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Possibility of a Double Well Potential in the Proton Bridge of Visual Pigments and Bacteriorhodopsin

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The resonance Raman effect is examined in the case of vibrations which are governed by a double well potential. It is shown that in such a case the intensity of a resonance Raman line depends on three factors: the relative populations of the two wells in the ground state and in the resonating electronic excited state and the displacement of the potential minima between the ground state and the excited state. Depending on the shapes of the potentials in the two states, the ground state populations might become unimportant as a factor determining intensities and the only observed Raman line might be due to the less populated well. It is suggested that such a situation is likely to exist for the proton bridge of the chromophore of visual pigments and bacteriorhodopsin, the Schiff bases of 11-*cis* and all-*trans* retinals respectively. This raises the question if resonance Raman spectra alone are capable of giving evidence of the extent of protonation of the chromophore in the ground state.

INTRODUCTION

Hydrogen bonds are ubiquitous in the living organism. They play a decisive role in shaping proteins and nucleic bases, link water molecules to each other and to other proton donors and acceptors. The chemistry of life cannot be understood without them.

Most of the H-bonds formed in biological media are weak or medium strong, their enthalpies are in the 10—30 kilojoules per mole range. We shall not be concerned with these in the present communication. Rather we will turn to the following question, intriguing to infrared spectroscopists: does the living body make use of strong H-bonds?

The answer can only be yes. Strong H-bonds must exist at every ionic or highly polar site. The phosphate groups in the polar heads of lipids, for example, must hold water molecules with H-bonds in the range of 50—100 kilojoules per mole. We are, however, aiming at more specific H-bond systems. In what follows the suggestion is made that there is a strong H-bond connected with the chromophore of visual pigments whose nature is not yet sufficiently understood.

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THE VISUAL CHROMOPHORE; ISOMERIZATION AND PROTONATION

As is well known the chromophore of visual pigments is the Schiff base of 11-*cis* retinal (Figure 1) (Reviews[1,2]). It is linked to a protein (opsin)
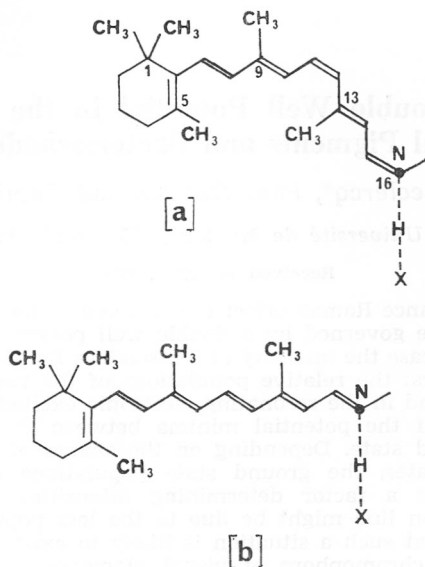

Figure 1. The hydrogen-bonded Schiff bases of visual pigments [a] and bacteriorhodopsin [b].

through the Schiff base linkage, forming rhodopsin. The structure and absorption spectrum of the chromophore is profoundly affected by its protein (and, perhaps, lipid) environment. Whereas, in a test-tube the Schiff base of 11-*cis* retinal has the maximum of its characteristic, intense, lowest singlet--singlet (π, π^*) band at about 370 nm, in the (human or bovine) visual pigment it is at about 500 nm. It varies a great deal from (animal) species to species, from about 400 to about 600 nm. Furthermore, to our present knowledge, in cones, which provide for color vision, the three different chromophores only differ by their molecular environment; they all contain the Schiff base of 11-*cis* retinal otherwise.

These color variations constitute an important problem for research on the initial photochemical step of vision. To explain the shift from 370 to 500 nm, first the Schiff base is supposed to be protonated. This could account for about half of the shift. The other half could then be attributed to the field of polar groups in the surrounding protein[3,4,5]. Both of these conditions have been the target of intensive research.

The strongest argument in favor of protonation of the Schiff base nitrogen comes from resonance Raman measurements. Oseroff and Callender[6] found a band at 1655 cm^{-1} in the spectrum of rhodopsin which they assigned to the stretching of the protonated C=NH$^+$ bond. This was subsequently confirmed by new measurements and by normal coordinate analysis[7,10]. More importantly, this band showed a bathochromic shift upon deuteration. 100 p. c. protonation of the Schiff base is, indeed, considered as an experimental fact by many researchers in the field of visual pigments. Connected with

this is the assumption that the primary photochemical event of vision is 11-*cis* to all-*trans* isomerization leading from rhodopsin to bathorhodopsin.

As to the additional polar groups whose effect is also needed to explain the location of the (π, π^*) absorption band, these are located, according to the results of Nakanishi and coworkers[11] in the $C_{12} - C_{14}$ region for rhodopsin and in the C_5 region, near the β-ionone ring for bacteriorhodopsin. This work was based on the successive saturation of the different double bonds in the chromophore. Earlier theoretical work by Wiesenfeld and Abrahamson[4] and by Mantione and Pullman[5] placed the additional polar groups near the β-ionone ring. The identity of these groups is not known; in all probablility they are carboxylate groups of the aminoacid residues of the surrounding protein.

