

TALKING POINT

The pH dependence of the apparent equilibrium constant, K' , of a biochemical reaction

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THE USE OF THE APPARENT equilibrium constant, K' , for a biochemical equation was recommended by an Interunion Commission on Biochemical Thermodynamics (IUPAC, IUB, IUPAB) in 1976¹. Recently, it has been shown^{2,3} that the criterion of equilibrium at specified temperature (T), pressure (P), pH and pMg is not the Gibbs energy, G , but a transformed Gibbs energy, G' , which leads to a transformed enthalpy, H' , and transformed entropy, S' . Since the apparent equilibrium constant K' is a function of T , P , pH, pMg and ionic strength (I), it leads to the transformed thermodynamic properties.

These new developments have led the IUBMB-IUPAC Joint Commission on Biochemical Nomenclature (JCBN) to set up a panel on Biochemical Thermodynamics to make recommendations for nomenclature and tables in biochemical thermodynamics.

We have written this article because we believe it important for biochemists to know that recommendations are planned, so that they have an opportunity to comment on them before they are accepted. Nevertheless, we emphasize that we are expressing here our own opinions as individuals, and not the official view of the Panel, IUBMB or IUPAC.

Biochemists need two types of reaction equations, chemical equations and biochemical equations, but the distinction is often not made clearly. Chemical equations balance atoms and charge as in



and are needed to discuss the details of the roles of the atoms of the reactants, enzyme and solvent in an enzyme-catalysed reaction. The equilibrium

Biochemical reactions can be discussed in terms of chemical equations or biochemical equations. Both are right and both are needed. Therefore, it is important to be able to distinguish between them at a glance. Sometimes the relations between the apparent equilibrium constant ' K' ' for a biochemical reaction and the equilibrium constant, K , for a reference reaction written as a chemical equation is simple, and sometimes it is complicated. This article will discuss several examples.

constant expression for this reaction is written as

$$K = \frac{[\text{ADP}^3-][\text{HPO}_4^{2-}][\text{H}^+]}{[\text{ATP}^4-](c^\circ)^2} \quad (2)$$

where c° is the standard state concentration, which is usually 1 M (Ref. 4). The $(c^\circ)^2$ in the denominator is required to make the equilibrium constant dimensionless so that its logarithm can be calculated in using $\Delta_r G^\circ = -RT \ln K$, where the subscript, r , denotes that the change in Gibbs energy is that for a reaction⁴. The solvent is omitted by convention when it is a reactant in dilute aqueous solutions. The value of the equilibrium constant depends on T , P and I . The pressure is generally understood, but the temperature and ionic strength must be specified; for example $K(25^\circ\text{C}, I = 0.25 \text{ M}) = 0.295$ (Ref. 5) for the reaction in eqn 1.

The equilibrium constant expression given in eqn 2 fails to answer the biochemist's question: to what extent does the hydrolysis of ATP to ADP and inorganic phosphate (P_i) occur at pH = 7 and pMg = 3? This question can be answered by using the biochemical equation



where ATP, ADP and P_i represent sums of species at the specified T , P , pH, pMg and I . The expression for the apparent

equilibrium constant K' for this biochemical reaction is given by

$$K' = \frac{[\text{ADP}][P_i]}{[\text{ATP}]c^\circ} \quad (4)$$

The c° in the denominator is required to make the apparent equilibrium constant dimensionless. The apparent equilibrium constant depends on T , P , pH, pMg and I ; for example, $K'(25^\circ\text{C}, \text{pH} = 7, \text{pMg} = 3, I = 0.25 \text{ M}) = 4.92 \times 10^5$ (Ref. 5). K' may depend on the concentrations of other free metal ions. Biochemical equations do not balance H, Mg, or charge when pH and pMg are specified, but they do balance other kinds of atoms. This is why words or abbreviations are used to represent the reactants in a biochemical equation, rather than atomic formulas. Conversely, chemical equations relate species, e.g. HPO_4^{2-} , which cannot exist at equilibrium except in the presence of other forms like $\text{H}_2\text{PO}_4^{2-}$ and MgHPO_4 . Since these two types of reaction equations mean different things, it is essential that they are easily recognized.

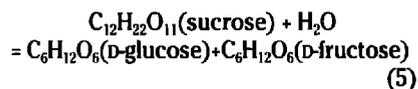
The relation between K' and K is sometimes simple, as in $K' = Kc^\circ/[\text{H}^+]$, or it may be complicated, as it is for ATP hydrolysis which is discussed below. In other cases these two equilibrium constants are equal. This can be confusing and so it is important to understand when the relation is simple and when it is complicated.

