

THERMODYNAMICS OF ENZYME-CATALYSED REACTIONS

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ABSTRACT

Since the pH is treated as an independent variable in biochemical thermodynamics, the Gibbs energy G does not provide the criterion for equilibrium, but the transformed Gibbs energy G' does. The standard transformed Gibbs energy of formation $\Delta_f G'^0$ of a reactant (sum of species) can be calculated at the desired temperature, pH, and ionic strength if the standard Gibbs energies of formation $\Delta_f G^0$ and standard entropies of formation $\Delta_f H^0$ of the species that make up the reactant are known. BasicBiochemData3 in MathSource provides species properties for 199 biochemical reactants and Mathematica programs for calculating apparent equilibrium constants K' and other transformed thermodynamic properties of enzyme-catalysed reactions are given. This database can be extended, and the number of reactions for which apparent equilibrium constants can be calculated increases exponentially with the number of reactants in the database.

DATABASE ON THERMODYNAMICS OF ENZYME CATALYSED REACTIONS

My main interest is in the thermodynamics of reactions catalysed by enzymes, but some of the things I am going to talk about apply to the kinetics of these reactions. There are two ways to discuss the thermodynamics of enzyme-catalysed reactions: (1) with chemical reactions written in terms of species (like ATP^+ , HATP^{3-} , and $\text{H}_2\text{ATP}^{2-}$) using the Gibbs energy G , enthalpy H , and entropy S . (2) with biochemical reactions written in terms of reactants (sums of species like ATP) at a specified pH and using the apparent equilibrium constant K' , the transformed Gibbs energy G' , the transformed enthalpy H' , and the transformed entropy S' . These transformed thermodynamic properties depend on the pH. Both ways are needed by biochemists. Chemical reactions are needed to discuss mechanisms of enzyme-catalysed reactions in terms of species. Biochemical reactions are needed to obtain a broader overview of enzyme-catalysed reactions at specified pH (and perhaps pMg). This is all explained in IUPAC/IUB Recommendations, 1994 [1].

Both chemical equations and biochemical equations are mathematical equations in the sense that chemical equations must balance numbers of atoms of all elements and electric charges. Biochemical equations must balance numbers of atoms of all elements except for hydrogen. Biochemical equations also do not balance electric charge. The reason why biochemical equations do not balance numbers of hydrogen atoms and electric charges is that it is assumed that the pH is held constant during the reaction. This can be done with a pHstat, but biochemists use a buffer to keep the pH nearly constant during an enzyme-catalysed reaction, and then, if they are determining an apparent equilibrium constant, they measure the composition and pH at equilibrium. The equilibrium composition corresponds with this pH, and so this is equivalent to using a pHstat.

Most enzyme-catalysed reactions produce or consume hydrogen ions. This leads to a new thermodynamic property, the change in binding of hydrogen ions $\Delta_r N_{\text{H}}$ in the reaction. $\Delta_r N_{\text{H}}$ will depend on the pH if any reactant has a pK in the pH range of interest (usually between pH 5 and pH 9). This property can be calculated using:

$$\Delta_r N_{\text{H}} = \sum v_i' \bar{N}_{\text{H}i} \quad (1)$$

where v_i' is the stoichiometric number for reactant i and $\bar{N}_{\text{H}i}$ is the average number of hydrogen atoms in reactant i . The prime is needed to distinguish v_i' from the stoichiometric numbers in the underlying chemical reactions. In making tables of thermodynamic properties of enzyme-catalysed reactions, $\Delta_r N_{\text{H}}$ is usually calculated using:

$$\Delta_r N_{\text{H}} = \frac{1}{RT \ln(10)} \frac{\partial \Delta_r G'^{\circ}}{\partial \text{pH}} \quad (2)$$

However, it can also be calculated using Equation 1. It should be noted that in the thermodynamics of biochemical reactions, $\Delta_r N_H$ is on the same level as $\Delta_r H^0$ and $\Delta_r S^0$ since all three of these properties are calculated by taking partial derivatives of $\Delta_r G^0$. The average number of hydrogen atoms in a biochemical reactant can be calculated using the binding polynomial [2, 3] for the reactant. This calculation requires the pK values of the reactant in the pH range of interest and the numbers of hydrogen atoms in the species of the reactant. The use of Equation 1 has the advantage over Equation 2 in that $\Delta_r G^0$ values for species in the reaction do not have to be known.

