View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by The University of Sydney: Sydney eScholarship Journals online

THE PROBABILITY ISOTHERM: AN INTUITIVE NON-EQUILIBRIUM THERMODYNAMIC FRAMEWORK FOR BIOCHEMICAL KINETICS

Brian Chapman[a], Jennifer A. Mosse[b], Jo-Ann Larkins[b]

Presenting Author: Brian Chapman (Brian.Chapman@monash.edu)
[a]Gippsland Medical School, Monash University, Churchill VIC 3842, Australia
[b]School of Applied Sciences and Engineering, Monash University, Churchill VIC3842, Australia

KEYWORDS: Second Law of Thermodynamics, free energy dissipation, entropy creation, entropy exchange, probability isotherm, rate isotherm, coupled reactions, exergonic reactions, endergonic reactions, shared intermediates

ABSTRACT

Widespread error exists in the 'thermodynamics' and/or 'bioenergetics' sections of most biochemical textbooks. Three typical examples are drawn from a premier pedagogical source and shown to encapsulate (1) confusion about entropy and reversibility, (2) confounding of coupled reactions with sequential reactions in misguided attempts to show how exergonic reactions might drive endergonic reactions, and (3) confusion about the proximity to equilibrium of living processes. A fresh approach is developed, based on the Second Law imperative that free energy be dissipated (identical to the requirement that entropy be created). This approach identifies a Probability Isotherm, being a probabilistic expression of the Second Law, relating molar free energy dissipation to the overall ratio of probability of forward reaction to backward reaction. By equating the Probability Isotherm to the Van't Hoff Isotherm, the overall probability ratio may be decomposed into an *intrinsic* probability ratio (the equilibrium constant) and an *extrinsic* probability ratio (dependent on composition). The Probability Isotherm is manifest kinetically as the Rate Isotherm, also thermodynamically determined even for kinetically complex reactions. The concept of 'bound energy' is introduced to complement 'free energy' in reconciling the Second Law imperative for free energy dissipation with the First Law requirement for total energy conservation.

Proceedings of the Australian Conference on Science and Mathematics Education, University of Melbourne, Sept 28th to Sept 30th, 2011, pages 169-174, ISBN Number 978-0-9871834-0-8.

INTRODUCTION

The stimulus for this paper derives from widespread error and confusion found in the 'thermodynamics' and/or 'bioenergetics' sections of many textbooks of biochemistry. While the discovery of such error is not new, its identification has not always led to greater insight. For example, Welch (1985) has noted a failure of rigour in the use of Gibbs free energy in biochemistry, and Abu-Salah (1992) has correctly noted the desirability of unifying the bioenergetic treatments of intermediary metabolism and membrane transport. However, our observation is that there is generally far too great an emphasis on biochemically irrelevant 'standard conditions'. Moreover, terms such as 'free energy', 'total energy', 'entropy' and 'reversible/irreversible' are rarely defined authoritatively or used consistently so as to express and exemplify their meanings in pedagogically useful ways.

To take two high profile instances from the current literature, we find that:

1. the current edition (5th) of *Lehninger Principles of Biochemistry* (Nelson & Cox, 2008; pp.22-23) introduces the concepts of *entropy*, *enthalpy* and *free energy*, in that order, without clarifying the important distinction between *entropy creation* and *entropy exchange*, and without establishing the essential identity between *entropy creation* and *free energy dissipation*.
2. the current edition (6th) of *Biochemistry* (Berg, Tymoczko & Stryer, 2007; p.210 and p.411) makes the common pedagogical mistake of confounding sequential reactions (as in metabolic pathways with shared intermediates) with truly coupled reactions. This leads them to highlight the completely erroneous heading 'A Thermodynamically Unfavourable Reaction Can Be Driven By a Favourable Reaction' (p. 411).

Regarding the second of these two instances, Nelson and Cox (2008; p.22) avoid this error by restricting the discussion to the coupling of two (partial) reactions into a single, truly coupled, reaction. With regard to the first instance, the treatment by Berg et al. (2007; p.12) is an improvement on that by Nelson and Cox (2008) while still falling just short of being pedagogically explicit about the required distinction and identity.

