

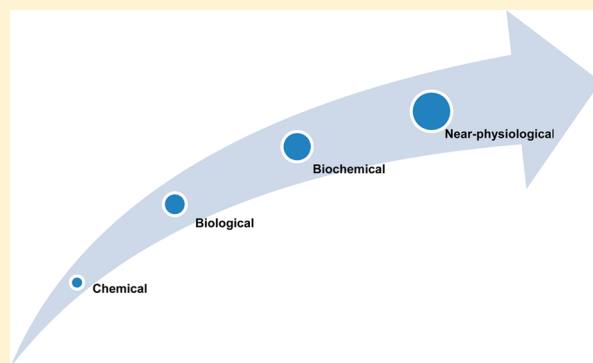
# Discovering Reliable Sources of Biochemical Thermodynamic Data To Aid Students' Understanding

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**ABSTRACT:** Students of physical chemistry in biochemical disciplines need biochemical examples to capture the need, not always understood, of a difficult area in their studies. The use of thermodynamic data in the chemical reference state may lead to incorrect interpretations in the analysis of biochemical examples when the analysis does not include relevant conditions to the biochemical state. The definition of a new reference state for biochemical reactions in 1994 still contends with the low number of published and consistent compilations of thermodynamic data. To fill the gap in the organized presentation of biochemical thermodynamic data, reliable sources of databases of biochemical thermodynamic data are discussed, along with textbooks and specialized academic sources.

**KEYWORDS:** *Second-Year Undergraduate, Chemoinformatics, Physical Chemistry, Textbooks/Reference Books, Bioenergetics, Thermodynamics*



As a basic discipline in chemical sciences spanning for many decades, chemical thermodynamics has been treated by a large number of pedagogical views, textbooks, and handbooks containing extensive data of thermodynamic properties of different compounds. On the contrary, biochemical thermodynamics lack of such diversity, because of difficulties in metabolite purification and identification, late application of thermodynamic principles to biochemical disciplines, and poor predictability owing to improper reference state definition. Despite these drawbacks, teaching and learning biological systems may benefit from the application of thermodynamic principles, including prediction of growth parameters and biomass yields in the optimization of biochemical production, and understanding of metabolic networks and their regulation (and by extension to nonequilibrium thermodynamics), which can provide useful information about the kinetics of biological systems.<sup>1</sup> Recognition of the incidence of pH and magnesium ions concentration, and particularly the advancements in analytical instrumentation that yielded valuable experimental data, encouraged the International Union of Biochemistry and Molecular Biology (IUBMB) to define a novel reference state for biochemical thermodynamic quantities<sup>2</sup> that greatly improves biochemically related predictions.

## ■ THE BEGINNINGS OF BIOCHEMICAL THERMODYNAMICS AND BEYOND

Three remarkable scientists significantly contributed to the actual state of biochemical thermodynamics. First, the systematic work carried out by Hans Krebs emphasized the need of reliable biochemical thermodynamic data to study respiration

and fermentation processes. His pioneering works from the 1950s ended in the 1957 book *Energy Transformations in Living Matter*<sup>3</sup> that included an appendix with the first table of standard Gibbs energies of formation of 88 species from enzyme-catalyzed reactions. Close analysis of tabulated data suggests the need of a different reference state for biochemical data, and the constriction pH 7 was included in the new definition of “biochemical reference state”.

In the late 1960s, the work of Robert A. Alberty showed the complex nature of the biochemical reactions and the incidence of  $Mg^{2+}$  concentration in the definition of the actual species present in living organisms. Such complexity was demonstrated in the classic works on adenosine phosphate species in the late 1960s.<sup>4</sup> The mixed influence of pH and pMg complicates the calculations of the thermodynamic properties, and Alberty proposed the application of the Legendre transforms<sup>5</sup> and computational programs to carry out all these calculations, including the thermodynamic properties of biochemical reactions from chemical data obtained under other experimental conditions. Due to the application of the Legendre transforms in the calculations, the new thermodynamic properties with pH and pMg constraints were termed “transformed thermodynamic properties”.

