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Chemical and biochemical thermodynamics reunification (IUPAC Technical Report)

Abstract: According to the 1994 IUBMB-IUPAC Joint Commission on Biochemical Nomenclature (JCBN) on chemical and biochemical reactions, two categories of thermodynamics, based on different concepts and different formalisms, are established: (i) chemical thermodynamics, which employ conventional thermodynamic potentials to deal with chemical reactions [1–3]; and (ii) biochemical thermodynamics, which employ transformed thermodynamic quantities to deal with biochemical reactions based on the formalism proposed by Alberty [4–7]. We showed that the two worlds of chemical and biochemical thermodynamics, which so far have been treated separately, can be reunified within the same thermodynamic framework. The thermodynamics of chemical reactions, in which all species are explicitly considered with their atoms and charge balanced, are compared with the transformed thermodynamics generally used to treat biochemical reactions where atoms and charges are not balanced. The transformed thermodynamic quantities suggested by Alberty are obtained by a mathematical transformation of the usual thermodynamic quantities. The present analysis demonstrates that the transformed values for $\Delta_r G'^0$ and $\Delta_r H'^0$ can be obtained directly, without performing any transformation, by simply writing the chemical reactions with all the pseudoisomers explicitly included and the elements and charges balanced. The appropriate procedures for computing the stoichiometric coefficients for the pseudoisomers are fully explained by means of an example calculation for the biochemical ATP hydrolysis reaction. It is concluded that the analysis reunifies the "two separate worlds" of conventional thermodynamics and transformed thermodynamics.

Keywords: chemical equilibrium; magnesium; Thermodynamics.

CONTENTS

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1 Introduction

In a chemical reaction, all species involved are explicitly considered and their atoms and charges balanced. At equilibrium, the thermodynamic (or standard) equilibrium constant, K, for the chemical reaction is

$$K = \prod_i \left(\frac{\gamma_i\, c_i}{c_i^0} \right)_{\text{eq}}^{\nu_i},$$

where γ_i and c_i are the activity coefficient and the concentration (molarity) of the ith chemical species (reactant or product), respectively; c_i^0 is the standard concentration of species i; and we assume that all c_i^0 are equal to 1 mol dm^{-3} (we assume that this does not influence the results presented here). This is the case for the "chemical" convention. It differs from the biochemical convention, which, for example, uses 10^{-7} mol dm^{-3} as the standard concentration for the species H$^+$. ν_i is the corresponding stoichiometric coefficient (positive for products and negative for reactants) and eq stands for equilibrium. K is therefore of dimension 1 ("dimensionless") and defined by the relation $\Delta_r G^0 = -RT \ln K$ [1–7].

A biochemical reaction involves many species and simultaneous ancillary reactions. Therefore, the determination of the equilibrium composition requires many equilibrium expressions and many conservation equations. A different approach has thus far proved more suitable.

Networks of biochemical reactions take place under steady-state conditions in living organisms. Steady state is the typical condition of the living systems that allows work to be done by chemical reactions at maximal efficiency, or, in other words, to increase entropy at the minimal rate [8]. Another typical feature of living systems is homeostasis, which has the effect of keeping the conditions at which biochemical reactions occur within a narrow range. For example, in a biochemical reaction, the concentration of certain ancillary chemical species, such as H$^+$ and Mg^{2+} ions, remains essentially constant.

The polyanionic nature of the molecules involved in biochemical reactions implies that these reagents may act as Lewis bases B and react with Lewis acids, such as H$^+$ and Mg^{2+}, to form adducts such as BH$^+$, BMg^{2+}, etc. Consequently, the biochemical reagents are often a mixture of different chemical species. Therefore, biochemical reactions require the use of an equilibrium constant, usually named the apparent equilibrium constant or conditional equilibrium constant. This is the equivalent of an equilibrium constant for a biochemical reaction, where reagents and products are written in terms of the sum of species instead of specific species [4–7]. It is valid at a given pH, temperature, and ionic strength.

Given the biochemical reaction a A + b B = c C + d D, where A, B, C, and D are biochemical reagents ("sum of species"), the conditional equilibrium constant is as follows (activity coefficients γ_i are assumed equal to 1 and are therefore omitted for sake of clarity, see below):

$$K' = \frac{\left([\text{C}]/c^0 \right)^c \left([\text{D}]/c^0 \right)^d}{\left([\text{A}]/c^0 \right)^a \left([\text{B}]/c^0 \right)^b} \tag{1}$$

where c^0 is the standard concentration [4–7] considered equal for all species (1 mol dm^{-3}, according to the "chemical convention"). The value of the conditional equilibrium constant K' depends, besides T, p, and I, also on pH and pMg (according to IUPAC convention, in this paper pH and pMg are defined as $-\log_{10} a_{\text{H}^+}$ and $-\log_{10} a_{\text{Mg}^{2+}}$, respectively [1–3]). In vivo, the biochemical reactions occur at constant pH and pMg.