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Cases where $K' = K$

The two types of equilibrium constants are equal when the reactants are not weak acids, do not bind metal ions and do not react to produce or consume H^+ . An example is the β -fructofuranosidase (invertase) reaction (EC 3.2.1.26). The chemical equation for this reaction can be written as



and the biochemical equation can be written as



In this case the two equilibrium constants are clearly equal. Of course, sugars do have ionizable groups, but we are usually not interested in the dissociations that occur to an appreciable extent only at pH 12 and above.

The two types of equilibrium constants are also equal for racemases because the acid dissociation constants of reactant and product are equal. An example is the reaction catalysed by alanine racemase [EC 5.1.1.1]. We could represent the chemical equation by



and the biochemical equation by



$K' = K = 1$ under all conditions.

For isomerizations of weak acids, the pK 's are different for the isomeric forms, but if the pK 's are well below the neutral region, $K' = K$ in the neutral region. For the reaction catalysed by maleate isomerase [EC 5.2.1.1], the chemical reaction can be written as



with equilibrium constant, K , and the biochemical equation can be written as



with apparent equilibrium constant K' (25°C , $\text{pH} = 7$, $I = 0.25 \text{ M}$) = $K(25^\circ\text{C}$, $I = 0.25 \text{ M}$).

Cases where $K' = K$

We have included the maleate isomerase reaction in the $K' = K$ cases above, but isomerization reactions of weak acids with pK' values in the neutral

region certainly have to be classified as $K' = K$. For example, if the pK values of glucose phosphate and fructose phosphate were equal, the effects of the ionization on the apparent equilibrium constant for the isomerization would cancel, but since they are slightly unequal the value of K' will depend somewhat on the pH in the neutral region.

Even for a phosphate ester hydrolysis, the pH dependence of K' may be small. For alkaline phosphatase [EC 3.1.3.1], the chemical equation can be written



with

$$K = \frac{[\text{ROH}][\text{HPO}_4^{2-}]}{[\text{ROPO}_3^{2-}]c^0} \quad (12)$$

The biochemical equation can be written



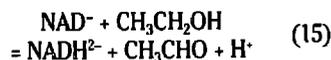
with

$$K' = \frac{[\text{ROH}][P_i]}{[\text{ROP}]c^0} = \frac{K\{1 + [H^+]/K_{aP}\}}{\{1 + [H^+]/K_{aRP}\}} \quad (14)$$

In this case, $K_{aRP} \approx K_{aP}$, and so K' is approximately equal to K . However, the difference will be important in careful work. Note that R can be adenosine. Thus the hydrolysis of adenosine monophosphate does not produce a significant amount of acid, unlike the hydrolysis of ATP.

Cases where $K' = Kc^0/[H^+]$ or $K[H^+]/c^0$

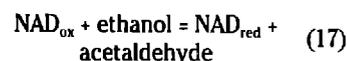
There are many reactions for which the relationship between K' and K is simple over a wide range of pH. The dependence on $[H^+]$ is linear for oxidoreductases with NAD as acceptor when the other reactants are not weak acids. For alcohol dehydrogenase [EC 1.1.1.1] the chemical reaction can be written



with

$$K = \frac{[\text{NADH}^+][\text{CH}_3\text{CHO}][\text{H}^+]}{[\text{NAD}^+][\text{CH}_3\text{CH}_2\text{OH}]c^0} \quad (16)$$

The equilibrium constant for this reaction is $K(25^\circ\text{C}$, $I = 0.25 \text{ M}) = 1.35 \times 10^{-11}$ (Ref. 6), independent of pH. The biochemical reaction can be represented by



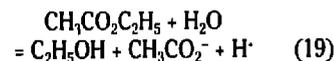
and the apparent equilibrium constant is defined by

$$K' = \frac{[\text{NAD}_{\text{red}}][\text{acetaldehyde}]}{[\text{NAD}_{\text{ox}}][\text{ethanol}]} \quad (18)$$

Thus, $K'(25^\circ\text{C}$, $\text{pH} = 7$, $I = 0.25 \text{ M}) = Kc^0(10^{-7} \text{ M}) = 1.35 \times 10^{-4}$. NAD^+ and NADH^+ each have pK values of around 4; but since these pK values are expected to be very nearly the same, these terms cancel in the expression for K' . Thus K' is inversely proportional to $[H^+]$ over essentially the whole pH range.

