The first and second laws of thermodynamics were known before Gibbs wrote his great paper “Equilibrium of Heterogeneous Substances” that was published in the Transactions of the Connecticut Academy in 1876 (1), but he completely changed the character of thermodynamics by showing it obeyed all the rules of calculus and introducing the chemical potential $\mu$ and what we now call the Gibbs energy, $G$. Obeying the rules of calculus means that there are many relationships between various thermodynamic properties. The introduction of the chemical potential made it possible to treat chemical reactions. The first law introduces the internal energy $U$ that provides the criterion for spontaneous change and equilibrium at constant volume $V$ and entropy $S$. The enthalpy $H$ is defined by

$$H = U + PV$$

(Eq. 1)

and it provides the criterion for spontaneous change and equilibrium at constant pressure and entropy. The Helmholtz energy $A$ is defined by

$$A = U - TS$$

(Eq. 2)

and it provides the criterion for spontaneous change and equilibrium at constant volume and temperature. This is beginning to look useful in the laboratory except that chemistry is usually carried out at constant pressure and temperature. Gibbs defined the property $G$, which we now call the Gibbs energy, as follows.

$$G = H - TS$$

(Eq. 3)

The Gibbs energy provides the criterion for spontaneous change and equilibrium at constant temperature and pressure. These three definitions are what we now refer to as Legendre transforms. Legendre transforms are the most significant concept in this short history. Note that Equations 1–3 each involve a product of conjugate variables that yields energy. Everybody knows that the variables in an equation can be changed by simply substituting an equation for one of the variables in terms of a new variable. Not everybody knows that a derivative can be introduced as a new variable, but Gibbs did. I wrote a review on Legendre transforms in 1994 (2), and later I chaired an IUPAC Committee that wrote a Technical Report on Legendre transforms (3). Two more Legendre transforms will be used in these Reflections.

Before going further, we must be clear about what thermodynamics can tell us about a reaction system. Callen (4) has written: “Prediction of the new equilibrium state is the central problem of thermodynamics.” Thermodynamic measurements on systems at equilibrium can be used to calculate properties that can be used to predict whether reactions in a system will go to the right or the left under a given set of conditions in addition to making it possible to calculate the equilibrium composition. It is not necessary to have measurements on the actual reactions in the system to do this because other pathways can be used to calculate the
equilibrium composition. Rather than tabulating equilibrium constants of chemical reactions, chemists recognized a long time ago that the most efficient way to store thermodynamic information on chemical reactions was to make tables of the standard Gibbs energies of formation, $\Delta_f G^0$, standard enthalpies of formation, $\Delta_f H^0$, and standard molar entropies, $S_m$, of species (5).

**Early Measurements of Equilibrium Constants of Enzyme-catalyzed Reactions**

Robert N. Goldberg has told me that the first reported measurement of the equilibrium constant of an enzyme-catalyzed reaction was published by Quastel and Woolf in 1926 (6) on the aspartate ammonia-lyase reaction.

\[
\text{Aspartic acid} = \text{fumaric acid} + \text{ammonia} \quad \text{(Eq. 4)}
\]

They summarized their experimental data with the following equation.

\[
K = \frac{[\text{fumaric acid}][\text{NH}_3]}{[\text{aspartic acid}]} = 0.040 \text{ at pH 7.4 and 37 °C} \quad \text{(Eq. 5)}
\]

Several more equilibrium constants were determined in the 1930s and even more in the 1940s, but it was not until 1953 when Burton joined Krebs (7) that the field began to get organized. In their article about the Gibbs energy changes associated with the individual steps of the tricarboxylic acid cycle, glycolysis, and alcoholic fermentation and with the hydrolysis of the pyrophosphate groups of adenosine triphosphate, they referred to an article by Alberty et al. (8), which notes the fact that the $\Delta G^0$ for the hydrolysis of ATP becomes practically independent of pH below 6.3. So how did I get involved with ATP? I was very fortunate to have worked on the wartime Medical Research Council project on plasma proteins centered at Harvard Medical School under the direction of Prof. Edwin Cohn from June 1944 to January 1947. I worked for Prof. J. W. Williams at the University of Wisconsin on plasma fractionation and submitted my Ph.D. thesis on the electrophoresis of plasma proteins in June 1947. As an instructor at the University of Wisconsin I looked for new ways to use the Tiselius moving boundary electrophoresis apparatus and measured amounts of AMP and ADP in commercial preparations of ATP that were just then becoming available. To understand the changes in mobility of ATP with pH, a couple of my first graduate students and I determined the pK values of these substances and the dissociation constants of their magnesium complex ions (8).