The amount of H$^+$ and Mg^{2+} bound to the chemical species of the reactants A and B is different from the amount of these ions bound to the chemical species of the products C and D. Ions H$^+$ and Mg^{2+} are thus produced or consumed during the course of the reaction. For this reason, chemical and biochemical reactions require a different thermodynamic formulation, since chemical equations are written in terms of specific ionic and elemental species and balance elements and charge, whereas biochemical equations are written in terms of biochemical reactants that consist of species in equilibrium with each other and do not balance elements that are assumed to be fixed, such as H$^+$ and Mg^{2+}. Therefore, when pH and pMg are specified, the conditional equilibrium constant K' for a biochemical reaction is written in terms of sums of species and can be used to

calculate a standard Gibbs energy of reaction $\Delta_r G'^0$, where the prime symbol (′) has been used to indicate that H^+ and Mg^{2+} ions concentrations are constant, but not at the standard value.

For example, in the cytosolic solution, the biochemical reactant ATP (adenosine 5′-triphosphate) is composed of the chemical species ATP^{4-}, $HATP^{3-}$, $MgATP^{2-}$, $MgHATP^-$, H_2ATP^{2-}, and Mg_2ATP. The biochemical reaction of the hydrolysis of ATP is formally written as:

$$ATP + H_2O = ADP + P_i \tag{2}$$

where ADP (adenosine 5′-diphosphate) is understood to be composed of the chemical species ADP^{3-}, $HADP^{2-}$, H_2ADP^-, $MgADP^-$, and $MgHADP$; and P_i (inorganic phosphate) is similarly understood to be composed of the chemical species PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, and $MgHPO_4$.

Alberty defined pseudoisomers as all the chemical species that form a biochemical reagent [4–7]. Referring to what is written above, the number of pseudoisomers of ATP are six, while those of ADP are five and those of P_i are four. The complex species are the adducts of these Lewis bases with the Lewis acids H^+ and Mg^{2+}. The equilibria between the pseudoisomers of ATP, ADP, and P_i are shown in Fig. 1.

However, $MgATP^{2-}$ is the active species in enzyme binding in the cellular active transport and the form responsible for the energy production and muscular contraction [9]. As a consequence, the biologically relevant ATP pseudoisomer is $MgATP^{2-}$ and the chemical reaction related to ATP hydrolysis is:

$$MgATP^{2-} + H_2O = MgADP^- + H_2PO_4^- \tag{3}$$
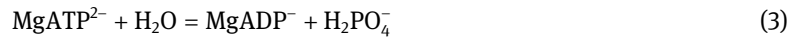
Taking into account the equilibrium $H_2PO_4^- \leftrightarrows H^+ + HPO_4^{2-}$, the equilibrium constant for the chemical reaction (3) can be written as:

$$K = \frac{\left([MgADP^-]/c^0\right)\left(\left[HPO_4^{2-}\right]/c^0\right)\left([H^+]/c^0\right)}{[MgATP^{2-}]/c^0} \tag{4}$$

The value of K depends on temperature T, pressure p, and ionic strength I of the solution. The conditional equilibrium constant for the biochemical reaction (2) is

$$K' = \frac{\left([ADP]/c^0\right)\left([P_i]/c^0\right)}{[ATP]/c^0} \tag{5}$$

Besides T, p, and I, its value depends also on pH and pMg. The corresponding values of the standard Gibbs energy of reaction can be obtained from the values of the equilibrium constants:

$$\Delta_r G^0 = -RT \ln K \tag{6}$$

$$\Delta_r G'^0 = -RT \ln K' \tag{7}$$

The $\Delta_r G'^0$, named the standard "conditional" Gibbs energy of reaction, is the reference state Gibbs energy of reaction for the biochemical reaction under the specific experimental condition of pH and pMg.

In the "Recommendations for nomenclature and tables in biochemical thermodynamics", the IUPAC-IUBMB Joint Commission on Biochemical Nomenclature (JCBN) states [7], "When pH and pMg are specified, a whole new set of transformed thermodynamic properties come into play. These properties are different from the usual Gibbs energy G, enthalpy H, and entropy S and they are referred to as the transformed Gibbs energy G', transformed enthalpy H', transformed entropy S'." As a consequence, two categories of
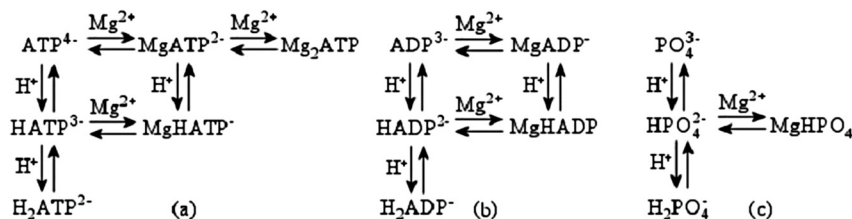

Fig. 1: Equilibria between the pseudoisomers of ATP (a), ADP (b) and P_i (c) (from. ref. 16).

thermodynamics based on different concepts and different formulations were established: (i) chemical thermodynamics, which employ conventional thermodynamic quantities to deal with chemical reactions; and (ii) biochemical thermodynamics, which employ transformed thermodynamic quantities to deal with biochemical reactions.
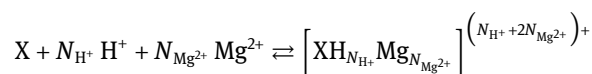
In his works, Alberty has shown how to obtain the transformed thermodynamic quantities $\Delta_r G'^0$, $\Delta_r H'^0$, and $\Delta_r S'^0$ from $\Delta_f G^0$, $\Delta_f H^0$, and $\Delta_f S^0$ of the specific chemical species [10, 11]. According to Alberty, $\Delta_r G$ and $\Delta_r G'$ provide the Gibbs energy of the chemical and biochemical reactions, respectively. However, it has been shown that $\Delta_r G = \Delta_r G'$ regardless of the reaction involved [12]. This fact has not been sufficiently divulged, and it is still not generally well known by the scientific community. It is worth underlining that this equality is restricted to Gibbs energy changes and does not apply to enthalpy or entropy changes.