These requirements of the thermodynamics of enzyme-catalysed reactions also apply to the kinetics because the complete steady-state rate equation for an enzyme-catalysed reaction must yield the same equilibrium composition for the reaction as thermodynamics. The apparent equilibrium constant K' for an enzyme-catalysed reaction can be calculated from kinetic parameters; this expression for the apparent equilibrium constant is referred to a Haldane equation. For some mechanisms there is more than one Haldane equation.

The calculations in biochemical thermodynamics are very complicated, but fortunately the application Mathematica[®] [4] is very convenient for making them. I have developed a database, written in Mathematica and called BasicBiochemData3 [5], that gives the standard Gibbs energies of formation $\Delta_f G^0$ and standard enthalpies of formation $\Delta_f H^0$ of species of biochemical interest at 298.15 K and zero ionic strength for 199 reactants of biochemical interest. Some of these species properties come from the NBS and CODATA thermodynamic tables that deal with chemical species, but for larger molecules of biochemical interest, species properties have to be calculated from experimental measurements of apparent equilibrium constants K' and enthalpies of enzyme-catalysed reactions. The experimental data in the literature has been summarized and evaluated (actually graded A, B, C) by Goldberg and Tewari in six survey papers in *J. Phys. Chem. Ref. Data* (1991 – 1999). They have summarized experimental data on about 500 different reactions involving about 1000 reactants. They have also established a web site on this experimental data [6]. We are indebted to Goldberg and Tewari for assembling all this literature data.

Mathematica is so useful that I have written a second book [3] this time in Mathematica, entitled “*Biochemical Thermodynamics; Applications of Mathematica.*” This makes it possible to intermingle explanations, programs, and calculations. It has a CD in the back with all the programs, data, and words. All the steps in calculating properties, making tables, and making figures are shown. We usually think of computer programs that calculate numbers, but Mathematica can do more. It can be used to derive equations that are too big to write out by hand.

BasicBiochemData3 provides $\Delta_f G^0$ values for species of 199 biochemical reactants, but $\Delta_f H^0$ are known for species for only 94 of these reactants. This database can be used to calculate standard transformed Gibbs energies of formation $\Delta_r G^0$ of these 199 reactants at 298.15 K in the pH range 5 to 9 and ionic strengths from zero to about 0.35 M. For the 94 reactants for which enthalpies are known, it is possible to calculate $\Delta_r G^0$ from 273.15 K to about 313.15 K.

These $\Delta_f G'^0$ have been used to calculate standard transformed Gibbs energies of reaction $\Delta_r G'^0$ and apparent equilibrium constants K' at 298.15 K and 0.25 M ionic strength for 229 enzyme-catalysed reactions [3], but the $\Delta_f G'^0$ can be used for even more reactions. When $\Delta_f H'^0$ are known for the species of a reactant, standard transformed Gibbs energies $\Delta_f G'^0$ and standard transformed enthalpies $\Delta_f H'^0$ of reactants can be calculated in the temperature range 273.15 K to about 313.15 K, pH values in the range 5 to 9, and ionic strengths from zero to about 0.35 M. This information has been used to calculate $\Delta_r G'^0$, $\Delta_r H'^0$, $\Delta_r S'^0$ and apparent equilibrium constants K' for 90 enzyme-catalysed reactions.

Since biochemists need thermodynamic properties at various temperatures, pH values and ionic strengths, tables and plots cannot satisfy these needs. Having a file of mathematical functions that give $\Delta_f G'^0$ of reactants does satisfy these needs, and BasicBiochemData3 makes available Mathematica programs and 774 mathematical functions for these properties. These functions can be added and subtracted to obtain changes in thermodynamic properties in biochemical reactions and apparent equilibrium constants. Plots can also be made to show how reaction properties depend on temperature, pH, and ionic strength. BasicBiochemData3.nb contains the functions of temperature, pH, and ionic strength that yield the standard transformed Gibbs energies of reaction for the 90 enzyme-catalysed reactions for which the effects of changing the temperature can be calculated.

