Cordes, W.P. Jencks, J. Am. Chem. Soc. 84 (1962) 4319–4328.
[28] W.P. Jencks, Chem. Rev. 72 (1972) 705–718.
[29] D.A. Jencks, W.P. Jencks, J. Am. Chem. Soc. 99 (1977) 7948–7960.
[30] W.P. Jencks, Chem. Rev. 85 (1985) 511–527.
[31] W.P. Jencks, Acc. Chem. Res. 9 (1976) 425–432.
[32] N. Gravitz, W.P. Jencks, J. Am. Chem. Soc. 96 (1974) 507–515.
[33] J.R. Gandler, W.P. Jencks, J. Am. Chem. Soc. 104 (1982) 1937–1951.
[34] I.H. Lee, Adv. Phys. Org. Chem. 27 (1992) 57–117.
[35] A. Williams, Adv. Phys. Org. Chem. 27 (1992) 1–55.
[36] E.R. Thornton, J. Am. Chem. Soc. 89 (1967) 2915–2927.
[37] G.S. Hammond, J. Am. Chem. Soc. 77 (1955) 334–340.
[38] H. Benedict, H.-H. Limbach, M. Wehlan, W.P. Fehlhammer, N.S. Golubev, R. Janoschek, J. Am. Chem. Soc. 120 (1998) 2939–2950.
[39] T. Steiner, W. Saenger, Acta Crystallogr. B50 (1994) 348–357.
[40] T. Steiner, J. Chem. Soc. Chem. Commun. (1995) 1331–1332.
[41] P. Gilli, V. Bertolasi, V. Ferretti, G. Gilli, J. Am. Chem. Soc. 116 (1994) 909.
[42] S.N. Smirnov, H. Benedict, N.S. Golubev, G.S. Denisov, M.M. Kreevoy, R.L. Schowen, H.-H. Limbach, Can. J. Chem. 77 (1999) 943–949.
[43] S.N. Smirnov, N.S. Golubev, G.S. Denisov, H. Benedict, P. Schah-Mohammedi, H.-H. Limbach, J. Am. Chem. Soc. 118 (1996) 4094–4101.
[44] H.B. Bürgi, J.D. Dunitz, Acc. Chem. Res. 16 (1983) 153–161.
[45] L. Pauling, J. Am. Chem. Soc. 69 (1947) 542–553.
[46] A. Warshel, J. Biol. Chem. 273 (1998) 27035–27038.

[47] W.R. Cannon, S.J. Benkovic, J. Biol. Chem. 273 (1998) 26257–26260.
[48] J.J. Perona, C.S. Craik, J. Biol. Chem. 272 (1997) 29987–29990.
[49] T.C. Liang, R.H. Abeles, Biochemistry 26 (1987) 7603–7608.
[50] M.D. Finucane, J.P.G. Malthouse, Biochem. J. 286 (1992) 889–900.
[51] C.S. Cassidy, J. Lin, P.A. Frey, Biochemistry 36 (1997) 4576–4584.
[52] P. Frey, S.A. Whitt, J.B. Tobin, Science 264 (1994) 1927–1930.
[53] R. Breslow, S.D. Dong, Y. Webb, R. Xu, J. Am. Chem. Soc. 118 (1996) 6588–6600.
[54] T.D. Meek, in: M.L. Sinnott (Ed.) Comprehensive Biological Catalysis, Vol. 1, Academic Press, London, 1997, pp. 327–344.
[55] T. Kubiak, R.J. Hondal, X. Yue, M.D. Tsai, K.S. Bruzik, J. Am. Chem. Soc. 121 (1999) 488–489.
[56] H.F. Gilbert, W.P. Jencks, J. Am. Chem. Soc. 104 (1982) 6769–6779.