In addition, it has been subsequently demonstrated that the 'transformed' thermodynamic quantities can be obtained without carrying out any 'transformation', but simply by considering the chemical potential of the H^+ and Mg^{2+} ions constant in the course of the reaction [13].

This means that the thermodynamic quantities G', H', and S' can also be obtained without any "transformation" and the classification into chemical thermodynamics and biochemical thermodynamics can be misleading. Mathematical clarification of the methods allows the two worlds of chemical and biochemical thermodynamics, which are often treated separately, to be reunified within the same thermodynamic framework. In the next sections, the two different methods will be described in detail.

2 Alberty's method

According to Alberty, the species formed by a given biochemical reagent constitute a pseudoisomer group [4–7]. Starting from this consideration, Alberty develops a procedure based on transformed Gibbs energies [4, 7, 10, 11]. Since the chemical species constituting the pseudoisomer group are at equilibrium with H^+ and Mg^{2+} ions ($\Delta\mu_{\text{reaction}} = 0$), *i.e.*

$$X + N_{H^+} H^+ + N_{Mg^{2+}} Mg^{2+} \rightleftharpoons \left[XH_{N_{H_+}} Mg_{N_{Mg^{2+}}} \right]^{\left(N_{H^+} + 2N_{Mg^{2+}} \right)+}$$

the chemical potential μ_i of the ith complex species $\left(\text{i.e.} \left[XH_{N_{H_+}} Mg_{N_{Mg^{2+}}} \right]^{\left(N_{H^+} + 2N_{Mg^{2+}} \right)+} \right)$ is

$$\mu_i = \mu_X + N_{H^+} (i)\, \mu_{H^+} + N_{Mg^{2+}} (i)\, \mu_{Mg^{2+}} \tag{8}$$

where μ_x is the chemical potential of the free species (ATP^{4-}, ADP^{3-}, and PO$_4^{3-}$ if we take, for example, the pseudoisomers of ATP, ADP, and P$_i$ reported in Fig. 1); and $N_{H^+} (i)$, $N_{Mg^{2+}} (i)$ are, respectively, the number of H^+ and Mg^{2+} ions bound to ith complex species (all the species bound to H^+ and Mg^{2+} if we take, for example, the pseudoisomers of Fig. 1). The chemical potentials μ_i are adjusted by subtracting $N_{H^+} (i)\, \mu_{H^+}$ and $N_{Mg^{2+}} (i)\, \mu_{Mg^{2+}}$.

$$\mu_i^{'} = \mu_i - N_{H^+} (i)\, \mu_{H^+} - N_{Mg^{2+}} (i)\, \mu_{Mg^{2+}} = \mu_x \tag{9}$$

The chemical potentials, μ', have been named transformed chemical potentials by Alberty and are equal, as shown by Eq. (9), to the chemical potential, μ_X, of the free species. All the chemical species of a pseudoisomer group have the same transformed chemical potential, which is the chemical potential of the free species. The transformed chemical potential, expressed as a function of the concentration (mol dm^{-3}) for an ideal solution [4–7] is:

$$\mu_i^{'} = \mu_i^0 + RT\, \ln\left(c_i/c^0\right) - N_{H^+} (i)\, \mu_{H^+} - N_{Mg^{2+}} (i)\, \mu_{Mg^{2+}} \tag{10}$$

At a given pH and pMg, the chemical potentials of H^+ and Mg^{2+} ions have a defined value that can be subtracted from the standard potential:

$$\mu_i^{'0} = \mu_i^0 - N_{H^+} (i)\, \mu_{H^+} - N_{Mg^{2+}} (i)\, \mu_{Mg^{2+}} \tag{11}$$

Substituting in Eq. (10), one obtains

$$\mu_i^{'} = \mu_i^{'0} + RT \ln(c_i/c^0) \tag{12}$$

The standard transformed chemical potential, $\mu_i^{'0}$, can be replaced, in the calculations, by the experimental quantity $\Delta_f G_i^{'0} = \Delta_f G_i^0 - N_{H^+}(i)\mu_{H^+} - N_{Mg^{2+}}(i)\mu_{Mg^{2+}}$ [14]. The transformed standard Gibbs energy of formation $\Delta_r G^{'0}$ of the jth isomer group is calculated using

$$\Delta_f G_j^{'0} = -RT \ln \sum_{i=1}^{N_{iso}} \exp\left(-\Delta_f G_i^{'0}/RT\right) \tag{13}$$

where N_{iso} is the number of pseudoisomers in the jth isomer group [4–7]. As a consequence, $\Delta_r G^{'0}$ for a biochemical reaction, understood as a reaction involving biochemical reagents, each constituted by a pseudoisomer group, is given by the difference between the transformed standard Gibbs energy of formation of the products and that of the reactants [4–7]. For reaction (2), we have

$$\Delta_r G^{'0} = \Delta_f G_{ADP}^{'0} + \Delta_f G_{P_i}^{'0} - \Delta_f G_{ATP}^{'0} - \Delta_f G_{H_2O}^{'0} \tag{14}$$

3 Thermodynamics of biochemical reactions

An alternative procedure to the one proposed by Alberty for the study of the thermodynamics of a biochemical reaction has been developed and can be used without having to apply transformations of the Gibbs energy [12, 13, 15, 16].