For bacteriorhodpsin[12], whose chromophore is the Schiff base of all-*trans* retinal, the problems of protonation and additional polar perturbers are similar.

The essence of the theory described in this section is then 100% protonation of the Schiff base and *cis-trans* isomerization as the photochemical primary step.

ALTERNATIVE IDEAS AND FURTHER DEVELOPMENTS

The above described and seemingly satisfactory views on the photochemical part of the mechanism of vision were challenged by our team.[13-20] The following reasons can be invoked.

1) A wealth of infrared spectroscopic data and other physico-chemical evidence shows that whenever a nitrogen base is protonated the proton remains hydrogen-bonded to the »original« proton donor, or in other words, there must be a counter-ion, X^-:

$$C=N^+ \!-\! H \text{ --- } X^- \tag{1}$$

or, if the original proton donor was itself protonated:

$$C=N^+ \!-\! H \text{ --- } X \tag{2}$$

This was emphasized in our first publications.[14,15]

As a consequence of this a tautomeric equilibrium is likely to exist at the Schiff base nitrogen:

$$C=N \text{ --- } H\!-\!X \rightleftharpoons C=N^+ \!-\! H \text{ --- } X^- \tag{3}$$

or

$$C=N \text{ --- } H\!-\!X^+ \rightleftharpoons C=N^+ \!-\! H \text{ --- } X \tag{4}$$

2) In the (π, π^*) singlet excited state (which is widely considered as the state in which the photochemistry of vision occurs) the Schiff base can be expected to be more basic than in its ground state. This expectation is based on Förster's experiments[21] and on Mathies' dipole moment measurements[22] in the ground and excited states.

Since this is so, chemical intuition would predict proton transfer in the excited state with only a H-bond on the Schiff base in the ground state like on the left hand side of eqs. (3) and (4).

3) Rentzepis and his co-workers[23-26] who studied the primary event by picosecond visible spectroscopy found evidence for proton transfer occuring

at a picosecond time scale. In addition, performing the experiment on deuterated rhodopsin gave indication of proton tunnelling.

While these mesurements, because of the global nature of electronic spectra, could not possibly locate the proton which is transferred, it is pleasant to chemical intuition to place it into the proton bridge on the Schiff base nitrogen.

Clearly, this could not occur if the Schiff base was a priori, ground state protonated (cf. the previous section).

4) Since protonation has been invoked to account for a part of the bathochromic shift from the Schiff base itself to the Schiff base integrated into rhodopsin the question then arises if this shift can be obtained without protonation. Semi-empirical quantum mechanical calculations by Leclercq[14] have shown that, indeed, it can. It is enough for this to subject the chromophore to the joint perturbation by a H-bond at the Schiff base nitrogen and a negative group, like a carboxylate ion, placed near the β-ionone ring (C_5). The location of the latter is not highly critical, however.

5) Abrahamson, Mateescu[27,28] and their co-workers have published ^{13}C NMR spectra and found that the signal was *not* what would be expected for a protonated nitrogen. More extensive work on this problem, using isotopic substitution on other atoms of the chromophore has been announced by these authors. It has to be pointed out in this respect that NMR evidence carries as much weight as Raman for the given problem.

6) Infrared spectroscopy would be better suited than Raman for the study of visual pigments. Indeed, the crucial vibrations are motions of strongly polar groups. Now, while it may seem to be hopeless to pick out the bands due to the chromophore from the sea of bands due to the surrounding protein, Siebert and his co-workers[29,30] scored important successes in this respect by using flash induced differential, kinetic infrared spectroscopy.

Their results obtained on the rhodopsin/metarhodopsin II pair are particularly significant. The former is supposedly protonated, while the latter is deprotonated (UV at 380 nm). If this is so, in the difference spectrum, the 1655 cm^{-1} peak should decrease and the 1622 cm^{-1} peak should increase in intensity upon bleaching. Instead a new band appeared at 1645 cm^{-1}. This is hardly compatible with a priori protonation. It could be understood, however, if we admit that only a fraction of rhodopsin molecules had been protonated. The remainder could have engaged in H-bonding as in eqs. (3) or (4) with proton transfer occurring as a result of photon absorption.

7) The quantum yield of the photochemical process is only 0.67. Furthermore, it is more efficient at liquid nitrogen than at room temperature, a fact which is hard to understand if the primary process is isomerization but is compatible with observation if it is proton transfer with a tunnelling mechanism.

THE POTENTIAL IN THE PROTON BRIDGE

The proton between the Schiff base nitrogen and the counter-ion is bound to play a crucial role in the mechanism of vision. It forms a H-bond between particles with net charges and must be expected to be a strong one, perhaps of the 50—100 kilojoules per mole range. Consequently it can exert a considerable stabilizing effect on the visual chromophore. This suggests the idea that the proton bridge must be split before isomerization can take place.

This view is further strengthened by Cooper's results[31] who found by photo-calorimetric measurements that the ground state energies of rhodopsin and bathorhodopsin differ by as much as about 140 kilojoules per mole. Whatever the exact structure of bathorhodopsin, even a full 11-*cis*/all-*trans* transformation could not account for 140 kilojoules.