I wanted to move into enzyme kinetics and was interested in the ideas of Prof. Pauling, so I arranged to spend the academic year of 1950–1951 at Cal Tech. I chose to work on fumarase because I was able to get one of the first Beckman DU spectrometers equipped with a strip chart recorder. Although I was primarily interested in rates, this research brought me into contact with thermodynamics in a couple of ways. In 1953 Bock and I (9) determined the apparent equilibrium constant for the reaction fumarate $+ \text{H}_2\text{O} = L$-malate as a function of pH and temperature. Because we were able to determine the Michaelis constants and maximum velocities in both the forward and reverse directions, we were able to confirm the Haldane relation between these kinetic constants and the equilibrium constant. The next year Frieden, Bock, and I (10) determined the binding of substrates by fumarase was diffusion-controlled (12).

**Production of the First Tables of Standard Thermodynamic Properties of Species of Biochemical Reactants**

In 1957 Krebs and Kornberg published “Energy Transformations in Living Matter” (13), and Burton was the author of the Appendix “Free Energy Data of Biological Interest.” Burton made a table of standard Gibbs energies of formation of species from chemical thermodynamic sources and from measurements of equilibrium constants of enzyme-catalyzed reactions. He used this table to calculate equilibrium constants of a number of reactions using the convention that the “$\Delta G’$ (for the reaction at pH 7) is identical with the $\Delta G^0$ except that the standard condition of H$^+$ ion is that of the pH specified (usually 7) instead of 1 molal activity (pH 0).” This works fine when the reactants are single species (like fumarate and L-malate at pH 7), but it does not work for a reactant like ATP at pH 7.
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I had a wonderful time working on enzyme kinetics with graduate students and postdoctoral fellows, but in 1961, I became Associate Dean of Letters and Science and in 1963 I became Dean of the Graduate School at the University of Wisconsin. At first I carried out a nearly normal research program, but I had less and less time to spend with my graduate students. In 1967 I moved to MIT to be Dean of the School of Science. Although I did not have a laboratory, I wanted to do some research, and the availability of help from the Computer Laboratory led me to write two theoretical papers on the hydrolysis of ATP (14, 15). Phillips and co-workers (16) had published earlier papers, and we were in pretty good agreement. By this time, the problem that Burton had with ATP was avoided by using a balanced reference reaction written in terms of species and a binding polynomial for each reactant. Guy nn and Veech (17) carried out a number of interesting studies (for example, studies of the reactions catalyzed by phosphate acetyltransferase and acetate kinase).

In the early 1970s, Edsall and others felt that it was a good time to seek international agreement on nomenclature and symbols for biochemical thermodynamics. A Commission was formed with Gutfreynd and Privalov representing the International Union of Biochemistry (IUB), Edsall and Jenckes representing the International Union of Pure and Applied Biophysics (IUPAB), Wadsö (Chairman) representing the International Union of Pure and Applied Chemistry, and associate member Biltonen. I was invited to join the Commission, but I was too busy to do it. I corresponded with the Commission and was basically in agreement with their recommendations (18). They recommended the symbol $K'$ for the apparent equilibrium constant and $\Delta G^o' = -RT\ln K'$ for the standard Gibbs energy of reaction at a specified temperature, pH, and ionic strength. As the field of biochemical thermodynamics continued to grow, four important additions and improvements were made. Wilhoit (19) extended the Burton table by including standard enthalpies of formation of species. Thauer and co-workers (20) included inorganic species and suggested that $\Delta G^o'$ can be calculated using $\Delta G^o + m\Delta G'(\text{H}^+)$, where $\Delta G'(\text{H}^+)$ is equal to the Gibbs energy of hydrogen ions at the experimental pH and is negative when more protons are consumed than formed. Goldberg and Tewari (21) made a very thorough evaluation of the literature data on the pentoses and hexoses. Miller and Smith-Magowan (22) reviewed and evaluated the data on the Krebs cycle and related compounds.