The fundamental equation for the Gibbs energy is given by

$$dG = -SdT + VdP + \sum_{i=1}^{N} \mu_i\, dn_i \tag{15}$$

where the differential of the amount of species i is dn_i and N is the number of different kinds of species in the system. When making calculations in chemical thermodynamics, μ_i can be replaced with $\Delta_f G_i$, the Gibbs energy of formation of species i. At constant T and p, substituting μ_i with $\mu_i^0 + RT \ln(c_i/c^0)$, where c^0 is the standard concentration, one obtains

$$dG = \sum_{i=1}^{N} \mu_i\, dn_i = \sum_{i=1}^{N} \left(\mu_i^0 + RT\ln\frac{c_i}{c^0}\right) dn_i \tag{16}$$

In a biochemical reaction, however, pH and pMg remain constant and, consequently, their chemical potential remains constant. For this general approach, the considered equilibria are for a "dilute ideal" solution, in which the activity coefficients of all reactants and products are considered equal to one [13, 16]. Although these conditions cannot be expected to hold rigorously in a biological system, this approximation simplifies the following formal development and calculation of the thermodynamic quantities without compromising their validity. The problem of calculating and using the activity coefficients has been extensively discussed elsewhere [12, 17, 18]. Equation (16) can, therefore, be rewritten as follows:

$$dG^{'} = \sum_{i=1}^{N-2} \left(\mu_i^0 + RT\ln\frac{c_i}{c^0}\right) dn_i + \left(\mu_{H^+}^0 - RT\,(pH)\ln 10\right) dn_{H^+} + \left(\mu_{Mg^{2+}}^0 - RT\,(pMg)\ln 10\right) dn_{Mg^{2+}} \tag{17}$$

where $N - 2$ are the chemical species that make up the reactants and products of the biochemical reaction ($N = 17$ for reaction (2)). The prime symbol (′) indicates that the chemical potential of the H^+ and Mg^{2+} ions has a determined and constant value. By analogy with the terminology used for the equilibrium constant K' of the biochemical reactions, the Gibbs energy G' is called "conditional" Gibbs energy.

As reported above, a biochemical reagent is a kind of "sum of chemical species". Indicating the biochemical reagents with the letter j and the individual chemical species of these biochemical reagents with

the letter i, the intrinsic mole fraction $f_{i,j}$ of the chemical species i related to the biochemical reagent j is given by:

$$f_{i,j} = n_{i,j}/n_j \tag{18}$$

where $n_{i,j}$ is the amount of the chemical species i of the biochemical reagent j and n_j is the total amount of the biochemical reagent j. For example, referring to the equilibrium (a) in Fig. 1: biochemical reagent: ATP; individual chemical species: ATP^{4-}, $HATP^{3-}$, H_2ATP^{2-}, $MgATP^{2-}$, $MgHATP^-$ and Mg_2ATP; $n_{i,j}$ refers to $n_{ATP^{4-},ATP}$, $n_{HATP^{3-},ATP}$, $n_{H_2ATP^{2-},ATP}$, $n_{MgATP^{2-},ATP}$, $n_{MgHATP^-,ATP}$, $n_{Mg_2ATP,ATP}$; $n_j = n_{ATP^{4-},ATP} + n_{HATP^{3-},ATP} + n_{H2ATP^{2-},ATP} + n_{MgATP^{2-},ATP} + n_{MgHATP^-,ATP} + n_{Mg_2ATP,ATP}$. The detailed procedure for the calculation of $n_{i,j}$ is reported in [13]. Placing $v_{i,j} = f_{i,j}$ or $v_{i,j} = -f_{i,j}$ when the chemical species i is a product or a reagent of the reaction, $\Delta_r G'$ of a biochemical reaction, when the extent of reaction is 1 mol at constant pH and pMg, is given by

$$\Delta_r G' = \sum_{j=1}^{N_B} \sum_{i=1}^{N(j)} v_{i,j}\left(\mu_{i,j}^0 + RT \ \ln\frac{f_{i,j}c_j}{c^0}\right)+ $$
$$+ v_{H^+}\left(\mu_{H^+}^0 - RT\,(\text{pH})\ln 10\right) + v_{Mg^{2+}}\left(\mu_{Mg^{2+}}^0 - RT\,(\text{pMg})\ \ln 10\right) \tag{19}$$

where N_B is the number of biochemical reactants and products involved in the biochemical reaction, $N(j)$ is the number of chemical species of the biochemical reagent j, $\mu_{i,j}^0$ is the standard chemical potential of the chemical species i, and c_j is the concentration of the biochemical reagent j. The stoichiometric numbers v_{H^+} and $v_{Mg^{2+}}$ are equal to the numerical value of the amount of ions H^+ and Mg^{2+} that is consumed ($v < 0$) or produced ($v > 0$) when the extent of the biochemical reaction is 1 mol.