It is important to emphasize the importance of ionic strength in the thermodynamics of enzyme-catalysed reactions. According to the Debye-Huckel theory, the logarithm of the activity coefficient of an ion in water is proportional to its charge squared. This means that the ionic strength effect for the species ATP^{4-} is 16 times that for a chloride ion, a huge effect.

I often see MgATP in the biochemical literature, but this is not a species or a reactant. To treat the effect of magnesium ions, a further Legendre transform is required to introduce pMg as an independent variable. People determining apparent equilibrium constants of reactions in the presence of magnesium ions often give the total magnesium concentration, but it is $\text{pMg} = -\log[\text{Mg}^{2+}]$, where $[\text{Mg}^{2+}]$ is the free concentration, that affects the value of K' . The effects of pMg on the hydrolysis of ATP, ADP, and AMP have been calculated, but this is about the only series for which there is sufficient information about the dissociation of magnesium complex ions. The effect of Mg^{2+} sometimes cancels because both reactants and products bind Mg^{2+} .

STOICHIOMETRY OF ENZYME-CATALYSED REACTIONS

In making thermodynamic calculations on biochemical reactions, it is necessary to be very careful about stoichiometry (for example, see Equation 1). It is assumed that when a reactant is made up of species with different numbers of hydrogen atoms, these species are in equilibrium at a specific pH. I think that biochemists understand this pretty well for reactants like ATP, but not for reactants like carbon dioxide and ammonia. Many biochemical reactions are balanced on the web with CO_2 or NH_3 , but I do not think this is very

appropriate for considering reactions in a living cell where there is no gas phase. In aqueous phases, carbon dioxide is made up of four species: CO_2 , H_2CO_3 , HCO_3^- , and CO_3^{2-} . I represent this sum of species in the aqueous phase as CO_2tot , for which the transformed thermodynamic properties depend on the temperature, pH, and ionic strength. When $\text{CO}_2(\text{gas})$ is replaced with CO_2tot in a reaction equation, a H_2O has to be added on the other side of the reaction to balance oxygen atoms. In the aqueous phase ammonia is made up of NH_3 and NH_4^+ . I represent this sum of species by ammonia, for which the transformed thermodynamic properties depend on the temperature, pH, and ionic strength. These comments apply to other gases that dissolve in water and exist in the aqueous phase in different protonated forms.

Hydrogen ions should never appear in balanced biochemical equations because it is understood that the pH is held constant during the approach to equilibrium by adding or removing hydrogen ions. I am not advocating the use of pHstats, but what I am saying is that biochemists interpret determinations of apparent equilibrium constants and enthalpies of reaction as if they were carried out in a pHstat.

The abbreviations NAD^+ and NADH are a problem because this seems to indicate that hydrogen atoms and electric charges are to be balanced on the two sides of the biochemical equation, but they are not. I favour using NAD_{ox} and NAD_{red} instead. These remarks apply to other complicated coenzymes that exist in oxidized and reduced forms.

In my new book I have always written reactions in the direction in which they have apparent equilibrium constants greater than unity at pH 7 and 0.25 M ionic strength. In the 229 reactions in my book for which apparent equilibrium constants are calculated at 298.15 K, 78 are written in the opposite direction from the EC list.

FUTURE DEVELOPMENTS USING THE DATABASE OF GOLDBERG AND TEWARI

Many more species data can be obtained from the database surveyed by Goldberg and Tewari [6]. When the apparent equilibrium constant has been determined for an enzyme-catalysed reaction, there is the potential for calculating $\Delta_f G^0$ for the species of a reactant. It is necessary to say „there is the potential“ because the following conditions have to be met: (1) The $\Delta_f G^0$ of all of the species of all of the reactants, but the reactant of interest, are needed. (2) If the reactant of interest has pK values in the range of approximately 5 to 9, these pK values are needed. (3) The experiments have to be carried out carefully and reported accurately. These are pretty demanding requirements.