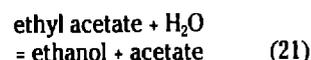
For the purposes of this article we use NAD_{ox} and NAD_{red} for the oxidized and reduced forms of NAD at a specified pH, but we emphasize that this usage does not conform to current recommendations of IUBMB. The use of NAD^+ as the symbol for either a species that actually has a negative charge in the pH range of interest or a reactant in a biochemical equation is contrary to principles we feel need to be adopted. Before proposing that they should become formal recommendations we should welcome the views of biochemists. These can be addressed directly to R. A. Alberty as convener of the Panel on Biochemical Thermodynamics.

The apparent equilibrium constant for the hydrolysis of ethyl acetate is inversely proportional to $[H^+]$ above about pH 5.5. A chemical equation for the hydrolysis of ethyl acetate by a carboxylesterase [EC 3.1.1.1] is



$$K = \frac{[\text{C}_2\text{H}_5\text{OH}][\text{CH}_3\text{CO}_2^-][\text{H}^+]}{[\text{CH}_3\text{CO}_2\text{C}_2\text{H}_5](c^0)^2} \quad (20)$$

The biochemical equation is



and the apparent equilibrium constant is defined by

$$K' = \frac{[\text{C}_2\text{H}_5\text{OH}][\text{CH}_3\text{CO}_2^-] + [\text{CH}_3\text{CO}_2\text{H}]}{[\text{CH}_3\text{CO}_2\text{C}_2\text{H}_5]c^0} = \frac{[\text{C}_2\text{H}_5\text{OH}][\text{CH}_3\text{CO}_2^-](1 + [H^+]/c^0K_a)}{[\text{CH}_3\text{CO}_2\text{C}_2\text{H}_5]c^0} = \frac{Kc^0}{[H^+]} \left(1 + \frac{[H^+]}{c^0K_a}\right) \quad (22)$$

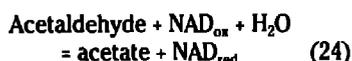
In the neutral pH range, $[H^+] \ll K_a c^0$, and so K' is inversely proportional to $[H^+]$.

Cases where $K' = K([H^+]/c^0)^{\Delta n(H^+)}$ where $\Delta n(H^+)$ is a positive or negative integer

This type of dependence is shown by the apparent equilibrium constant K' for the aldehyde dehydrogenase reaction [EC 1.2.1.3] at pH values above the pK for the weak acid involved. A chemical reaction is

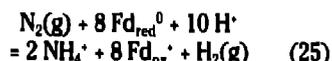


The biochemical reaction can be written



Thus $K' = K(c^0)^2/[H^+]^2$. $K(25^\circ\text{C}, I = 0.25 \text{ M}) = 4.76 \times 10^{-5}$ and $K'(25^\circ\text{C}, \text{pH} = 7, I = 0.25 \text{ M}) = 4.76 \times 10^9$ (R. A. Alberty, unpublished). Note that the biochemical reaction goes to the right at pH 7, even though the equilibrium constant for the reaction in eqn 23 is much less than 1.

This raises the question as to whether even greater pH dependences are to be found. The answer to this question is certainly yes. This is especially likely to happen when a number of chemical steps are catalysed within an enzyme-substrate complex without dissociating intermediates or when a large number of electrons are transferred in an oxidoreductase reaction. The chemical reaction for the fixation of nitrogen by nitrogenase can be represented by



where ferredoxin is represented by Fd and g indicates gas. The equilibrium constant for this reaction is calculated to be $K(25^\circ\text{C}, I = 0 \text{ or } 0.25 \text{ M}) = 1.40 \times 10^{91}$ (R. A. Alberty, unpublished). The dependence of the equilibrium composition on the pH is striking. The biochemical reaction can be written as



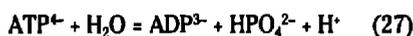
with apparent equilibrium constant $K' = K(c^0/[H^+])^{10}$. The value of K' at 25°C , $\text{pH} = 7$, $I = 0 \text{ or } 0.25 \text{ M}$ is equal to $K(c^0/10^{-7} \text{ M})^{10} = 1.40 \times 10^{11}$. Nitrogenase also catalyses the hydrolysis of ATP, and sometimes the hydrolysis of about 16 ATP to ADP and P_i is added to the reaction given in

eqn 26. However, the ratio of ATP hydrolysed to nitrogen fixed depends on temperature and pH, and is therefore not stoichiometric.

Cases where the exponent in the relation $K' = K([H^+]/c^0)^{\Delta n(H^+)}$ changes in the neutral pH range

In the previous examples given in this article, there was a simple relationship between the apparent equilibrium constant and the equilibrium constant; but when one or more reactants have pK values in the neutral pH range, this is not the case. Examples of reactants that have pK values in the neutral pH range are inorganic phosphate, pyrophosphate, organic phosphates, CO_2 and citrate.