The standard chemical potential $\mu_{i,j}^0$ can be replaced by the experimental quantity $\Delta_f G_{i,j}^0$. Equation (14) becomes

$$\Delta_r G' = \sum_{j=1}^{N_B} \sum_{i=1}^{N(j)} v_{i,j}\left(\Delta_f G_{i,j}^0 + RT \ \ln\frac{f_{i,j}c_j}{c^0}\right)$$
$$+ v_{H^+}\left(\Delta_f G_{H^+}^0 - RT\,(\text{pH})\ \ln 10\right) + v_{Mg^{2+}}\left(\Delta_f G_{Mg^{2+}}^0 - RT\,(\text{pMg})\ \ln 10\right) \tag{20}$$

When the concentrations c_j of the biochemical reagents is equal to c^0, Eq. (20) provides the conditional standard Gibbs energy value for the biochemical reaction:

$$\Delta_r G'^0 = \sum_{j=1}^{N_B} \sum_{i=1}^{N(j)} v_{i,j}\left(\Delta_f G_{i,j}^0 + RT \ \ln f_{i,j}\right)$$
$$+ v_{H^+}\left(\Delta_f G_{H^+}^0 - RT\,(\text{pH})\ \ln 10\right) + v_{Mg^{2+}}\left(\Delta_f G_{Mg^{2+}}^0 - RT\,(\text{pMg})\ \ln 10\right) \tag{21}$$

The intrinsic mole fractions $f_{i,j}$ are calculated with a procedure previously described [15]. In the following, X stands for the free species (ATP^{4-}, ADP^{3-}, etc.) of the biochemical reactant X_j (ATP, ADP, etc.) and $[XH_hMg_m]^{(h+2m)+}$ for a generic complex species of X (where h and m indicate, respectively, the number of H^+ and Mg^{2+} ions bound to X species; ionic charges from now on are omitted for simplicity). Remembering Eq. (9), one can write the following equality:

$$\mu_X^0 + RT \ \ln\left(c_X/c^0\right) = \mu_{XH_hMg_m}'^0 + RT \ \ln\left(c_{XH_hMg_m}/c^0\right) \tag{22}$$

Rearranging this equation, one obtains

$$\frac{c_{XH_hMg_m}}{c_X} = \exp\left[\left(\mu_X^0 - \mu_{XH_hMg_m}'^0\right)/RT\right] \tag{23}$$

Substituting μ_X^0 with $\Delta_f G_X^0$ and $\mu_{XH_hMg_m}'^0$ with

$$\Delta_f G_{XH_hMg_m}'^0 = \Delta_f G_{XH_hMg_m}^0 - h\Delta_f G_{H^+} - m\Delta_f G_{Mg^{2+}} \tag{24}$$

one obtains

$$\frac{c_{XH_hMg_m}}{c_X} = \exp\left[\left(\Delta_f G_X^0 - \Delta_f G_{XH_hMg_m}'^0\right)/RT\right] \tag{25}$$

For a 1 M solution of the biochemical reactant X, one has:

$$\sum_{i=1}^{N(j)} c_i = 1 \text{ M} \tag{26}$$

Dividing both members by c_X and taking the reciprocal one obtains:

$$\frac{1}{\sum_{i=1}^{N(j)} c_i / c_X} = \frac{1}{\sum_{i=1}^{N(j)} \exp\left[\left(\Delta_f G_X^0 - \Delta_f G_i'^0\right) / RT\right]} \tag{27}$$

Once c_X is obtained, the concentration of the complex species is calculated according to equation (25):

$$c_{XH_hMg_m} = c_X \exp\left[\frac{\Delta_f G_X^0 - \Delta_f G_{XH_hMg_m}'^0}{RT}\right] \tag{28}$$

The concentrations of the chemical species have been calculated for a 1 M solution of the biochemical reagent and their numerical value corresponds to their intrinsic mole fraction.

The stoichiometric coefficients ν_{H^+} and $\nu_{Mg^{2+}}$ are numerically equal to the difference between the amount of H^+ and Mg^{2+} ions bound to the reacting chemical species minus that bound to those produced. If we indicate with $N_{i,j}(H^+)$ and $N_{i,j}(Mg^{2+})$ the number of H^+ and Mg^{2+} ions bound to the ith adduct of the biochemical reactant/product j, the coefficients of these ions are:

$$\nu_{H^+} = -\sum_{j=1}^{N_B} \sum_{i=1}^{N(j)} N_{i,j}(H+)\nu_{i,j} \quad \text{and}$$

$$\nu_{Mg^{2+}} = -\sum_{j=1}^{N_B} \sum_{i=1}^{N(j)} N_{i,j}(Mg^{2+})\nu_{i,j} \tag{29}$$

If the value of ν_{H^+} is positive, that means that H^+ ions are released during the biochemical reaction, while if it is negative, they are consumed. The same goes for the Mg^{2+} ions.

Once the stoichiometric coefficients of all the chemical species participating in the reaction are obtained, the standard enthalpy of reaction can be easily calculated:

$$\Delta_r H'^0 = \sum_{i=1}^{N} \nu_i \Delta_f H_i'^0 \tag{30}$$

4 Thermodynamics of ATP hydrolysis

In this paragraph we report, as a numerical example of application of Eq. (21), the calculation of $\Delta_r G'^0$ for ATP hydrolysis (reaction (2)) at pH = 7 and pMg = 3.