These two instances may be found replicated to greater or lesser degrees throughout the pedagogical literature for the past forty years. To justify this sweeping statement with sufficient examples would quickly generate an article far too lengthy and tedious to be helpful. Instead, we may content ourselves with the three prototypical examples presented immediately below; they are taken from one of the least problematic sources to be found – Lehninger's original (1970) textbook, *Biochemistry; the molecular basis of cell structure and function*. They are prototypical in that they have at least found resonance in, if not having actually inspired, much of subsequent bioenergetics pedagogy.

EXAMPLE 1: CONFUSION ABOUT ENTROPY AND REVERSIBILITY

We read on p.291, paragraph 3:

> Processes proceeding with an increase in entropy are termed irreversible. Processes occurring without change in entropy are called reversible. All "real" processes occurring in our physical world, including the process of life, are irreversible.

This quote is pedagogically weak on three grounds:

- it focuses on the arcane concept of entropy and, having introduced the concept, fails to make the crucial distinction between *entropy creation* and *entropy exchange*;
- it loosely fails to specify the universe as the essential locus for entropy increase (creation) in spontaneous processes – and note that, given the correct locus, "processes occurring without change in entropy" are, in fact, *not occurring at all*;
- it uses the word "irreversible" in far too sweeping a generalisation that is biochemically misleading – it does not distinguish between the mega-concept of "life" as an irreversible process (e.g., the life of an organism) and the more restricted concepts of biochemical reactions (e.g., the formation of ATP from ADP and P_i by mitochondrial aerobic metabolism or cytoplasmic glycolysis, as distinct from the formation of ADP and P_i from ATP by various transport ATPases, myosin ATPases, etc.). Lehninger's whole concept of the ATP/ADP system as an 'obligatory intermediate carrier of phosphate groups originating from high-energy phosphate compounds above ATP on the thermodynamic scale to acceptor molecules that form low-energy phosphate compounds below ATP on the scale' (p.302 paragraph 3) implies the essentially *reversible* nature of ATP synthesis/breakdown according to the micro-location within the cell in which the ATP/ADP system is being engaged (i.e., according to the other processes with which it is stoichiometrically coupled or linked – see *Example 2* and the relevant section on exergonic and endergonic reactions below).

EXAMPLE 2: CONFOUNDING COUPLED REACTIONS WITH SEQUENTIAL REACTIONS

One of the most pervasive errors to be found is typified on pages 297 and 306 where the words 'coupled' and 'linked' are used in relation to *sequential* reactions that share a common intermediate. This error is almost always introduced in a misapplied attempt to illustrate how endergonic reactions (i.e. reactions requiring an input of free energy) might be driven by exergonic reactions (i.e. reactions involving the dissipation of free energy). This error – absent from Nelson and Cox (2008) while present in Berg et al. (2007), as noted above – is dealt with under a separate heading below.

EXAMPLE 3: CONFUSION ABOUT THE PROXIMITY TO EQUILIBRIUM OF LIVING PROCESSES

On p.309 we read that

> ... living cells are open systems; i.e., they do exchange matter with their surroundings. Furthermore, they are never totally in equilibrium. A living cell at any given moment exists in a steady state in which the rate of input of matter equals the rate of output of matter.

However, on p.310, in the relevant part of the summary, we read: 'Living cells are open systems which exchange both matter and energy with their surroundings; they exist in steady states, far from equilibrium.' Clearly, there is confusion in the confounded descriptions 'never totally in equilibrium' (suggesting almost, or not quite, in equilibrium) and 'far from equilibrium'. Furthermore the 'steady state' condition is being required to take far more weight than it can possibly bear. Living creatures are rarely in a steady state, even when asleep. Even the cytoplasm, far from being a quasi-quiescent 'soup', is actually a hive of molecular industry, with different enzymes constantly being activated and/or expressed, molecular assembly lines and conveyor belts coming into existence or disappearing according to need. Also, the nature of living cells as open systems is not a significant issue in thermodynamic and kinetic analysis of biochemical phenomena. It has been shown elsewhere that the errors involved in approximating whole animals to closed systems are negligible fractions of the energy changes that actually occur (Chapman, 1989).