At any rate, it is clear that the position of the proton within the proton bridge that is, the potential governing its motions therein is of decisive importance. The shape of the potential in strong H-bonds received a great deal of attention (for a review see Hadži and Bratos[32], Novak[33]). The potential may contain one or two wells. It can be asymmetrical single well (as), asymmetrical double well (ad), symmetrical broad single well (ss) or symmetrical double well (sd).

Unfortunately the identity of the proton donor has not so far been established. In all likelihood, however, it is an amino-acid residue, in particular histidine, or aspartic or glutamic acid. Unprotonated histidine is a weak base like the Schiff base. Thus, if a protonated histidine molecule formed the proton bridge with the chromophore this would lead to a (BHB)$^+$ type (where B stands for base) situation in which the proton is placed near the midpoint of the (N --- H$^+$ --- N) unit. The two wells are unlikely to be of equal depths since donor and acceptor are different molecules (although there are known examples for this[32]). In view of their similar basicity the two wells may have slightly different depths both being significantly populated with a possibility of tunnelling.

Should the proton donor be aspartic or glutamic acid, a proton bridge would be formed with the proton »hesitating« between N --- H—O and N$^+$—H --- O$^-$. The pK_a difference between these acids and the Schiff base is of the order of 4 units. According to Zundel's practical rule[34,35] this is just about the limit at which protonation usually occurs. So there again the proton can be expected to place itself near the midpoint. In both cases a potential with only one flat minimum would be conceivable. This would amount to a complete disappearance of the potential barrier between the two wells which seems to us a less probable assumption in view of the relatively weak acids and bases and not extremely short H-bond distances which are involved.

The proposal is now put forward that a double well potential governing the motions of the proton in the bridge is compatible with all known experimental facts about visual pigments including the resonance Raman spectrum. It is then necessary to examine certain aspects of the resonance Raman effect for a system with a double well potential.

COMMENTS ON THE SELECTIVITY OF THE RESONANCE RAMAN EFFECT

Let us consider a molecule of n atoms having no symmetry in its ground state g. This molecule has $3n - 6$ »totally symmetrical« vibrations in its state g. We shall assume that the Born-Oppenheimer approximation is valid for both the state g and the »resonating« electronic excited state e. For simplicity's sake it will be further assumed that it is sufficient to involve one excited state and that the dipole transition moment between g and e, M_{ge}, is a slowly varying function of the nuclear coordinates (polarization subscripts will be omitted). Then the total scattering intensity, I_s, from a given initial vibrational level i of state g to the final vibrational level j of the same state g may be written as follows[36-39]

$$I_S = \frac{8\,\pi\,\omega_S{}^4\,I_L}{9\,c^4\,\hbar^2}\,[M_{ge}]^4\left[\,\Sigma_v\,\frac{\langle\,j\,|\,v\,\rangle\,\langle\,v\,|\,i\,\rangle}{\omega_{iv}-\omega_L+i\,\Gamma_v}\,\right]^2 \tag{5}$$

where M_{ge} is evaluated at the equilibrium geometry of g; the sum Σ_v is extended over all vibrational levels of state e, i. e.

$$\Sigma_v \equiv \Sigma_{v_1}\,\text{---}\,\Sigma_{v_k}\,\text{---}\,\Sigma_{v_{3n-6}}$$

v_k being the quantum number of the kth normal vibration and Γ_v is the damping term.

Following a treatment by Peticolas[40], we give the Raman active mode under study the subscript 1 and we limit our investigation to the related fundamental Raman line,

$$i \equiv \{0, 0 \ldots, 0\}; \quad j \equiv \{1, 0, \ldots, 0\}$$

The sum in (5) may be rewritten as

$$\Sigma_{v_1}\,\text{---}\,\Sigma_{v_{3n-6}}\,\frac{\langle\,1_1\,|\,v_1\,\rangle\,\langle\,v_1\,|\,0_1\,\rangle\,\Pi_{k=2}^{3n-6}\,|\,\langle\,v_k\,|\,0_k\,\rangle\,|^2}{\omega_{ge}^{0-0}+\Sigma_{k=1}^{3n-6}\,v_k\,\omega_k-\omega_L+i\,\Gamma_v} \tag{6}$$

where ω_{ge}^{0-0} is the 0—0 frequency in the band due to transition from g and e and ω_k the frequency of the kth normal vibration in state e in cm^{-1}. The following notations are introduced:

$$|\,i\,\rangle \equiv \Pi_{k=1}^{3n-6}\,|\,0_k\,\rangle \tag{7}$$

$$|\,j\,\rangle \equiv |\,1_1\,\rangle\,\Pi_{k=2}^{3n-6}\,|\,0_k\,\rangle \tag{8}$$

$$|\,v\,\rangle \equiv \Pi_{k=1}^{3n-6}\,|\,v_k\,\rangle \tag{9}$$

All this supposes that the normal coordinates of vibration in g and in e are identical (Figure 2).
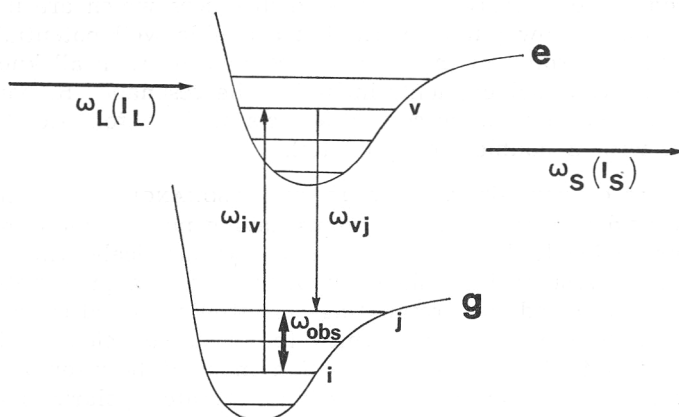