The first column of Table 1 shows the list of the chemical species (reactants and products) involved in the hydrolysis reaction. In the second column the values of $\Delta_f G^0$ ($T = 298.15$ K, $I = 0.25$ M) of all chemical species implied in the biochemical reaction are reported [7, 11, 13]. At first, we calculate the standard conditional Gibbs energies of formation $\Delta_f G_i'^0$. As shown in eq. (24), we need to know the Gibbs energy of formation $\Delta_f G$ of H^+ and Mg^{2+} ions [7, 11, 13].

$$\Delta_f G(H^+) = \Delta_f G^0(H^+) - RT \text{ pH ln10} = (-0.81 - 39.96) \text{ kJ mol}^{-1} = -40.77 \text{ kJ mol}^{-1} \tag{31}$$

$$\Delta_f G(Mg^{2+}) = \Delta_f G^0(Mg^{2+}) - RT \text{ pMg ln10} = (-458.54 - 17.12) \text{ kJ mol}^{-1} = -475.66 \text{ kJ mol}^{-1} \tag{32}$$

These values, reported in the last two rows of the table's column 8, must then be subtracted from the standard Gibbs energies of formation $\Delta_f G_i^0$ to get the standard conditional Gibbs energies of formation $\Delta_f G_i'^0$. The values of $N_i(H^+)$ and $N_i(Mg^{2+})$ are reported in column 3 and 4, respectively, and the values of $\Delta_f G_i'^0$, calculated according to Eq. (24), are reported in column 5. The values of $\exp\left[\left(\Delta_f G_X^0 - \Delta_f G_i'^0\right)/RT\right]$ (see Eq. (27)) are reported in column 6. The concentrations of each chemical species XH_hMg_m (indicated as c) were calculated according to equations (27) and (28) and reported in column 7. These concentrations, each divided by the standard
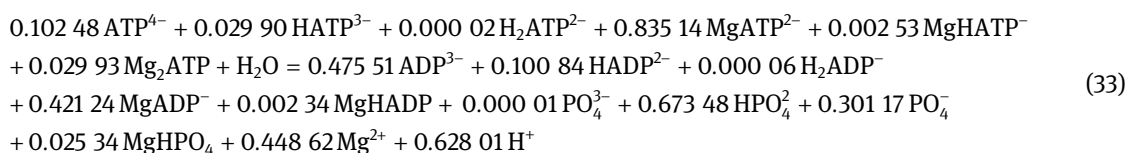
Table 1: The chemical species (reactants and products) involved in the hydrolysis reaction are reported in col. 1. The values reported in the other columns are calculated according to the corresponding equations reported in the text.

Species	$\Delta_f G^0$ (kJ mol^{-1})	$N(H^+)$	$N(Mg^{2+})$	$\Delta_f G'^0$ (kJ mol^{-1})	exp	$c = fc^0$ (mol dm^{-3})	$\Delta_f G$ (kJ mol^{-1})	v	$v\Delta_f G$ (kJ mol^{-1})
ATP^{4-}	−2781.06	0	0	−2781.06	1	0.102 48	−2786.70	−0.102 48	285.57
HATP^{3-}	−2818.77	1	0	−2778.00	0.291 74	0.029 90	−2827.47	−0.029 90	84.53
H$_2$ATP^{2-}	−2841.42	2	0	−2759.89	0.000 20	0.000 02	−2868.23	−0.000 02	0.06
MgATP^{2-}	−3261.92	0	1	−2786.26	8.149 65	0.835 14	−3262.37	−0.835 14	2724.54
MgHATP$^-$	−3288.31	1	1	−2771.88	0.024 70	0.002 53	−3303.13	−0.002 53	8.36
Mg$_2$ATP	−3729.33	0	2	−2778.00	0.292 09	0.029 93	−3738.03	−0.029 93	111.89
ADP^{3-}	−1913.42	0	0	−1913.42	1	0.475 51	−1915.26	0.475 51	−910.73
HADP^{2-}	−1950.34	1	0	−1909.57	0.212 07	0.100 84	−1956.03	0.100 84	−197.25
H$_2$ADP$^-$	−1972.79	2	0	−1891.26	0.000 13	0.000 06	−1996.79	0.000 06	−0.12
MgADP$^-$	−2388.78	0	1	−1913.12	0.885 87	0.421 24	−2390.92	0.421 24	−1007.16
MgHADP	−2416.67	1	1	−1900.24	0.004 92	0.002 34	−2431.69	0.002 34	−5.69
PO$_4{}^{3-}$	−1031.80	0	0	−1031.80	1	0.000 01	−1059.55	0.000 01	−0.01
HPO$_4{}^{2-}$	−1099.34	1	0	−1058.57	48977.9	0.673 48	−1100.32	0.673 48	−741.05
H$_2$PO$_4{}^-$	−1138.11	2	0	−1056.58	21902.0	0.301 17	−1141.08	0.301 17	−343.66
MgHPO$_4$	−1566.87	1	1	−1050.44	1842.49	0.025 34	−1575.98	0.025 34	−39.93
H$_2$O	−237.19	2	0					−1	237.19
H$^+$	−0.81	1	0			1.00E-07	−40.77	0.62801	−25.60
Mg^{2+}	−458.54	0	1			1.00E-03	−475.66	0.44862	−213.39