Any one, or all three, of the examples just given may be found replicated in abundance throughout the pedagogical literature. To counter this widespread confusion it is necessary to re-visit the Second Law of thermodynamics and examine what it has to say about the relationship between *free energy* and the *direction* and *rate* of a biochemical process. The present paper affords a fresh approach by focussing on the essentially probabilistic nature of the Second Law. The development will be pursued intuitively rather than formally, although the results will be related to concepts that are already familiar from more formal, traditional derivations.

FREE ENERGY AND THE PROBABILITY ISOTHERM

Consider a molecular system that can exist in either one of two states ***A*** or ***B***, the equation of state transition being given as

$$\boldsymbol{A \rightarrow B} \tag{1}$$

The likelihood of ***B***'s being formed from ***A*** relative to the likelihood of ***A***'s being formed from ***B*** is always and everywhere determined by the Second Law of thermodynamics, which expresses the molar free energy change of isothermal transformation from state ***A*** to state ***B***, $\Delta \boldsymbol{G}_{\boldsymbol{A \rightarrow B}}$, thus:

$$\Delta \boldsymbol{G}_{\boldsymbol{A \rightarrow B}} = \boldsymbol{-RT} \ln \frac{\boldsymbol{p}_{\boldsymbol{A \rightarrow B}}}{\boldsymbol{p}_{\boldsymbol{B \rightarrow A}}} = \boldsymbol{-RT} \ln \frac{\boldsymbol{p}_{\boldsymbol{f}}}{\boldsymbol{p}_{\boldsymbol{b}}} \tag{2}$$

where ***R*** and ***T*** have their usual meanings and the argument of the logarithm is the relative probability ratio for forward to backward state transition, shown in two equivalent forms.

Note that this is not a *kinetic expression* of the Second Law; it is a *probabilistic expression* that holds under all conceivable isothermal conditions and is specifically independent of

- the presence or absence of catalyst, and
- whether the mechanism of state transition is elementary or complex.

In short, as an expression of the Second Law, the Probability Isotherm is as aloof from mechanism as is the more familiar Van't Hoff Isotherm given by

$$\Delta \boldsymbol{G}_{\boldsymbol{A \rightarrow B}} = \Delta \boldsymbol{G}^{\boldsymbol{o}}{}_{\boldsymbol{A \rightarrow B}} - \boldsymbol{RT} \ln \frac{[\boldsymbol{A}]}{[\boldsymbol{B}]} \tag{3}$$

where $\Delta \boldsymbol{G}^{\boldsymbol{o}}{}_{\boldsymbol{A \rightarrow B}}$ is the standard molar free energy of state transition at unit concentrations of ***A*** and ***B***, and [***A***] and [***B***] are the molar concentrations of ***A*** and ***B***. Note that equation (3) is notated unconventionally (with a minus sign preceding the second term on the right hand side and with the logarithm inverted accordingly) in order to mesh better with the intuitive approach used here.

Given that

$$\Delta \boldsymbol{G}^{\boldsymbol{o}}_{\boldsymbol{A \rightarrow B}} = -\boldsymbol{RT} \ln \boldsymbol{K}_{\boldsymbol{eq}} \tag{4}$$

where $\boldsymbol{K}_{\boldsymbol{eq}}$ is the equilibrium constant, independent of catalyst, composition and mechanism, the overall probability ratio, determined in the Probability Isotherm, may be decomposed into *intrinsic* and *extrinsic* probability ratios as follows:

$$\Delta \boldsymbol{G}_{\boldsymbol{A \rightarrow B}} = \boldsymbol{-RT} \ln \frac{\boldsymbol{p}_{\boldsymbol{f}}}{\boldsymbol{p}_{\boldsymbol{b}}} = -\boldsymbol{RT} \ln \frac{\boldsymbol{p}^{\boldsymbol{o}}_{\boldsymbol{f}}}{\boldsymbol{p}^{\boldsymbol{o}}_{\boldsymbol{b}}} - \boldsymbol{RT} \ln \frac{[\boldsymbol{A}]}{[\boldsymbol{B}]} \tag{5}$$

where $\boldsymbol{p}^{\boldsymbol{o}}_{\boldsymbol{f}}$ and $\boldsymbol{p}^{\boldsymbol{o}}_{\boldsymbol{b}}$ are the intrinsic probabilities of transition in the forward and reverse directions, respectively, independent of catalyst, composition and mechanism.