Figure 2. Discrete resonance Raman scattering. g: electronic ground state; e: resonant electronic exicited state; i: initial; j: final vibrational states in g; v: vibrational state in e; ω_L, I_L: frequency and intensity of the incident light (laser); ω_S, I_S: frequency and intensity of the scattering light.

In this type of treatment the molecular model which is usually assumed is the independent mode, displaced harmonic oscillator approximation, i. e., the potential curves of the g and e states are assumed to be harmonic and the vibrational frequencies equal for any normal mode. Then, if $\Delta \xi_k$ represents the shift between g and e of the dimensionless normal coordinate $\xi_k = 2 \pi \sqrt{\mu_k \nu_k / h}\, Q_k$ associated with the kth normal coordinate and μ_k the reduced mass, the terms in the numerator of equation (6) are, according to Inagaki and coworkers[41], given by

$$| \langle v_k | 0_k \rangle |^2 = F (v_k, \Delta \xi_k) \tag{10}$$

$$\langle 1_1 | v_1 \rangle \langle v_1 | 0_1 \rangle = G (v_1, \Delta \xi_1) \tag{11}$$

where

$$F (v, \Delta) \equiv \Delta^{2v}\, e^{-\Delta^2/2} / (2^v\, v!) \tag{12}$$

$$G (v, \Delta) \equiv \Delta^{2v-1} (\Delta^2 - 2v)\, e^{-\Delta^2/2} / (\sqrt{2}\, 2^v\, v!) \tag{13}$$

In particular, if $\Delta \xi_k$ is small,

$$| \langle v_k | 0_k \rangle |^2 \propto (\Delta \xi_k)^{2v_k} (= \delta_{ov} \text{ if } \Delta \xi_k = 0)$$

$$\langle 1_1 | v_1 \rangle \langle v_1 | 0_1 \rangle \propto \Delta \xi_k (= 0 \text{ if } \Delta \xi_k = 0) \tag{15}$$

the principal terms of the $\langle 1 | v \rangle \langle v | 0 \rangle$ type bein those with $v = 0$ and 1. The intensity, I_S, is then proportional to $\Delta \xi^2$ and therefore, weak.

If $\Delta \xi_k$ is large, amouting, for example, to a 5 to 10% fluctuation in the length of a bond, the intensity is high although the contribution of a given term is usually difficult to evaluate.

The fact that is often disregarded is that when $\Delta \xi_k$ is *very* large the intensity can only be weak since $| G (v, \Delta) |^2$ becomes a rapidly decreasing function after reaching its maximum (or its successive maxima are weaker and weaker; see for example Figure 8 in reference[41]). This disregard is evidently due to the fact that in the displaced harmonic oscillator model the shift $\Delta \xi_k$ is assumed to be small or moderately large but not very large. As it is shown below this is essential when at least one of the potential curves is double well.

RESONANCE RAMAN SPECTRA IN THE CASE OF A DOUBLE WELL POTENTIAL

To our knowledge theories of resonance Raman spectra have not so far been applied to systems having one (or more) double well potential. This means that the interpretation of RR spectra has always implied the displaced harmonic oscillator approximation, either explicitly or implicitly. Our aim is to show that this limitation is subject to criticism and that, if pursued too far, it may lead to artefacts.

The vibrational energy levels and wave functions for potential curves with two minima have been the object of many investigations.[42-45] In the case of symmetrical or slighly asymmetrical double wells — which are of greatest interest for the present study — a promising way of obtaining the energy levels and associated wave functions is to analyse conditions in terms of the energy levels and wave functions of two harmonic oscillators relating to the two wells. In this approximation the first levels are correctly described by linear combinations of the type

$$\Phi_{v+}(q) \approx a_{v+}\, \varphi_{Av}(q-q_A) + b_{v+}\, \varphi_{Bv}(q-q_B) \quad (\text{with } a_{v+} \cdot b_{v+} > 0) \qquad (16)$$

$$\Phi_{v-}(q) \approx a_{v-}\, \varphi_{Av}(q-q_A) + b_{v-}\, \varphi_{Bv}(q-q_B) \quad (\text{with } a_{v-} \cdot b_{v-} < 0) \qquad (16')$$