Standard state: T = 298.15 K, p = 100 kPa, I = 0.25 M; exp stands for $\exp\left[(\Delta_f G_X^0 - \Delta_f G_i'^0)/RT\right]$.

concentration $c^0 = 1$ M, are simply the intrinsic mole fraction f_i of each chemical species in respect to the corresponding parent biochemical species (i.e. f_i is the amount of substance of a specific chemical species (pseudoisomer) XH_hMg_m divided by the sum of all (h, m) species that together form the pseudoisomer group XH_hMg_m). The $\Delta_f G_i = \Delta_f G_i^0 + RT \ln f_i$ of each chemical species is reported in column 8. The stoichiometric numbers v_i, reported in column 9, are negative for reactants and positive for products. The stoichiometric numbers v_{H^+} and $v_{Mg^{2+}}$ are calculated according to Eq. (29), $v_{H_2O} = -1$, while for the other chemical species v_i is numerically equal to $c = f_i c^o$. Then, in column 10 the products $v_i\Delta_f G_i$ are reported; according to Eq. (21), the sum of these values is −32.45 kJ mol^{-1}, *i.e.* the standard conditional Gibbs energy of hydrolysis of ATP.

The stoichiometric numbers, $v_{i,j}$, of Eq. (20), reported in column 9 of the table, refer to the chemical species, i, j, that are all the individual reactants/products involved in the biochemical reaction (col. 1). We can write the corresponding chemical equation for the hydrolysis of ATP in which the reactants/products are the chemical species reported in column 1 and in which the stoichiometric numbers v are the values reported in column 9 of Table 1:

$$
\begin{aligned}
&0.102\,48\,ATP^{4-} + 0.029\,90\,HATP^{3-} + 0.000\,02\,H_2ATP^{2-} + 0.835\,14\,MgATP^{2-} + 0.002\,53\,MgHATP^- \\
&+ 0.029\,93\,Mg_2ATP + H_2O = 0.475\,51\,ADP^{3-} + 0.100\,84\,HADP^{2-} + 0.000\,06\,H_2ADP^- \\
&+ 0.421\,24\,MgADP^- + 0.002\,34\,MgHADP + 0.000\,01\,PO_4^{3-} + 0.673\,48\,HPO_4^2 + 0.301\,17\,PO_4^- \\
&+ 0.025\,34\,MgHPO_4 + 0.448\,62\,Mg^{2+} + 0.628\,01\,H^+
\end{aligned}
\tag{33}
$$

This chemical equation is written in terms of specific ionic species and balanced elements and charge and it has been named the balanced biochemical reaction [13]. Using the balanced biochemical reaction approach, the calculations needed to obtain $\Delta_r N(H^+) = v_{H^+}$ and $\Delta_r N(Mg^{2+}) = v_{Mg^{2+}}$ are trivial, while the Alberty's procedure [14] involves much more complex calculations.

As already shown [11], at constant pH and pMg, when the chemical species of each biochemical reagent are in equilibrium with each other, the $\Delta_r G$ of any chemical reaction between various chemical species has the same value of the $\Delta_r G'$ of the biochemical reaction. It would be difficult to measure the concentration of the

single chemical species involved in the energy-producing chemical reaction, while it is relatively easy to measure the total concentration of the biochemical reactants.

In the case of ATP hydrolysis, $MgATP^{2-}$ is the active species [9] and, as a consequence, the actual reaction of ATP hydrolysis, *in vivo*, is the chemical reaction (3). In the muscle cytosol, we observe [9]:

$$[ATP] = 8 \times 10^{-3}\, M \quad [ADP] = 20 \times 10^{-6}\, M \quad [Pi] = 4 \times 10^{-3}\, M.$$

Using the stoichiometric numbers, v, reported in Table 1 and the procedure reported in detail in the reference publication [12], we obtain:

$$\left[MgATP^{2-}\right] = \left(0.835\,14 \times 8 \times 10^{-3}\right) M = 6.681\,12 \times 10^{-3}\, M$$
$$\left[MgADP^{-}\right] = \left(0.421\,24 \times 20 \times 10^{-6}\right) M = 8.4248 \times 10^{-6}\, M$$
$$\left[H_2PO_4^{-}\right] = \left(0.301\,17 \times 4 \times 10^{-3}\right) M = 1.204\,68 \times 10^{-3}\, M$$

The $\Delta_r G$ of the chemical reaction (3) at $T = 298.15$ K, $p = 10^5$ Pa, $I = 0.25$ M, pH = 7 and pMg = 3 is:

$$\Delta_r G = \Delta_r G^0 + RT\, \ln\, Q = \left(-27.781 + 2.478\,97\, \ln \frac{8.4248 \times 10^{-6} \times 1.204\,68 \times 10^{-3}}{6.681\,12 \times 10^{-3}} \right) kJ\, mol^{-1}$$
$$= -60.992\, kJ\, mol^{-1}$$

At the same conditions of T, p, I, pH, and pMg, the $\Delta_r G'$ of the biochemical reaction (2) is

$$\Delta_r G' = \Delta_r G'^0 + RT\, \ln\, Q' = \left(-32.452 + 2.478\,97\, \ln \frac{2 \times 10^{-5} \times 4 \times 10^{-3}}{8 \times 10^{-3}} \right) kJ\, mol^{-1} = -60.992\, kJ\, mol^{-1}$$

The assertion that the $\Delta_r G$ of any chemical reaction between various chemical species has the same value of the $\Delta_r G'$ of the biochemical reaction is verified. It is worth underlining that it has also been demonstrated that $\Delta_r G$ can be calculated either in terms of activities or concentrations, giving the same value, under the assumption that all activity coefficients are constant [12].