In parallel to Alberty's work, Robert Goldberg from NIST (National Institute of Standards Technology) made extensive measurements on biochemical reactions, providing high quality data that supported further calculations. Besides his own work,

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Goldberg's contribution also included systematic review of carefully selected data based on their quality; he compiled them in successive reviews published between 1993 and 2007.<sup>6</sup>

### AMENDING THE REFERENCE STATES

The need for different restrictions to data tables lead to the consideration of different reference states. First, the old biochemical reference state is now known as the biological reference state. In substitution, the new biochemical reference state takes into account the conditions pH 7 and pMg 3, and an ionic strength of 0.25 M or null, but still at a temperature of 25 °C. A further refinement includes the physiological temperature 37 °C and an ionic strength of 0.25 M, defining the near-physiological standard state (Table 1).<sup>2a</sup>

**Table 1. Reference States for Thermodynamic Data**

Reference State	Constraints	Nomenclature Example
Chemical	1 bar, 25 °C	$\Delta_r G_m^\circ$ (molar formation Gibbs energy)
Biological	1 bar, 25 °C, pH = 7	$\Delta_r G_m^{\prime\circ}$ (molar formation Gibbs energy)
Biochemical	1 bar, 25 °C, pH = 7, pMg = 3, $I = 0/0.25$ M	$\Delta_r G_m^{\prime\prime\circ}$ (molar transformed formation Gibbs energy)
Near-physiological	1 bar, 37 °C, pH = 7, pMg = 3, $I = 0.25$ M	$\Delta_r G_{m,310k}^{\prime\prime\circ}$ (molar transformed formation Gibbs energy)

The change between chemical and biological reference states is straightforward, as the only difference between both rests in the consideration of a fixed concentration of H<sup>+</sup> ions at 10<sup>-7</sup> M (pH 7). Hence, for a given reaction:



the Gibbs energy of reaction in the different reference states are related by

$$\Delta_r G_{m,298K}^{\prime\prime\circ} = \Delta_r G_{m,298K}^{\circ} \mp \nu RT \ln 10^{-7} \quad (2)$$

which at 298 K is

$$\Delta_r G_{m,298K}^{\prime\prime\circ} = \Delta_r G_{m,298K}^{\circ} \pm (39.93 \text{ kJ mol}^{-1})\nu \quad (3)$$

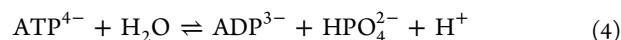
where  $\nu$  represents the stoichiometric number for H<sup>+</sup> ions, and the “±” sign correspond to H<sup>+</sup> ions as reactant or product, respectively.<sup>7</sup>

Nevertheless, the constriction of pH 7 does not take into account the distribution of phosphate and ATP species at such

a pH value. This refinement involves consideration of several simultaneous equilibria and the addition of Mg<sup>2+</sup> complex equilibria, resulting in a highly complicated system that should be evaluated computationally.

Once the conditions of pH, pMg, and ionic strength along with the temperature and pressure are fixed, the chemical equation representing the process under study can be substituted by a biochemical equation, for which each species represents all related species present under such constrained conditions.<sup>8</sup>

The incidence of the reference state on the thermodynamic properties (Table 2) is illustrated with the hydrolysis of ATP, whose chemical equations with (eq 5) and without Mg<sup>2+</sup> ions (eq 4) and the biochemical eq 6 are



Notice that the nomenclature for biochemical equations is different from those of chemical equations. A complete list of rules for writing biochemical equations have been summarized in the literature.<sup>2a</sup>

Molar Gibbs energy values in the chemical reference state are positive in almost all conditions, with the exception of non-null ionic strength and Mg<sup>2+</sup> concentration. This is the clearest example of how the consideration of an erroneous reference state for a biochemical reaction leads to incorrect conclusions, as this particular reaction is considered the key one in providing energy for metabolic reactions. Notice that the single consideration of pH 7 in the biological reference state changes the Gibbs energy sign to negative, now in accordance with the importance of ATP hydrolysis in biochemical processes. Also notice that this change is mainly made up by the change in the sign in the entropic  $T\Delta_r S_m^\circ$  term owing to the dilution of H<sup>+</sup> at a higher pH.<sup>4c</sup>

Finally, the biochemical and near-physiological reference states add further refinement to the calculated values in the biological reference state, but with less impact.