where φ_{Av} and φ_{Bv} are wave functions of the harmonic oscillators located on A and B respectively (Figure 3). The first levels are split into doublets, the splitting being slight compared with the average separation between the doublets (at least when ΔU is much smaller than U_{max}; see Figure 3).
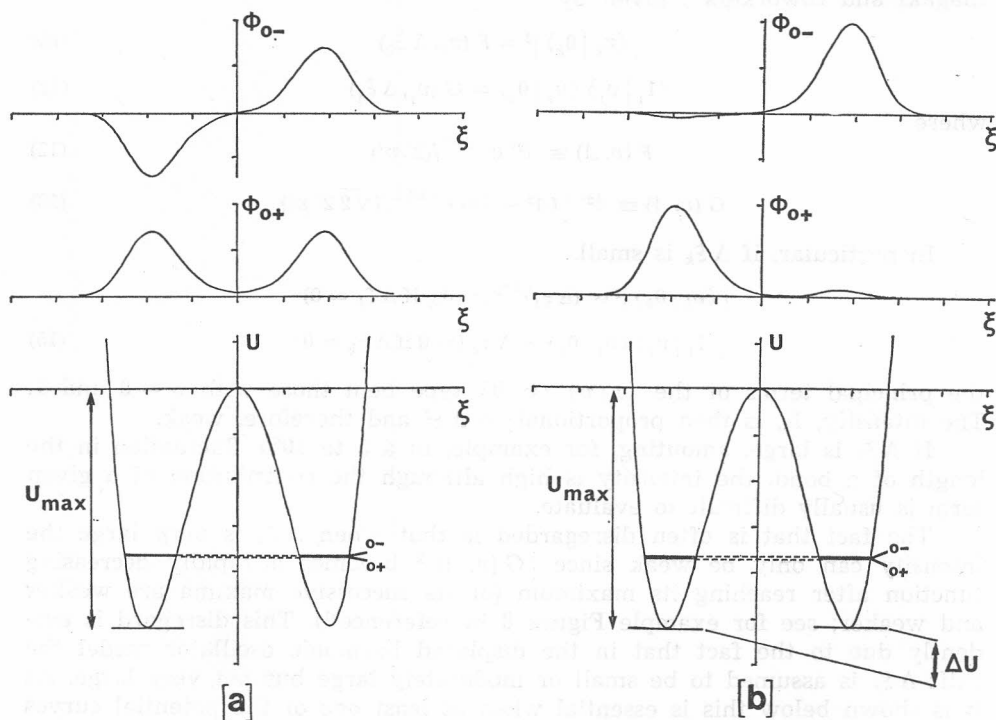

Figure 3. Representation of the first eigenfunctions of typical double well potentials. [a] A symmetric potential: the stationary states $|0+\rangle$ and $|0-\rangle$ correspond to delocalized protomeric structures. [b] A (slightly) asymmetric potential: $|0+\rangle$ and $|0-\rangle$ correspond to localized protomeric structures.

The situation can then be analyzed statistically in terms of the populations relating to the two wells. These will be equal for symmetrical potential curves and different otherwise, the population of the deeper well becoming preponderant as ΔU increases. The dynamic behavior of the system can be described in terms of tunnelling between the two wells. We restrict ourselves here to the first aspect which leads to a simple presentation of facts. The dynamic aspects will be dealt with in a later publication. Also for simplicity's sake we limit our analysis to a system with two variables. More exactly, from now on, having in mind the proton bridge of visual pigments, we assume that for both the ground state g and the electronic excited state e, one of the generalized coordinates q is the normal coordinate of the N—H stretching vibration which is governed by the double well potential and Q the Schiff base C=N vibration which is observed in the resonance spectrum at about

1655 cm^{-1} and is associated with a simple harmonic potential. If, in addition, we admit that the coupling terms $k_{nm} q^n Q^m$ are, in a first approximation, negligible compared to terms containing q^n and Q^2 of the Hamiltonian, the vibrational wave function $\Phi_{v,V}(q, Q)$ for the first vibrational levels $\{v, V\}$, can be written as:
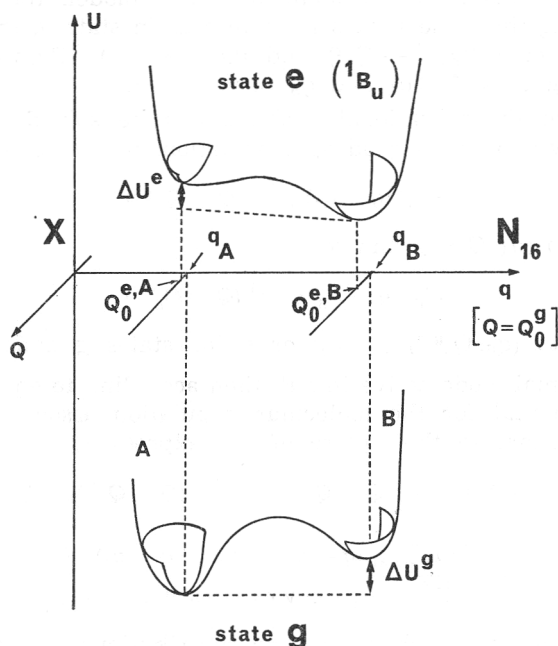

Figure 4. An oscillator (q) governed by a double well potential and coupled with a harmonic oscillator (Q): a possible situation for visual pigments and bacteriorhodopsin (Q stands for $C_{15} = N_{16}$ stretching).

$$\Phi_{v,V}(q, Q) \approx [a_v \varphi_{Av}(q - q_A) + b_v \varphi_{Bv}(q - q_B)] \varphi_V^{(K)}(Q - Q_0) \tag{17}$$

(subscripts ($\pm$) are omitted)

where $\varphi_V^{(K)}(Q - Q_0)$ is the solution for quantum number V of the harmonic oscillator whose potential is $^1/_2 K(Q - Q_0)^2$. (In (16) and (16′) the approximation is best for $v = 0$; as will appear subsequently these are the only ones that are needed for our purposes.)