There is an exception to requirement (1) that should be used sparingly: when there are two reactants in an enzyme-catalysed reaction for which thermodynamic properties are not known, $\Delta_f G^0 = 0$ can be assigned to one species of one of these two reactants. This was done by Alberty and Goldberg (1992) with the ATP series when $\Delta_f G^0(\text{adenosine}^0) = 0$ was

adopted as a convention of the thermodynamic table. This made it possible to calculate $\Delta_f G^0$ for all the other species in the ATP series. When this convention is used, the adenosine moiety must appear on both sides of a biochemical reaction. After Boeiro-Goates and coworkers (2001) determined the standard entropy of adenosine(cryst) using the third law method, they were able to calculate $\Delta_f G^0$ (adenosine⁰) in aqueous solution with respect to the elements in their reference states, and this changed the $\Delta_f G^0$ of all the species in the ATP series by the same amount. It did not change the apparent equilibrium constants that had been calculated earlier for reactions involving the ATP series. But now it is possible to explore the thermodynamics of the formation of adenosine and adenine all the way back to the elements. The convention that $\Delta_f G^0 = 0$ for one species is especially useful for reactants in oxidoreductase reactions because the oxidized form is on one side of the equation and the reduced form is always on the other side. Similar remarks apply to the use of the convention that $\Delta_f H^0 = 0$ for one of the species of the reactant.

Enzymes are making it possible to learn about the thermodynamics of large molecules in aqueous solution because they catalyse very specific reactions rapidly. The thermodynamics of these large molecules could never have been determined classically because without catalysts complicated mixtures are obtained.

FACTORS THAT FAVOUR THE EXTENSION OF THE DATABASE ON SPECIES

In looking ahead to the future of biochemical thermodynamics I want to point out that as species properties of reactants are added to the database, the number of reactions for which apparent equilibrium constants can be calculated increases exponentially. ATP participates in 41 of the 229 reactions for which I have made calculations at 298.15 K, and urea is involved in one. The “average” reactant is involved in about 6 reactions. Thus we can expect that when a new reactant is added to the database, K' can be calculated for about 6 additional reactions. This leads to an exponential increase in the number of enzyme-catalysed reactions for which apparent equilibrium constants can be calculated. Many equilibrium constants that can be calculated are so large that they cannot be measured directly with today's technology.

Oxidoreductase reactions are a striking example of this. The table of standard apparent reduction potentials of half reactions can be used to calculate apparent equilibrium constants for any pair of half reactions. If the table of standard apparent reduction potentials contains N different half reactions, the number R of different reactions for which K' can be calculated is given by $R = N(N-1)/2$. The current table of standard apparent reduction potentials in BasicBiochemData3 contains 60 half reactions, and so K' can be calculated for $60 \times 59 / 2 = 1770$ oxidoreductase reactions. Of course enzymes are not known for all of these reactions, but I am sure that enzymes will be found for more of them.

Another reason for this exponential increase in the number of reactions with known K' is coupling. Transferase reactions couple two oxidoreductase reactions or two hydrolase reactions, and so knowledge of K' for oxidoreductase reactions and hydrolase reactions yield K' for transferase reactions that have not been studied. Lyase reactions are coupled by definition, and so their K' values can be obtained by multiplying the K' for the two or three reactions coupled by the lyase reaction.

INDEPENDENCE OF THE REACTIVITIES OF SOME GROUPS IN LARGE MOLECULES

Since many reactants in enzyme-catalysed reactions are rather large molecules, the chemical thermodynamic properties of various groups may be nearly independent, especially at zero ionic strength. As an example of this, when Boeiro-Goates and coworkers obtained $\Delta_f G^0$ and $\Delta_f H^0$ of inosine by calorimetric methods, they calculated the $\Delta_f G^0$ and $\Delta_f H^0$ of all the species of ITP, IDP, and IMP on the assumption that the phosphate pK values and the chemical equilibrium constants for phosphatase reactions and nucleosidase reactions are the same in the ITP series as in the ATP series. This does not mean that the standard transformed Gibbs energies of formation of reactants in the two series are different by a constant increment because the pK values of the purine rings in the two series are different. The pK values for the purine rings are 4.68 for ATP, 4.36 for ADP, 3.99 for AMP, compared with 10.09 for ITP, 9.56 for IDP, and 9.63 for IMP.