Learning about Legendre Transforms

Jeffries Wyman had been working on the thermodynamics of the equilibria involving hemoglobin since before 1948, and he and Stanley Gill published their book on binding and linkage in 1990 (23). Ligand binding was considered to be a separate field, but researchers on enzyme-catalyzed reactions recognized that some ideas like linkage $(\partial N_p/\partial \text{pMg}) = (\partial N_{\text{Mg}}/\partial \text{pH})$ applied in both fields. Wyman understood how to use Legendre transforms, and he and Stanley Gill discussed them in the last chapter of their book. I had heard of Legendre transforms, but I did not learn about them from Wyman. To show how I learned to use Legendre transforms, I have to write about my leaving the Dean’s Office in 1982 and going back to teaching physical chemistry.

Except for my papers on ATP hydrolysis in 1968 and 1969, my administrative responsibilities had kept me too busy to do any research or even to stay current with research on enzyme kinetics and the thermodynamic of enzyme-catalyzed reactions. In thinking about starting research again, I knew I wanted to use computers to study complicated reaction systems, but I practically had to start over in research. The oil shock of 1972 made me worry about what the world was going to do when the petroleum supplies began to decrease. I applied to the Department of Energy to work on coal liquefaction, and I was turned down. However, they were willing to support me to work on petroleum processing, and so I began to learn about hydrocarbons and global ways of making calculations on systems with literally millions of different molecules. I learned how to calculate standard Gibbs energies of formation of isomer groups, such as the decanes, assuming the isomers are rapidly interconverted. I learned to use Benson’s group additivity method to estimate thermodynamic properties of gas molecules for which there was no experimental data. I also learned how to use matrices in calculations of equilibrium compositions of very large systems of reactions by using computer programs. In 1988 I joined with Irwin Oppenheim to apply statistical mechanics to these systems (24). When the partial pressure of ethylene is held constant, a semigrand ensemble can be used to calculate the equilibrium distribution of alkyl benzenes, and Irwin and I began to realize that in our calculations we were inventing a new thermodynamic potential, that is we were using...
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a Legendre transform. My "eureka" moment came when I realized that these new thermodynamic concepts applied to biochemistry because the pH is treated as an independent variable, and so the Gibbs energy $G$ does not provide the criterion for spontaneous change and equilibrium. I applied to NIH for support and have been funded for the past decade to make a new type of biochemical thermodynamic table and new types of thermodynamic calculations on biochemical reaction systems.

**Transformed Thermodynamic Properties of Biochemical Reactants and Reactions**

To define a transformed Gibbs energy $G'$ of a biochemical reaction system at specified temperature, pH, and ionic strength that does provide the criterion for spontaneous change and equilibrium, we have to use a Legendre transform, that is we have to continue the process described in Equations 1–3. This time we have to subtract from the Gibbs energy $G$ the product of conjugate variables involved with the pH. The specified chemical potential of hydrogen ions $\mu(H^+)$, which is related to the pH, has to be multiplied by its conjugate variable, the total amount of hydrogen atoms in the system that is represented by $n_s(H)$. Here $n_s$ stands for the amount of a component, which is something that is conserved (25). The transformed Gibbs energy $G'$ is defined as follows (26, 27).

$$G' = G - n_s(H)\mu(H^+)$$  \hspace{1cm} \text{(Eq. 6)}$$

The chemical potential of hydrogen ions at a specified pH is given by

$$\mu(H^+) = \mu(H^+) + RT\ln[H^+] = \mu(H^+) - RT\ln(10)pH$$  \hspace{1cm} \text{(Eq. 7)}$$

where $\mu(H^+)$ is the chemical potential of hydrogen ions at unit activity. The amount of the hydrogen component in the system is given by $n_s(H) = \Sigma N_{Hj}(j)n(j)$, where $N_{Hj}(j)$ is the number of hydrogen atoms in species $j$ and $n(j)$ is the amount of species $j$. The Gibbs energy of the system is additive as shown by $G = \Sigma \mu n(j)$, and the transformed Gibbs energy is additive as shown by $G' = \Sigma \mu'(n(j)$, where $\mu'(j)$ is the transformed chemical potential of species $j$.