We continue to admit that the potential has a quadratic dependence on Q. Then the coupling terms will cause Q_0 and the force constant K to depend on q. To simplify further we suppose that:

a) in state g, only K depends on q, i.e. $K = K_A$ for $q = q_A$; $K = K_B$ for $q = q_B$ (where K_B is not very different from K_A); the $|0+\rangle$ and $|0-\rangle$ stationary states are localized on wells A and B, respectively, (the most realistic hypothesis if the proton donor and acceptor are not identical because even a very small value of $\Delta U/U_{max}$ leads to such a situation), α_0^g and β_0^g being the coefficients of the expansion of the initial wave packet on $|0+\rangle$ and $|0-\rangle$;

b) in state e the dependence of the potential on q is similar to that in state g with the same values of q_A and q_B for the two wells whose dissymmetry, however, may be different (the deeper well can be on the same side with a different depth, the two wells may be of equal depths, the deeper well may be on the other side); the dependence on Q is in conformity with the usual hypothesis for the displaced harmonic oscillator model, the force constants K_A and K_B having the same values in state e as in state g, the displacements of the minima being $(Q_0^{e,A} - Q_0^g)$ and $(Q_0^{e,B} - Q_0^g)$ (Figure 4; sections of $U(q, Q)$ according to Q for q_A and q_B respectively).

Clearly, since the vibrational problem can be solved locally for each well $\Phi_{v,V}(q, Q)$ will be, in a good approximation, proportional to the product

$$\varphi^u_{Av}(q - q_A)\, \varphi_V^{(k_A)}(Q - Q_0^{u,A}) \tag{18}$$

for (q, Q) close to $(q_A, Q_0^{u,A})$, and to

$$\varphi^u_{Bv}(q - q_B)\, \varphi_V^{(k_B)}(Q - Q_0^{u,B}) \tag{19}$$

for (q, Q) close to $(q_B, Q_0^{u,B})$ ($u \equiv g$ or e for states g and e, respectively).

If Q is a normal mode active in RR, then according to eq. (6) the intensity I_S of its fundamental for the molecular population associated with well A will be proportionnal to the square of the expression

$$\Sigma_{v\pm}\ \Sigma_V \langle \varphi_1^{(K_A)}(Q - Q_0^g) \,|\, \varphi_V^{(k_A)}(Q - Q_0^{e,A}) \rangle \langle \varphi_V^{(k_A)}(Q - Q_0^{e,A}) \,|\, \varphi_0^{(k_A)}(Q - Q_0^g) \rangle \times \tag{20}$$

$$\times\ \frac{|\langle a_{v\pm}^e \varphi_{Av}(q - q_A) \,|\, a_0^g\, \varphi_{A0}(q - q_A) \rangle|^2}{\omega_{ge}^{0-0} + \omega_{v\pm} + V\,\omega_V - \omega_L + i\,\Gamma_{\{v\pm,\,V\}}}$$

where $\Sigma_{v\pm}$ includes, for each v, the two contributions of eqs. (16) and (16'), and $\omega_{v\pm}$ is the frequency in cm^{-1} associated with the $v\pm$ level of the q mode. For the molecular population associated with well B the same expression holds if A and a are replaced by B and b, respectively.

A few comments are in order.

a) The only value of v that enters these expressions is 0. This follows from the simplifying assumption that q_A has the same value in states g and e; for slightly different values the terms with $v = 0$ will still be preponderant. These are the terms for which eqs. (16) and (16') are the most accurate.

b) The analysis of the first two terms in eq. (20) follows from the preceeding discussion; the essential point for our problem is that, *for weak bands*, this term is proportionnal, as found above, to $|Q_0^{e,A} - Q_0^g|^2$ for the population of A and $|Q_0^{e,B} - Q_0^g|^2$ for the population of B (if Q is not the dimensionless normal coordinate, it is proportionnal to it).

This leads us to the important result that

$$\frac{I_S(A)}{I_S(B)} \approx \frac{(a_{0\pm}^e)^2\,(a_0^g)^2\,|Q_0^{e,A} - Q_0^g|^2}{(b_{0\pm}^e)^2\,(\beta_0^g)^2\,|Q_0^{e,B} - Q_0^g|^2} \tag{21}$$

in the case of a discrete resonance with one (or more) vibrational state of e with 0+ (or 0—) quantum number for the q mode

$$\frac{I_S(A)}{I_S(B)} \approx \frac{(a_{0+}^e/\Delta\omega_+ + a_{0-}^e/\Delta\omega_-)^2 (\alpha_0^g)^2 \, |Q_0^{e,A} - Q_0^g|^2}{(b_{0+}^e/\Delta\omega_+ + b_{0-}^e/\Delta\omega_-)^2 (\beta_0^g)^2 \, |Q_0^{e,B} - Q_0^g|^2} \quad (22)$$

(where $\Delta\omega_\pm = \omega_{ge}^{0-0} + \omega_{0\pm} - \omega_L + i\,\Gamma_{\{v\pm, v\}}$), in the case of a resonance with vibrational states of e with 0+ *and* 0— quantum numbers for the q mode.

