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# Effect of binding polynomials on feasibility study of biochemical reactions

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**Abstract:** In a typical biochemical reaction at specified pH, each metabolite in a particular reaction may be available as an equilibrated mixture of different charged ions and it is termed as "metabolite species". At equilibrium, each metabolite is represented as pseudoisomer group of metabolite species. At a particular pH, pMg, ionic strength I, T and P, the sum of the metabolite species taking part in a biochemical reaction were considered for establishing the stoichiometry. The transformed Gibbs free energy change of reaction ($\Delta_r \boldsymbol{G'^0}$) was calculated and compared with standard Gibbs free energy change of reaction ($\Delta_r \boldsymbol{G^0}$). The results indicated that there is difference in values of $\Delta_r \boldsymbol{G'^0}$and $\Delta_r \boldsymbol{G^0}$. Hence, it is shown that the thermodynamic property *G* is not sufficient to provide a criterion for the spontaneity of biochemical reaction. *G′* must be minimized rather than *G* at equilibrium in case of biochemical reactions at constant pH and pMg.

**Keywords :** Transformed and standard Gibbs free energy change of reaction; glucose to G6P hydrolysis; pH and pMg; ionic strength.

## Introduction

To describe chemical and biochemical reactions, there are two different equations available. Mass (element) and charge balanced chemical species are considered in chemical reactions, whereas in biochemical reactions, consideration of the sum of species of a biochemical reactant which is at equilibrium and the elemental balance such as hydrogen and magnesium are fixed at a constant pH and pMg[1]. Based on these concepts, there is an existence of two different types of application of thermodynamic concepts to chemical and biochemical systems. In chemical thermodynamics, conventional thermodynamic properties are used to analyze a particular chemical reaction and in biochemical thermodynamics, the transformed or unconventional thermodynamic properties are applied to biochemical reactions[2]. A biochemical reactant contains a sub system of metabolite species based on its reaction with Lewis acids such as $H^+$ , $Mg^{2+}$, $Na^+$ and $K^+$. The corresponding equilibrium constant for the chemical and biochemical reactions are *K* and *K′* (conditional or apparent equilibrium constant) which is written as the sum of the equilibrium constants of metabolite species[3].

IUBMB-IUPAC (International Union of Biochemistry and Molecular Biology-International Union of pure and applied chemistry) Joint Commission on Biochemical nomenclature (JCBN) stated that when pH and pMg are specified in a biochemical reaction, Gibbs free energy *G* was referred as transformed Gibbs free energy (*G′*) (Moss, 1994). Moreover, the transformed Gibbs free energy *G′* in a biochemical reaction, not *G* is minimized at equilibrium at constant pH and pMg and hence, *G′* and not *G* provides the condition for feasible chemical change in a biochemical reaction[4].

In the present study, the transformed Gibbs free energy of reaction ($\Delta_r G'^0$) was calculated based on the change in binding of hydrogen and magnesium ions in a biochemical reactant using conventional thermodynamic properties at a particular ionic strength (*I*), pH and pMg.

## Materials and Methods

### Thermodynamic analysis of metabolic reaction network

Thermodynamic feasibility of the chemical reactions are calculated based on the concept of Gibbs free energy change of a reaction. In an enzyme catalyzed reaction, the substrates are combined together to form a product. There are two types of reactions available such as chemical reactions and biochemical equations. In case of chemical reactions, both the element and charge must be balanced. The biochemical reactions are written in terms of biochemical reactants at a specified pH[5,6]. At a constant pH condition, the number of hydrogen ions in a particular biochemical reactant is constant. The conventional thermodynamic property does not signify the biochemical systems precisely. Therefore, transformed thermodynamic properties were established for the calculation of Gibbs free energy change of a reaction[2]. In each metabolic pathway, a consecutive set of biochemical reactions are available which result in the synthesis of final product. In this study, the transformed Gibbs energy change of the conversion of glucose to glucose 6- phosphate was calculated by considering all the metabolite species formed for each reactant and product by combining with $H^+$ and $Mg^{2+}$.

### Standard transformed Gibbs free energy of formation of a metabolite and its species

Literature reports are available on the influence of pH, ionic strength and binding polynomials on apparent equilibrium constants of biochemical reactions[7]. During the course of metabolism, most of the acids are synthesized as fermentation end products and have weak acidic functional groups whose $pK_a$ values are nearer to the physiological pH, and hence resulting in losing protons. Thus, dissociated weak acids result in the formation of deprotonated metabolite species. This kind of interaction is mainly due to two reasons (i) depending on the ionic force present in the biochemical media, the interactions between the solutes can create non-ideal behavior in the solution and thus the solution becomes electrostatic in nature; (ii) in most of the biochemical reactions, one of the metabolite species is mostly involved in the reaction resulting in the formation of enzyme-substrate complex which is stabilized by electrostatic interactions. In typical biochemical reactions at specified pH, each metabolite in a particular reaction may be available as an equilibrated mixture of different charged ions and it is termed as "metabolite species". At equilibrium, each metabolite is represented as pseudoisomer group of metabolite species[6].