### THERMODYNAMIC DATA SOURCES

Most databases containing thermodynamic properties of compounds refer to the chemical reference state, as happens with most physical chemistry textbooks. The difficulties in

**Table 2. Thermodynamic Properties for the Reaction of Hydrolysis of ATP, under Different Reference States**

Reference State (Conditions)	$\Delta_r G_m^\circ / (\text{kJ mol}^{-1})$	$\Delta_r H_m^\circ / (\text{kJ mol}^{-1})^a$	$\Delta_r S_m^\circ / (\text{kJ mol}^{-1} \text{K}^{-1})$
Chemical (298 K, $I = 0$ , pH = 0, pMg = 0)	3.06	-20.5	-0.0791
Chemical (298 K, $I = 0.2$ , pH = 0, pMg = 0)	1.17	-19.7	-0.0699
Chemical (298 K, $I = 0$ , pH = 0, pMg = 3)	11.7	-23.0	-0.1164
Chemical (298 K, $I = 0.2$ , pH = 0, pMg = 3)	-3.89	-6.28	-0.0079
Chemical (310 K, $I = 0$ , pH = 0, pMg = 3)	13.1	-23.0	-0.0032
Biological <sup>b</sup> (298 K, $I = 0$ , pH = 7, pMg = 0)	-35	-20	0.050
Biological <sup>b</sup> (298 K, $I = 0$ , pH = 7, pMg ≠ 0)	-29	-25	0.013
Biological (310 K, $I = 0$ , pH = 7, pMg = 0)	-31	-20	0.034
Biochemical (298 K, $I = 0$ , pH = 7, pMg = 3)	-37.6	—	—
Biochemical (298 K, $I = 0.25$ M, pH = 7, pMg = 3)	-36.0	-28.2	0.0435
Near-physiological (310 K, $I = 0.25$ M, pH = 7, pMg = 3)	-36.3	-28.2	0.0262

<sup>a</sup> $\Delta_r H_m^\circ$  values are considered as independent of the temperature in the range 298–310 K. <sup>b</sup>Data taken from ref.9 after correction for print errors in the signs.

finding thermodynamic data under relevant biochemical conditions were considered by IUPAC/IUBMB, which suggested, for biochemical data compilation, the use of the biochemical reference state, including transformed molar formation enthalpies and Gibbs energies, absolute molar entropies, and molar calorific capacities.<sup>2b</sup> These recommendations are relatively new; hence, they are not widely followed. As a consequence, the only reliable source of biochemical thermodynamic data comes from specialized books or dedicated Web pages that compile existing data.

### Textbooks

The absence of thermodynamic properties data for biochemical compounds in textbooks for chemical and engineering students is partially solved in physical chemistry textbooks intended for biology or biochemistry students. Among them, *Physical Chemistry for the Life Sciences* by Atkins and De Paula includes in its second edition<sup>7</sup> an appendix with thermodynamic data (referring to the chemical reference state) for 64 biochemical compounds arranged according their nature (amino acids, sugars, a few peptides, and nucleotide bases). Another book used for biology or biochemistry students is *Physical Chemistry for the Biosciences* by Chang,<sup>10</sup> which uses the biological reference state. Finally, the recently published edition of Hammes' *Physical Chemistry for the Biological Sciences*<sup>11</sup> makes no distinction about the standard states, despite recognizing its importance.

More specialized books are also available. Particular attention should be given to *Biothermodynamics: The Role of Thermodynamics in Biochemical Engineering*, in which the final chapter includes a very detailed metabolic data analysis in the biochemical reference state at near-physiological conditions.<sup>12</sup>

### Specialized Books

Most biochemical thermodynamic data are already compiled in Alberty's books, with editions from 2003,<sup>13</sup> 2006,<sup>14</sup> and 2011.<sup>15</sup> An excellent and very complete compilation of data in the biological reference state is available;<sup>9</sup> however, a note of caution should be given warning of some print errors we have noticed (see the footnote in Table 2).

Systematic data compilation for biochemical thermodynamic data from enzyme-catalyzed reactions in the chemical reference state was done by Goldberg between 1993 and 2007.<sup>6</sup> Most of these data served for Alberty's work on the calculation of the transformed thermodynamic data, comprising a total of 199 biochemical compounds with their corresponding transformed Gibbs energy of formation at pH 7.<sup>14</sup> Of these compounds, there are only 80 data points for the corresponding transformed enthalpy of formation,<sup>14</sup> enabling for the calculation of the transformed Gibbs energy in the near-physiological reference state, according to<sup>16</sup>

$$\Delta_r G'_{m,310K} = \frac{310}{298} \Delta_r G'_{m,298K} + \left(1 - \frac{310}{298}\right) \Delta_r H'_{m,298K} \quad (7)$$

The compilation is completed with 80 data points for the transformed absolute entropies.

### Web Sites

Web pages accessed through the Internet can be conveniently arranged in two groups: one corresponding to databases maintained regularly by specialized institutions (universities, governmental agencies), and the other to the freely accessible Web pages that should be checked for accuracy.