DISCUSSION

According to (21) and (22), for a vibration with normal coordinate Q, the intensity of the band originating with one of the wells, say A, is a function of its population in the ground state g, of its population in the electronic
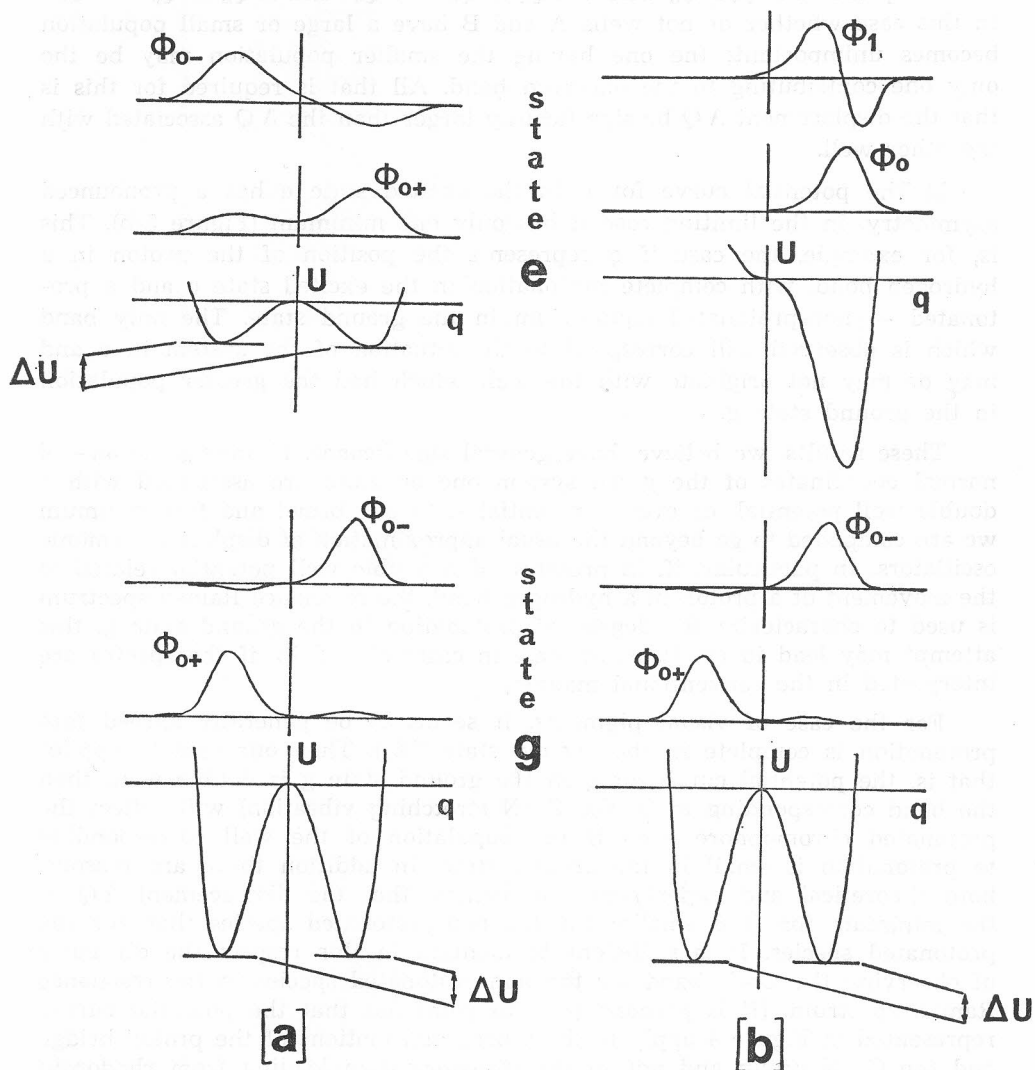

Figure 5. Two typical cases in resonance Raman scattering when a »normal« mode is governed by a double well potential (see text).

excited state e, as well as the square of the displacement of the minimum of the potential curve for Q from g to e.

It is instructive to develop, in a qualitative manner, the following two special cases:

a) the potential curve for q in the state e has a small barrier; the roles of q_A and q_B are interchanged (i. e. the well B is the deeper one), and the resonance is with a single discrete state, with $0+$ quantum number for the q mode (Figure 5-a). The products $(a_0^e)^2 (a_0^g)^2$ and $(b_0^e)^2 (\beta_0^g)^2$ may be nearly equal. Then, the ratio of the intensities I_S (A) and I_S (B) depends essentially on the square of $\Delta Q_A / \Delta Q_B$ where $\Delta Q_A \equiv Q_0^{e,A} - Q_0^g$ and $\Delta Q_B \equiv Q_0^{e,B} - Q_0^g$. In this case whether or not wells A and B have a large or small population becomes unimportant; the one having the smaller population may be the only one contributing to the observed band. All that is required for this is that the displacement ΔQ be significantly larger than the ΔQ associated with the other well.

b) The potential curve for q in the excited state e has a pronounced asymmetry, in the limiting case it has only one minimum (Figure 5-b). This is, for example, the case if q represents the position of the proton in a hydrogen bond, with complete protonation in the excited state e and a pro-tonated — non-protonated equilibrium in the ground state. The only band which is observed will correspond to the situation of the system in e and may or may not originate with the well which had the greater population in the ground state g.