5 Conclusions

The presence of two categories of thermodynamics based on different concepts and different formalisms has often been presumed in the literature: (i) chemical thermodynamics, which make use of the conventional thermodynamic properties and is only suitable to deal with chemical reactions; and (ii) biochemical thermodynamics, which make use of transformed thermodynamic properties and is only suitable to deal with biochemical reactions.

The complexity of biochemical reactions entails a conceptual and experimental problem to determine experimentally the energy released by a specific enzymatic reaction and to assess the associated thermodynamic properties.

The analysis above demonstrates that biochemical reactions, which occur at constant pH and pMg, can be conveniently described by conventional thermodynamics [16]. The two worlds of chemical and biochemical thermodynamics should be viewed as being within the same consistent thermodynamic framework.

Biochemists and analytical chemists are used to dealing with chemical equilibria occurring in a solution at constant pH and having reagents that are the "sum of species". The corresponding equilibrium constant, K', is referred to as *conditional* [19, 20] whereas $\Delta_r G'$, $\Delta_r H'$, and $\Delta_r S'$ have been so far termed, according to Alberty, transformed thermodynamics properties. We suggest the unification of the terminology using the appellation conditional for both K' and $\Delta_r G'$, $\Delta_r H'$, and $\Delta_r S'$.

The prime symbol is used to indicate that the equilibrium constant and the thermodynamic properties refer to reactions that occur, not only at constant T, p, and I, but also at certain fixed pH and pMg values. The term *transformed* thermodynamics should be abolished and *conditional* thermodynamics used instead.

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References

[1] M. B. Ewing, T. H. Lilley, G. M. Olofsson, M. T. Ratzsch, G. Somsen. *Pure Appl. Chem.* **66**, 533–552 (1994).

[2] IUPAC Green Book-Physical Chemistry Division. *Quantities, Units and Symbols in Physical Chemistry*, Blackwell Scientific Publications, Oxford (1988), 2nd ed. (1993).

[3] IUPAC-Physical Chemistry Division. Manual of symbols and terminology for physicochemical quantities and units. Appendix IV, *Pure Appl. Chem.* **54**, 1239–1250 (1982).

[4] R. A. Alberty. *Pure Appl. Chem.* **66**, 1641–1666 (1994). Revised by IUBMB in R. A. Alberty, A. Cornish-Bowden, R. N. Goldberg, G. G. Hammes, K. Tipton, H. V. Westerhoff. *Biophys. Chem.* **155**, 89–203 (2011).

[5] R. A. Alberty. *Biophys. J.* **65**, 1243–1254 (1993).

[6] K. C. Vinnakota, F. Wu, M. J. Kushmerick, D. A. Beard. *Methods Enzymol.* **454**, 29–68 (2009).

[7] G. P. Moss (1994) IUBMB-IUPAC Joint commission on Biochemical Nomenclature (JCBN), recommendations for nomenclature and tables in biochemical thermodynamics. Available: http://www.chem.qmul.ac.uk/iubmb/thermod/.

[8] A. Katchalsky, P. F. Curran. *Non Equilibrium Thermodynamics in Biophysics*, Harvard University Press, Cambridge, Massachusetts (1965).

[9] S. Iotti, C. Frassineti, A. Sabatini, A. Vacca, B. Barbiroli. *Biochim. Biophys. Acta* **1708**, 164–177 (2005).

[10] R. A. Alberty, *Biophys. Chem.* **42**, 117–131 (1992).

[11] R. A. Alberty, *Biophys. Chem.* **43**, 239–254 (1992).

[12] S. Iotti, A. Sabatini, A. Vacca. *J. Phys. Chem. B* **114**, 1985–1993 (2010).

[13] A. Sabatini, A. Vacca, S. Iotti. *PLoS One* **7**, e29529 (2012).

[14] R. A. Alberty. *Biochim. Biophys. Acta* **1207**, 1–11 (1994).

[15] A. Sabatini, M. Borsari M, L. M. Raff, W. R. Cannon, S. Iotti. *Chem. Int.* **41**(2), (2019). https://doi.org/10.1515/ci-2019-0211. http://www.iupac.org/project/2017-021-2-100.

[16] S. Iotti, L. Raff, A. Sabatini. *Biophys. Chem.* **221**, 49–57 (2017).

[17] P. Hoffmann, T. Maskow, C. Held, G. Sadowski. A thermodynamic investigation of the glucose-6-phosphate isomerization, *Biophys. Chem.* **195** (2014) 22–31.

[18] C. Held, G. Sadowski. Thermodynamics of Bioreactions, *Ann. Rev. Chem. Biomol. Eng.* **7**, 395–414 (2016).

[19] A. Ringbom. *J. Chem. Ed.* **35**, 282–288 (1958).

[20] J.-L. Burgot. *Ionic Equilibria in Analytical Chemistry*, Springer, New York (2012).