Substituting these relations into Equation 6 leads to the following equation.

$$\Delta_j G'_0(j) = \Delta_j G_0(j) + RT\ln[j] - N_{Hj}(j)[\Delta_j G_0(H^+) - RT\ln(10)pH]$$  \hspace{1cm} \text{(Eq. 8)}$$

In writing this equation, chemical potentials have been replaced by Gibbs energies of formation $\Delta_j G_0$, as is customary in dealing with experimental data. The remarkable thing about this equation is that it turns out that the transformed Gibbs energies of formation $\Delta_j G'_0$ for the various protonated forms of a reactant like ATP are equal at a specified pH when the species are at chemical equilibrium! The standard Gibbs energies of formation $\Delta_j G_0$ of the species ATP$^4^-$, HATP$^5^-$, and $H_2$ATP$^2^-$ with respect to the elements at 298.15 K and zero ionic strength are $-2768.10$, $-2811.48$, and $-2838.18$ kJ mol$^{-1}$ (28, 29). When these species are at equilibrium at pH 5.90 and 0.25 M ionic strength, their concentrations are $2.133 \times 10^{-3}$, $7.806 \times 10^{-3}$, and $6.568 \times 10^{-5}$ M, respectively, in a 0.01 M solution of ATP. The transformed Gibbs energy of formation $\Delta_j G'_0$ calculated using Equation 8 is $-2382.35$ kJ mol$^{-1}$ for each of the three species. Therefore, we can say that the transformed Gibbs energy of the sum of the three species, referred to as ATP, is $-2382.35$ kJ mol$^{-1}$ at 298.15 K, pH 5.90, and 0.25 M ionic strength when the ATP concentration is 0.01 M. At equilibrium at a specified pH, the three species of ATP are pseudoisomers, that is they have the same transformed Gibbs energies of formation just like isomers have the same Gibbs energies of formation when they are in equilibrium.

The equation for the standard transformed Gibbs energy of formation of species $j$ is obtained using Equation 8 with $\lfloor j \rfloor = 1.000$ M.

$$\Delta_j G'_0(j) = \Delta_j G_0(j) - N_{Hj}(j)[\Delta_j G_0(H^+) - RT\ln(10)pH]$$  \hspace{1cm} \text{(Eq. 9)}$$

Thus if we know the standard Gibbs energy of formation of a species $\Delta_j G_0(j)$, its standard transformed Gibbs energy of formation $\Delta_j G'_0(j)$ can be calculated at a specified temperature, pH, and ionic strength. The next question is "How do you calculate the standard transformed Gibbs energy of formation $\Delta_j G'_0$ of a reactant $i$, like ATP, at a specified temperature, pH, and ionic strength?" The answer is

$$\Delta_j G'_0(i) = -RT\ln\sum_{j=1}^{N_i}\exp(-\Delta_j G_0(j)/RT)$$  \hspace{1cm} \text{(Eq. 10)}$$
where \( N_s \) is the number of species in the pseudoisomer group. The standard transformed Gibbs energy of formation of a reactant \( \Delta_f G^0(i) \) is a very important property because if it can be expressed as a function of temperature, \( \text{pH} \), and ionic strength, the standard transformed enthalpy of formation \( \Delta_f H^0(i) \), standard transformed entropy of formation \( \Delta_f S^0(i) \), and average number of hydrogen ions bound by reactant \( i \) can be calculated by simply taking partial derivatives. When \( \Delta_f G^0(i) \) is known for all the reactants in an enzyme-catalyzed reaction, the apparent equilibrium constant \( K' \), standard transformed enthalpy of reaction \( \Delta_r H^0 \), standard transformed entropy of reaction \( \Delta_r S^0 \), and the change in binding of hydrogen ions in the reaction can be calculated at the desired temperatures, \( \text{pH} \)s, and ionic strengths.