Thus, the *intrinsic probability ratio*, given by

$$\frac{\boldsymbol{p}^{\boldsymbol{o}}_{\boldsymbol{f}}}{\boldsymbol{p}^{\boldsymbol{o}}_{\boldsymbol{b}}} = \boldsymbol{K}_{\boldsymbol{eq}} \tag{6}$$

expresses the intrinsic likelihood or probability of forward transition relative to backward transition, regardless of catalyst, composition and mechanism. Applied anthropomorphically to state transition (1), it expresses how desperately ***A***, in being ***A***, *wants* to become ***B*** instead, relative to the *desire* of ***B***, in being ***B***, to become ***A*** instead. By contrast, the ratio $[\boldsymbol{A}]/[\boldsymbol{B}]$ is an *extrinsic probability ratio*,

independent of catalyst and mechanism, but dependent on composition. Together, the two probabilities multiply to give the overall probability ratio, thus:

$$\frac{p_f}{p_b} = \frac{p_f^o}{p_b^o} \cdot \frac{[A]}{[B]} = e^{-\left(\frac{\Delta G_{A \to B}}{RT}\right)} \qquad (7)$$

making the overall probability ratio a function of composition, but independent of catalyst and mechanism.

There are three possibilities of interest regarding equation (2), all being independent of catalyst and mechanism:

a. $\Delta G_{A \to B} < 0$: In this case, $p_f > p_b$ and the overall probability is for net state transition in the forward direction. Given such a direction for overall probability, the reaction is described as being ***exergonic***.

b. $\Delta G_{A \to B} > 0$: In this case, $p_f < p_b$ and the overall probability is for net state transition in the backward direction. Given such a direction for overall probability, the reaction is described as being ***endergonic***.

c. $\Delta G_{A \to B} = 0$: In this case, $p_f = p_b$ and the overall probability is for no net state transition in either direction; this is the condition of ***thermodynamic equilibrium***.

FREE ENERGY AND THE RATE ISOTHERM

The kinetic manifestation of the thermodynamically determined probability ratio of equation (7) is the *rate ratio*, thermodynamically determined thus:

$$\Delta G_{A \to B} = -RT \ln \frac{r_{A \to B}}{r_{B \to A}} = -RT \ln \frac{r_f}{r_b} \qquad (8)$$

where the probabilities (p) have been replaced by their kinetic manifestations as rates (r).

The absolute values of the unidirectional reaction rates will, of course, depend on the nature and degree of activity of any catalyst that might be present, on the mechanism of reaction, whether elementary or complex and, if complex, whether branched or unbranched. However, the *ratio* of the rates is thermodynamically determined, irrespective of catalyst or mechanism.

Equations (2) and (8) are thus non-equilibrium thermodynamic equations that apply under all conditions, whether at equilibrium, near equilibrium or far from equilibrium. Moreover, under non-equilibrium conditions, they apply generally to both steady states and non-steady states. [While it is true that the *net* rate ($r_f - r_b$) of a complex multi-step reaction is not defined other than in the steady state, the overall *unidirectional* rates remain defined at all times and under all conditions by the methods previously summarised by Boudart (1976) for unbranched complex reactions and extended by Wagg (1987) to *all* situations with 'no theoretical or practical limit to the complexity of the reaction for which a solution is required' (Wagg, 1987; p.376).]

Equations (2) and (8) express the molar free energy being instantaneously dissipated at any given moment. They are as important to the kinetic analysis of all possible biochemical reactions, whether elementary or complex, as the Van't Hoff isotherm given in equation (3) is to thermodynamic analysis. [Note that the *molar* free energy dissipation is the rate of dissipation per mole of reaction. This is not to be confused with the rate of dissipation per unit time, which may be undetectably slow for an uncatalysed reaction.]
As with the earlier discussion of the probability ratio, there are three possibilities of interest regarding the rate ratio, independent of catalyst and mechanism:

a. $\Delta G_{A \to B} < 0$

In this case, $r_f > r_b$ and so the state change is proceeding forwards spontaneously. Given such a direction for net change, the reaction is described as being ***exergonic***.

b. $\Delta G_{A \to B} > 0$

In this case, $r_f < r_b$ and so the state change is proceeding backwards spontaneously. Given such a direction for net change, the reaction is described as being ***endergonic***.

c. $\Delta G_{A \to B} = 0$

In this case, $r_f = r_b$ and so the state change shows no net procession in either direction; this is the condition of ***thermodynamic equilibrium***.