This kind of reasoning has been applied to put the guanosine triphosphate series, xanthosine triphosphate series, cytidine triphosphate series, uridine triphosphate series, and thymidine triphosphate series in the next version of BasicBiochemData3. This has required the introduction of the conventions that $\Delta_f G^0$ (guanosine, 298.15 K, $I=0$) = 0 and $\Delta_f G^0$ (cytidine, 298.15 K, $I=0$) = 0, but it is not necessary to introduce the conventions that $\Delta_f G^0$ (xanthosine, 298.15 K, $I=0$) = 0, $\Delta_f G^0$ (uridine, 298.15 K, $I=0$) = 0, and $\Delta_f G^0$ (thymidine, 298.15 K, $I=0$) = 0.

I do not want to over-emphasize this idea of exponential growth because a lot of work is required to analyse the current experimental data summarized by Goldberg and Tewari, and unfortunately not many new measurements of apparent equilibrium constants and enthalpies of reactions are being made today.

Transformed thermodynamic properties are also needed in the discussion of protein-ligand binding [7].

KINETICS OF SYSTEMS OF ENZYME-CATALYSED REACTIONS

For a system of enzyme-catalysed reactions, thermodynamics can do two things if the species properties are known for all of the reactants: (1) At a given temperature, pH, and ionic strength, and given concentrations of the reactants in the system, thermodynamics can tell the direction in which each enzyme-catalysed reaction will go. This information is provided by the following calculation of the transformed Gibbs energy of reaction at species concentrations $[i]$ of reactants:

$$\Delta_r G' = \Delta_r G'^0 + RT \ln \prod [i]^{v_i} \quad (3)$$

where Π indicates a product involving all the reactants. When $\Delta_r G'$ is negative, the reaction goes to the right. (2) Thermodynamics can also be used to calculate the equilibrium concentrations that will be reached at long times. These are the concentrations that will make $\Delta_r G' = 0$ for all the reactions. The equilibrium concentrations cannot be calculated analytically, but the Newton-Raphson method makes it possible to use a computer to iterate to the equilibrium concentrations. Two short programs in Mathematica make it possible to do this by specifying the stoichiometric number matrix, a list of apparent equilibrium constants, and the initial concentrations of the reactants.

I do not think that biochemists have sufficiently appreciated how much thermodynamics can help in understanding the series and cycles of enzyme-catalysed reactions. The steady-state rate law for a reaction can be used to calculate a small change in the concentration of each reactant. This changes the concentrations, and so thermodynamics can be used again to tell the directions of the reactions. When this process is continued with small steps the equilibrium concentrations will be reached. This equilibrium composition can be checked by comparing it with the equilibrium composition calculated using thermodynamics. Mathematica provides NDSolve that yields numerical solutions to systems of differential equations. This program yields interpolation functions for each reactant that can be plotted. In the absence of Michaelis constants and other kinetic parameters, calculations can always be made when concentrations of reactants are low in comparison with Michaelis constants.

WEBMATHEMATICA

I want to close by describing a recent development that promises to make complicated thermodynamic calculations available to biochemists who do not have the Mathematica application in their computer and do not know how to use Mathematica. webMathematica makes it possible to put up a web site that has boxes to fill in with input for a calculation. When the „Compute“ button is clicked, the problem is solved in a server that has Mathematica and BasicBiochemData3, and the numerical result or plot appears. Such a web site can be used to calculate the apparent equilibrium constant for an enzyme-catalysed reaction at the desired temperature, pH, and ionic strength. In the first box, the user would type in the names of reactants and their stoichiometric numbers. In the second box, the user would

type in the names of products and their stoichiometric numbers. Then the desired temperature, pH and ionic strength would be typed in. When the „Compute“ button is clicked, the server on the web that has Mathematica and BasicBiochemData3 will make the calculation and present the desired apparent equilibrium constant on the computer screen. Wolfram research has placed about 40 of these html files on the web as examples, and these sites can be run by going to <http://www.wolfram.com>, clicking on webMathematica. You do not need Mathematica in your computer to do this. At MIT we are working on the html file to make the calculation of apparent equilibrium constants I have just described.

CONCLUSION

I hope that in the future it will be possible for biochemists to calculate K' , $\Delta_r G^0$, $\Delta_r H^0$, $\Delta_r S^0$, and $\Delta_r N_H$ for a much larger number of enzyme-catalysed reactions at desired temperatures, pH values, and ionic strengths. I believe that these properties will contribute to the understanding of both the thermodynamics and the kinetics of individual enzyme-catalysed reactions and networks of reactions.

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