Thus when you specify the \( \text{pH} \) you open up a whole new world of thermodynamics in which you are dealing with reactants (sums of species) rather than species. Because magnesium ions play a role like hydrogen ions, the definition of \( G' \) in Equation 6 can be extended by subtracting a term \( n_{(\text{Mg})} \mu(\text{Mg}^{2+}) \) to make \( \text{pMg} \) also an independent variable. Since I was an officer in IUPAC at the time, I organized a committee to make recommendations about this new nomenclature (30).

Standard transformed Gibbs energies of formation and standard transformed enthalpies of formation can be calculated as functions of temperature, \( \text{pH} \), and ionic strength from the species properties in the tables mentioned earlier. Literature data on apparent equilibrium constants and standard transformed enthalpies of enzyme-catalyzed reactions can be used to extend these tables of species data. Goldberg, Tewari, and co-workers have surveyed the literature for measurements of these thermodynamic properties, evaluated them, and published six reports on these valuable thermodynamic data. Their first report was published in 1992 and the most recent in 1999 (31). These reports provide thermodynamic information on about 900 reactants.

The most efficient way to store thermodynamic data on enzyme-catalyzed reactions is to use small matrices with a row for each species containing \( \{\Delta_f G^0(j), \Delta_f H^0(j), z(j), N_{\text{H}}(j)\} \), where \( z(j) \) is the charge number and \( N_{\text{H}}(j) \) is the number of hydrogen atoms in species \( j \). The calculation of standard transformed thermodynamic properties at the specified temperature, \( \text{pH} \), and ionic strength is sufficiently complicated that a computer with a mathematical application is required. Mathematica® (32) is excellent for this because of its symbolic capabilities and its facilities for making plots and tables and taking partial derivatives. A table (BasicBiochemData2, library.wolfram.com/information/MathSource/797) of the properties of the species of 131 biochemical reactants has been placed on the web so that these data can be downloaded by anyone with Mathematica on their computer. This package also contains programs for using the data.

If the small matrix can be constructed for a reactant, it can be used to express \( \Delta_f G^0(i) \) of reactant \( i \) as a function of temperature, \( \text{pH} \), and ionic strength. This function is very important because all the other standard transformed thermodynamic properties can be calculated as a function of these variables by simply taking partial derivatives with a computer.

This process of making Legendre transforms can be taken a step further in considering systems of biochemical reactions like glycolysis and the tricarboxylic acid cycle (33). In a living cell, the concentrations of coenzymes tend to be in steady states because they are involved in many reactions. When this is the case, a further transformed Gibbs energy \( G'' \) of a reaction system can be defined by use of the following Legendre transform.

\[
G'' = G' - \Sigma n_{(\text{coenz})} \mu'(\text{coenz})
\]

(Eq. 11)

When this is applied to glycolysis, it can be shown that all the thermodynamics of the system can be represented by \( C_6 = 2C_3 \), where \( C_6 \) is the sum of the reactants with six carbon atoms and \( C_3 \) is the sum of the reactants with three carbon atoms. One of the advantages of Legendre transforms is that no information is lost, and so even the equilibrium concentrations of species can be calculated by using this one reaction.

Because thermodynamics can tell us whether a given enzyme-catalyzed reaction will go to the right or the left under specified conditions, we can see which reactions can be coupled with other reactions to store energy or provide energy for synthesis of needed reactants. As thermodynamic tables grow, the number of reactions between reactants in the table grows exponentially. Existing literature data will make it possible to greatly extend current tables, but more equilibrium studies are needed on reactants for which there is currently no literature data.
Reflections: Thermodynamics of Enzyme-catalyzed Reactions

This essay has been about the most fundamental concepts in the thermodynamics of biochemical reactions, but the actual calculations and results are described in detail in my book “Thermodynamics of Biochemical Reactions” (34).

Conclusion

I feel very fortunate that I worked on petroleum thermodynamics long enough to learn how to use Legendre transforms. When I came back to biochemistry I saw it with new eyes. I could see previously missed opportunities. Some of the new equations look like the equations of chemical thermodynamics, but they are different because they apply to sums of species like ATP rather than species. When the pH is specified there are more thermodynamic properties to consider and more relations between them. I think the moral is that sometimes in research it is a good idea to spend some time in another field because you may come back and see your previous field in a new light.

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