We may now complete our kinetic interpretation of the Van't Hoff isotherm by noting that the intrinsic probability ratio given in equation (6) is identical to the conventional ratio of rate coefficients, thus

$$\frac{p_f^o}{p_b^o} = \frac{k_f}{k_b} = K_{eq} \qquad (9)$$

where k_f and k_b are the forward and backward rate coefficients, respectively. For complex multi-step processes the overall unidirectional rate coefficients are derived using the methods implied by Boudart (1976) and Wagg (1987). However, while the unidirectional intrinsic probabilities are independent of composition, the unidirectional rate coefficients will generally vary with composition. Nonetheless, their *ratio* will always be thermodynamically determined and independent of composition.

THE SECOND LAW REQUIREMENT FOR FREE ENERGY DISSIPATION

The Probability Isotherm given in equation (2) and its kinetic equivalent – the Rate Isotherm given in equation (8) – both express the Second Law requirement that, for a process to take place spontaneously in a net forward direction, free energy must be dissipated, i.e., $\Delta G < 0$. This requirement is identical to saying that entropy must be created, because isothermal free energy dissipation implies entropy creation. In isothermal processes the dissipated free energy (that is, the created entropy) appears as heat exchanged with the surroundings. Note that *entropy creation* is not to be confused with *entropy exchange* – a distinction that may be partly clarified by introducing the concept of 'bound energy'.

THE RELATION BETWEEN FREE ENERGY, TOTAL ENERGY AND 'BOUND ENERGY'

The First Law of Thermodynamics dictates that, when a process such as the state transition given in equation (1) occurs, energy is conserved; this means that the change in total energy, ΔE (or total enthalpy, ΔH) appears either as heat (Q) or work (W), thus:

$$\Delta E = Q + W \qquad (10)$$

where ΔE is the total energy gained by the system from its surroundings, Q is the heat gained by the system from its surroundings, and W is the work done by the surroundings on the system. If ΔE is negative then total energy is lost from the system to its surroundings. [Note that the errors involved in ignoring the pressure-volume changes that distinguish enthalpy from energy, as classically defined, are negligible for all practical purposes in biochemistry (Chapman, 1989).]

The Second Law relates only to the free energy component (ΔG) of the total energy change (ΔE), i.e., the component that is free to do work. The remainder of the total energy change that is *not* free to do work may be usefully termed the 'bound energy' component, classically designated as $T \cdot \Delta S$, where T is the absolute temperature and ΔS is the entropy gained by the system from its surroundings by the process of *entropy exchange*. The Second Law requirement that free energy be dissipated means that not all of the free energy change may be conserved as work. Noting that thermodynamic efficiency is defined as the proportion, ε , of free energy conserved as work, the Second Law requires that $\varepsilon < 1$.
Thus, for a spontaneous process in the forward direction, the simultaneous requirements of the First and Second Laws are brought together by noting that

$$\Delta E = Q + W = \Delta G + T \cdot \Delta S \qquad (11)$$

where $W = \varepsilon \cdot \Delta G$ and $Q = (1-\varepsilon) \cdot \Delta G + T \cdot \Delta S$.

This useful conceptual division of total energy into 'free energy' (free to do work) and 'bound energy' (not free to do work, but rather bound to the material transformation involved in the state transition) is not entirely without its own intuitive difficulties. For example, the total energy changes involved in compression or expansion of a gas, or the generation or dissipation of a transmembrane electrochemical gradient, are approximately zero relative to the large and almost opposite changes in free and bound energy!

CAN EXERGONIC REACTIONS DRIVE ENDERGONIC REACTIONS?