A chemical equation for the hydrolysis of ATP can be represented by



and the equilibrium constant is defined by

$$K = \frac{[\text{ADP}^{3-}][\text{HPO}_4^{2-}][\text{H}^+]}{[\text{ATP}^+](c^0)^2} \quad (28)$$

The biochemical equation can be written as



and the apparent equilibrium constant is given by

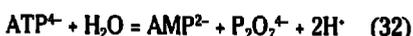
$$\begin{aligned} K' &= \frac{[\text{ADP}][P_i]}{[\text{ATP}]c^0} \\ &= \frac{Kc^0 (1 + [H^+]/K_{\text{ADP}}c^0)(1 + [H^+]/K_{\text{P}}c^0)}{[H^+](1 + [H^+]/K_{\text{ATP}}c^0)} \end{aligned} \quad (30)$$

if only the pK values in the neutral region are included. As a very rough approximation, we can say that the terms for ATP and ADP cancel out so that

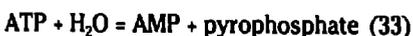
$$K' = \frac{Kc^0}{[H^+]} \left(1 + \frac{[H^+]}{K_{\text{P}}c^0}\right) \quad (31)$$

Thus, when $[H^+] \ll K_{\text{P}}c^0$, K' is inversely proportional to $[H^+]$.

For the hydrolysis of ATP to give pyrophosphate, a chemical equation is



The biochemical equation can be written as



and it is readily shown that at pH values above the highest pK for pyrophosphate

$$K' = K(c^0)^2/[H^+]^2 \quad (34)$$

Note that the hydrolysis of AMP to adenosine and inorganic phosphate does not yield any H^+ at high pH. Thus acid production depends on what kind of a bond is being cleaved.

Discussion

Many reactions are represented in the current biochemical literature by hybrids of chemical equations and biochemical equations; e.g. $\text{ATP} + \text{H}_2\text{O} = \text{ADP} + P_i + \text{H}^+$. This is unfortunate because these hybrid equations do not lead to equilibrium constant expressions that can be used for a discussion of the thermodynamics of these reactions or the change in binding of H^+ with pH. When you see a chemical reaction, you know how to write the expression for the equilibrium constant, K , and so it would be advantageous to be able to write the expression for the apparent equilibrium constant, K' , for a biochemical reaction by looking at the biochemical equation. The changes required in order to reach this goal are not large, but they do require the introduction of symbols (words or abbreviations) for biochemical reactants at an arbitrary pH and concentration of free metal ions that are bound by reactants. In general these abbreviations should not contain H because this suggests an attempt to balance H, but H_2O is an exception.

In general the roles of biochemical reactions in a living cell can be discussed by use of biochemical reactions, and this is the way that biochemical reactions are experienced in the laboratory, where the amount of ATP consumed in a reaction is measured, rather than the amounts of a specific species. Chemical reactions are useful in discussing the details of the catalytic process, but when reactants with pK values in the vicinity of 7 are involved, it is important to recognize that acid dissociations occur at the same time.

The amount of H^+ produced by a reaction is important experimentally because it determines the dependence of K' on pH. Conversely, the determination of the dependence of K' on pH yields the value of $\Delta n(H^+)$ in that pH range.

These discussions have all been concerned with reactions catalysed by a single enzyme, but they also apply to the net reaction catalysed by several

enzymes. Two or more biochemical equations can be added together and the apparent equilibrium constant K' for the net reaction is the product of the apparent equilibrium constants of the reactions that are added. But biochemical reactions and chemical reactions cannot be added to obtain an equation that has an equilibrium constant, since net biochemical reactions may have a high dependence on pH.

In these discussions we have ignored the binding of Mg^{2+} and other metal ions as a simplification, but the discussion of the effects of pMg on biochemical equilibria parallels the discussions of pH. Here we have avoided using the transformed thermodynamic properties ΔG° , ΔH° and ΔS° as a simplification, but it is clear that they are functions of pH and pMg in contrast to ΔG° , ΔH° and ΔS° , which are not.

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PROTEIN SEQUENCE MOTIFS

Transcriptional activator components and poxvirus DNA-dependent ATPases comprise a single family

Certain yeast and *Drosophila* proteins in the SNF2 family are thought to be essential components of multiprotein complexes involved in transcriptional activation¹. These proteins appear to contain motifs characteristic of RNA- and DNA-dependent helicases and other nucleic acid-dependent ATPases. However, the detection of these motifs can be subjective. For example, none of the five studies that reported helicase motifs agreed on the presence or exact locations of all seven motifs in subsets of the same proteins.