Among the first group, the NIST offers the Standard Reference Database 74,<sup>17</sup> based on the reviewed data systematically compiled since 1993.<sup>18</sup> Data include apparent equilibrium constants and calorimetrically determined molar enthalpies of reaction, summarizing more than 1000 published papers containing data of more than 400 different enzyme-catalyzed reactions. Most of the data refer to the chemical reference state, and many of them are measured according to the latest official recommendations,<sup>2b</sup> allowing for obtaining data in the biochemical and near-physiological reference states.

eQuilibrator (version 2.0) is a Web-based platform for biochemical thermodynamic calculations.<sup>19</sup> Data come from two sources. The first one from formation energies computed at different pH and ionic strength levels, mainly from Alberty's work. The other source is from reaction energies at different pH and ionic strength levels, using the component group contribution method to obtain tabulated data.<sup>20</sup> Data from both sources cannot be mixed, as they are not consistent with each other. Unfortunately, only Gibbs energies are compiled, not data for enthalpy and entropy state functions.

Databases searching property and bibliographic databases constitute the ultimate data sources for thermodynamic data. Among them, REAXYS, PubMed, Web of Science, SciFinder, and NIST provide complete data searching by chemical compound, even with detailed information on the experimental conditions employed to obtain such data. However, the conditions employed are not always the same; even more, these conditions often do not correspond exactly to a defined reference state. Most data could be classified under the chemical reference state ( $P = 1$  bar) for temperatures different from 298 K, or variable pH and ionic strength conditions. Hence, these data inconsistencies limit their use for further thermodynamic calculations.

Finally, Wikipedia is a resource still often used by students. Searches using relevant keywords such as "biochemical thermodynamics", "thermodynamic database", and "biological thermodynamics" yield poor results, while searches using specific biochemical compounds face the same problems found in other general Web sites.

## TEACHING TIPS FOR USING BIOCHEMICAL THERMODYNAMIC DATA

Biochemistry and chemistry students can benefit from examining the use of different reference states for a given biochemical reaction (typically ATP hydrolysis), illustrating the importance of adequate selection of thermodynamic tables to analyze biochemical situations. As a general rule, any example used should clearly indicate the reference state used, in order to discuss the calculations in light of the application.<sup>8,21</sup>

Once a reliable data source is used, and consistent values are obtained (i.e., under the same reference state), several calculations can be done. The first one relates to the actual state of the cells, which requires knowledge of the actual concentration of metabolites in the cells in order to calculate the mass action law quotient  $Q'$ :

$$\Delta_r G'_m = \Delta_r G'^{\circ}_m + RT \ln Q' \quad (8)$$

These data are extremely difficult to obtain experimentally; therefore, relatively few works are reported in the literature. As a recent example, the absolute concentrations of more than 100 metabolites in *Escherichia coli* were reported,<sup>22</sup> which constitute the first important (in number) concentration database obtained under the same experimental conditions.

The previously mentioned calculation can be extended to the analysis of a whole metabolic route. Several examples on the use of transformed thermodynamic properties exist in the literature, including the glycolytic,<sup>16,23</sup> fatty acid degradation,<sup>24</sup> and pentose phosphate<sup>25</sup> pathways and the tricarboxylic acid,<sup>23b</sup> Calvin,<sup>25</sup> urea,<sup>26</sup> purine nucleotide,<sup>27</sup> citric acid,<sup>28</sup> and glyoxylate<sup>29</sup> cycles. Giving real examples to analyze under near-physiological conditions, instructors can use these data extensively in a university course on biochemical thermodynamics to give students an appreciation of the magnificent tools physical chemistry provides to help understand biochemical reactions.

## CONCLUSIONS

Incorporation of the reference state constraints depicted in Table 1 to biochemical data make calculations difficult. This is because of the need to consider simultaneous equilibria involving biochemical species and all possible complexes or existing forms depending on pH and pMg values. Nevertheless, calculated thermodynamic properties can be used without the knowledge of how they are obtained.<sup>16</sup> Thus, it is only necessary to have all these data collected in reliable and accessible tables. Such data compilations already exist, yet they are not directly accessible through widely recognized databases usually employed in chemical research and analysis. The main problem still is the inconsistencies in the use of a common reference state, a problem recognized by the IUBMB.<sup>2</sup> This is a typical problem in transition times, when old criteria are replaced by new criteria. In the meantime, biochemical data sources covered in this paper should help students use consistent data for biochemical thermodynamic calculations.

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### Notes

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