No they cannot. If an exergonic reaction takes place in the presence of an endergonic reaction, the former will run spontaneously forwards while the latter will run spontaneously backwards (and, in so running backwards, it will run exergonically). The technological trick of enzymes is to couple or link exergonic and endergonic processes stoichiometrically into a single *combined* process in which the independent partial reactions are *forbidden by the enzyme* while the combined process runs exergonically. This is precisely how active transport works and this view reflects the original sound thermodynamic foundations of Mitchell's chemiosmotic hypothesis (Mitchell, 1961).

Therefore, it is quite erroneous to speak of *sequential* reactions along a biochemical pathway being 'coupled' or 'linked' such that an exergonic step might 'drive' an endergonic step with which it shares a common intermediate. Every step in a biochemical pathway runs exergonically or not at all, just as every elementary state transition in a complex reaction runs exergonically or not at all.

CONCLUSION

The widespread misuse of words such as 'reversible' and 'irreversible' can be avoided by laying a foundational understanding of how the Second Law determines a *probability ratio* for forward and reverse directions of reaction. Although the Second Law has been expressed in numerous ways, the requirement that free energy be dissipated (and the identity of this to the requirement that entropy be created) is suggested as being most pedagogically useful for biochemists, particularly if applied to the concept of the Probability Isotherm of equation (2) and the Rate Isotherm of equation (8).

There is no excuse for perpetuating the error that sequential steps sharing common intermediates in biochemical pathways provide examples of 'coupling' whereby endergonic steps might be driven by exergonic steps. All processes, however complex or elementary, proceed exergonically or not at all.

The proximity to equilibrium of living processes is not something that can be categorised in any pedagogically useful way. Certain processes, such as active transport of calcium ions within resting skeletal muscle cells, may approach thermodynamic equilibrium very closely, while others may not. Within linear biochemical pathways, so-called rate-limiting steps will operate further away from equilibrium (with large probability ratios) than will non-rate-limiting steps (with small probability ratios).

Beyond these truisms there would seem to be little point in pursuing idle speculation as to whether or not living organisms 'produce entropy at a minimal rate by maintaining a steady state' (Katchalsky, 1965; p.201). The main thermodynamic imperative in which to frame our understanding is that free energy is dissipated always and everywhere, i.e., all biochemical processes operate exergonically. With regard to the history of discovery in physics, some have seen it as unfortunate that the electron turned out to be negative. Equally, it might seem unfortunate that the thermodynamic imperative for *free energy dissipation* was placed secondary to the law of *total energy conservation*; after all, it's the former that makes the world go round! At the very least, it is hoped that the present approach might dispel the unhelpful myth that thermodynamics has nothing to say about reaction rates.

ACKNOWLEDGEMENTS

This work was supported by a grant-in-aid from the Gippsland Medical School. We thank Ms Barbara Dungey of the Gippsland Medical School for helpful input after reading an early draft of this paper.

REFERENCES

Abu-Salah, K. M. (1992). Simplified unifying basis for teaching the applications of the laws of thermodynamics to biological systems. *Biochemical Education*, *20*, 99-103.

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2007). *Biochemistry* (6th ed.). New York: W.H. Freeman and Company.

Boudart, M. (1976). Consistency between kinetics and thermodynamics. *Journal of Physical Chemistry*, *80*, 2869-2970.

Chapman, J. B. (1989). *Basic electrophysiology of cells: A student's compan*ion. Melbourne: Desktop publication, Department of Physiology, Monash University.

Katchalsky, E. (1965), 'Non-equilibrium thermodynamics'. In R. Colbern (Ed.) *Modern Science and Technology*. New York: D. Van Nostrand Company, Inc., 194-201.

Lehninger, A. L. (1970). *Biochemistry: the molecular basis of cell structure and function*, New York: Worth Publishers.

Mitchell, P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature, 191*, 144-148.

Nelson, D. L., & Cox, M. M. (2008). *Lehninger principles of biochemistry* (5th ed.). New York: W.H. Freeman and Company.

Wagg, J. (1987). A Method for defining steady-state unidirectional fluxes through branched chemical, osmotic and chemiosmotic reactions. *Journal of Theoretical Biology*, *128*, 375-385.

Welch, G. R. (1985). Some problems in the usage of Gibbs Free Energy in biochemistry. *Journal of Theoretical Biology*, *114*, 433-446.