To clarify this situation, an objective approach to detection of very distant relationships was applied. Conserved regions from 560 known protein families are represented in a general database of over 2000 multiply aligned sequence segments or 'blocks' derived by a fully automated procedure². This database was searched using 69 known and proposed helicase sequences that belong to seven well-defined families to detect possible cross-family relationships. The basis for detection is that a sequence must align well with two or more blocks representing a family. With alignments to blocks that score among the top 0.5% in a search, one asks whether any of them is supported by correctly spaced alignments with other lower scoring blocks representing the same family. If so, the probability is calculated that these supporting block alignments are chance hits based on their ranks in the search.

These searches detected two unequivocal cross-family relationships. Proteins in the DE-H group (e.g. yeast PRP16 and *Drosophila* Maleless) are

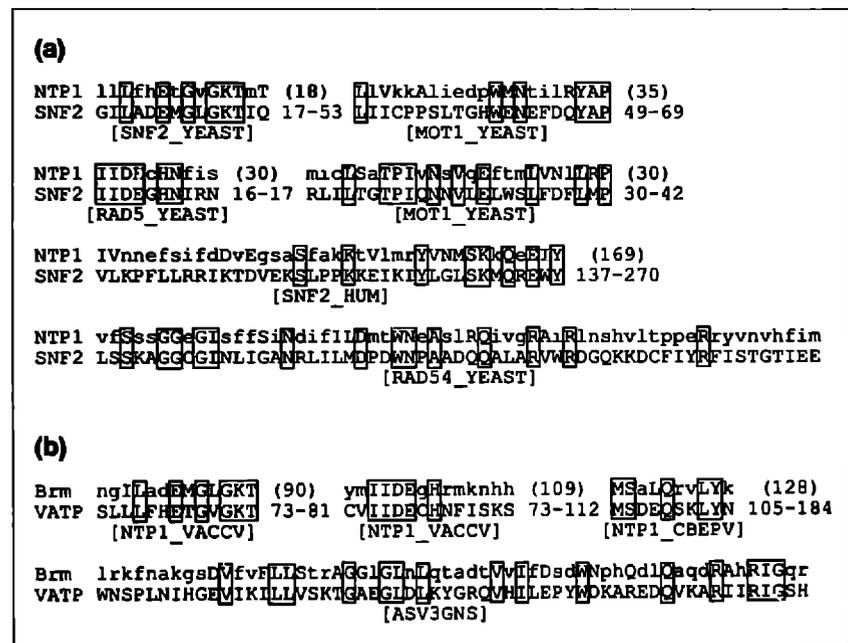


Figure 1

Alignments of query sequences against the most similar sequence segments from blocks detected in the searches. (a) Vaccinia NTP1 v. SNF2 blocks. (b) *Drosophila* Brm v. VATP blocks. The number in parentheses is the intervening distance in amino acids, with the range of distances between block segments for all family members shown below. Upper case letters indicate a match to any segment in the aligned block. SNF2 proteins are yeast SNF2, MOT1, RAD5, RAD54, STH1, chromosome 1L ORF1, *Drosophila* Brm and Lds and a human homolog. VATP proteins are NTP1_VACCV, NTP1_CBEPV, YKP4_KLULA, ETF1_VACCV, ETF1_FOWP1 and GenPept ORF ASVGNS. Details on the searching method are obtainable from the BLOCKS e-mail searcher: blocks@howard.fhcrc.org, Subject: help.

sufficiently similar to the positive-stranded viral RNA helicases that they comprise a single family³. For example, the bovine diarrhea virus P80 sequence aligns as well with blocks consisting of DE-H proteins ($P < 5 \times 10^{-9}$) as with blocks consisting of corresponding RNA helicases from related viruses ($P < 1 \times 10^{-8}$). The SNF2 group and the poxvirus DNA-dependent ATPases (the VATP group) comprise another single distinct family. For example, vaccinia NTP1 (a

VATP protein) detected all six SNF2 blocks ($P < 1 \times 10^{-10}$, Fig. 1a) and *Drosophila* Brm (an SNF2 family member) detected all four VATP blocks ($P < 2 \times 10^{-7}$, Fig. 1b). In contrast, no other cross-family relationships were reliably detected above the highest scoring background or negative control hits ($P < 10^{-3}$). Previous failures to detect global relationships outside the SNF2 group are understandable, since the single best alignment between NTP1 and one