These results, we believe, have general significance. If among the $3n - 6$ normal coordinates of the given system one or more are associated with a double well potential, or even a potential with one broad and flat minimum we are compelled to go beyond the usual approximation of displaced harmonic oscillators. In particular, if, in presence of a double well potential related to the movement of a proton in a hydrogen bond, the resonance Raman spectrum is used to characterize the degree of protonation in the ground state g, this attempt may lead to artefacts, at least in cases a) and b), if the spectra are interpreted in the conventional manner.

For the case of visual pigments, it seems to be generally agreed that protonation is complete in the excited state (^1B_u). Then our case b) applies that is, the potential curve for q in the ground state g is double well; then the band corresponding to Q (the C=N stretching vibration) will reflect the protonated chromophore even if the population of the well corresponding to protonation is small in the ground state. In addition there are reasons, both theoretical and experimental to believe that the displacement ΔQ of the minimum for Q is smaller for the non-protonated species than for the protonated species. It is sufficient to mention in this respect the difficulty of observing the C=N band for the non-protonated species in the resonance Raman spectrum. (It is perhaps good to point out that the potential curves represented in Figure 4 apply to the vibrational motions of the proton bridge and the C=N group and *not* to the transformation leading from rhodopsin to bathorhodopsin.)

CONCLUSION

A qualitative examination of the resonance Raman effect leads, in certain cases, to surprising predictions when a vibration is governed by a double well potential. At first sight the intensity of the resonance Raman line would be expected to reflect mainly the conditions at the more populated potential well but this is not necessarily so. In addition to the populations of the wells, the appearance of one or the other band in the RR spectrum will depend on the difference in position of the minima of the potential wells, for the vibration concerned, between the ground state and the excited state. Indeed, this latter condition may become predominant and it may render the relative populations unimportant. As a consequence it may happen in certain special cases that the less populated well becomes the only one to give rise to a band observable in the spectrum.

It is believed that this may well be the case of visual pigments. While the C=N band characteristic of a protonated Schiff base does appear at 1655 cm^{-1} this could be due to a minority or even small minority of the chromophore molecules and these may not be the ones with which the visual cycle originates. As has been stated in this paper, a wide range of infrared experience suggests that, with the probable proton donors, the motion of the proton in the salt (proton) bridge adjoining the Schiff base nitrogen is very likely to be governed by a double well potential. All this does not constitute a *proof*, however. All that we wish to say is that this possibility exists; indeed it is probable and it is not contradicted by the presence in the RR spectrum of a band at 1655 cm^{-1}, or its deuteration shift. Both may be due to the well closer to the nitrogen of the Schiff base, the »protonation well« with a low population, whereas the highly populated »non-protonation well« may not give rise to bands observable in the RR spectrum.

This double well mechanism would yield intuitive explanation for the primary photochemical step of vision which then could be proton transfer followed by isomerization, for the less than 100% output of the bleaching cycle and, perhaps, even for the appearance of hypsorhodopsin.

The possibility of a tautomeric equilibrium at the Schiff base nitrogen is obvious and it has been a frustrating thought that nature does not make any use of it.

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IZVLEČEK

Možnost dvojnega potencialnega minimuma v protonskem mostu v vidnih pigmentih in bakteriorodopsinu

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V članku je najprej podan kratek pregled, kjer je opisana današnja stopnja znanja o kemizmu vidne zaznave. Poseben poudarek je namenjen vlogi vodikovih vezi in prenosu protona pri tem procesu. Zaznava barvnih odtenkov, je pogojena z načelom treh barv — torej sprejemnikov, ki so občutljivi na tri osnovne barve in gre preko enega samega molekulskega sklopa, 11 — *cis* retinala (Schiffova baza), ki je povezan z beljakovinsko molekulo opsina. Ta služi kot sprejemnik svetlobe in sicer reagira na različne dele svetlobnega spektra v odvisnosti od okolja. Pri tem je bistvenega pomena stanje in obnašanje vodikove vezi, ki veže Schiffovo bazo s proteinskim skeletom — verjetno preko enega od aminokislinskih ostankov. Podrobno je obrazložena teorija za resonančni Ramanov efekt za nihanja, ki so podvržena vplivu potenciala z dvema potencialnima jamama. V tem primeru je intenziteta črte v Ramanovem spektru, ki se resonančno ojači odvisna od treh faktorjev: od relativne zasedenosti obeh potencialnih loncev v osnovnem elektronskem stanju in vzbujenem elektronskem stanju in od premikov njihovih minimumov. V nasprotju s pričakovanji je verjetno, da opazimo resonančno ojačanje Ramanove črte na račun nihanja protona v manj zasedenem loncu dvojnega potencialnega minimuma.