For example, consider the hydrolysis reaction of glucose 6-phosphate (G6P). The biochemical reaction is given in Eq. 1

$$Glu + ATP \rightarrow G6P + ADP \quad (1)$$

The chemical reaction of Eq. (1) is represented as,

$$Glu^0 + ATP^{4-} \rightarrow G6P^{2-} + ADP^{3-} + H^+ \quad (2)$$

where,

*G6P* : Glucose 6-phosphate includes both free species $G6P^{2-}$and all the complex species such as $HG6P^{1-}$ and *MgG6P*

*Glu* : Glucose

*ATP* : Adenosine tri phosphate includes both free species $ATP^{4-}$ and all the complex species such as $HATP^{3-}$, $H_2ATP^{2-}$, $MgATP^{2-}$, $MgHATP^{1-}$ and $Mg_2ATP$.

*ADP* : Adenosine di phosphate includes both free species $ADP^{3-}$ and all the complex species such as $HADP^{2-}$, $H_2ADP^{1-}$,$MgADP^{1-}$and $MgHADP$.

The probability of deprotonation of each metabolite species depends on the pH and $pK_a$[5].

**Effect of ionic strength on free energy change**

In a solution, thermodynamic properties are affected by the interactions between the metabolite species present in it. One of the factors which influence thermodynamic property of a system is ionic strength. Most of the biochemical reactions work in the ionic strength range of 0.1 M to 0.3 M. Hence the effects of the solution ionic strength must be considered for correction of the standard Gibbs free energy of formation. The standard Gibbs free energy of formation of metabolite species *i* within a pseudo isomer group[6]at specific ionic strength *I* is calculated using Eq. (3).

$$\Delta G^0_{f,i} = \Delta G^0_{f,i}(I=0) - \left(\frac{2.91842\, z_i^2 I^{1/2}}{(1+BI^{1/2})}\right) \text{ ---------- (3)}$$

where,

$\Delta G^0_{f,i}$ : Standard Gibbs free energy of formation of metabolite species *i* within a pseudo isomer group at ionic strength I (KJ $mol^{-1}$)

$\Delta G^0_{f,i}$ $(I=0)$ : Standard free energy of formation of metabolite species *i* at zero ionic strength (KJ $mol^{-1}$)

$z_i$ : Charge of a metabolite species *i*

$I$ : Ionic strength

$B$ : 1.6 $L^{1/2}mol^{1/2}$

**Methodology adopted for the construction of stoichiometry**

The following assumptions were made for the construction of stoichiometry are as follows. (i) Water activity is constant, (ii) temperature T, pressure P, ionic strength I, pH are constant, (iii) standard concentration $c^0$ is 1M, (iv) free and complex species of all biochemical reactants are at equilibrium with each other. The stoichiometry for the biochemical reaction was constructed with mass and charge balanced reactions involving both free and complex species of all the reactants and products in a reaction using procedure[2].

**Thermodynamic Data**

Standard Gibbs free energy of formation of metabolite species ($\Delta G^0_{f,i}$) involved in the conversion of glucose to glucose 6- phosphate were obtained from literature[8]. Gibbs free energy change of reaction ($\Delta_f G'^0$) is calculated using below Eq.

$$\Delta_f G'^0 = \sum_i v_i \Delta_f G'_i$$

## Results and discussion

**Construction of stoichiometry for biochemical reactions**

Before constructing the stoichiometry of a biochemical reaction, it was assumed that the concentration of free and complex species of all the biochemical reactants are in 1M at constant pH and ionic strength[2]. The biochemical and chemical reactions are given in Eq. (1) & (2).

Step 1: The mass balance equation for each of the biochemical reactants present in Eq. (1) are given below.

$$[G6P] = [G6P^{2-}] + [HG6P^{1-}] + [MgG6P]$$

$$= [G6P^{2-}] + K_{HG6P^{1-}}[G6P^{2-}]\,[H^+] + K_{MgG6P}[G6P^{2-}]\,[Mg^{2+}]$$

$$= [G6P^{2-}]\{1 + K_{HG6P^{1-}}[H^+] + K_{MgG6P}[Mg^{2+}]\}$$

$$[G6P] = B_{G6P}[G6P^{2-}] \quad (4)$$

$$[ATP] = [ATP^{4-}] + [HATP^{3-}] + [H_2ATP^{2-}] + [MgHATP^{-}] + [MgATP^{2-}] + [Mg_2ATP]$$

$$= [ATP^{4-}] + K_{HATP^{3-}}[ATP^{4-}]\,[H^+] + K_{H_2ATP^{2-}}[ATP^{4-}][2H^+] + K_{MgHATP^{-}}[ATP^{4-}][Mg^{2+}][H^+] + K_{MgATP^{2-}}[ATP^{4-}][Mg^{2+}] + K_{Mg_2ATP}[ATP^{4-}][2Mg^{2+}]$$

$$= [ATP^{4-}]\{1 + K_{HATP^{3-}}[H^+] + K_{H_2ATP^{2-}}[2H^+] + K_{MgHATP^{-}}[Mg^{2+}][H^+] + K_{MgATP^{2-}}[Mg^{2+}] + K_{Mg_2ATP}[2Mg^{2+}]\}$$

$$[ATP] = B_{ATP}[ATP^{4-}] \quad (5)$$

$$[ADP] = [ADP^{3-}] + [HADP^{2-}] + [H_2ADP^{-}] + [MgHADP] + [MgADP^{-}]$$

$$= [ADP^{3-}] + K_{HADP^{2-}}[ADP^{3-}]\,[H^+] + K_{H_2ADP^{-}}[ADP^{3-}][2H^+] + K_{MgHADP}[ADP^{3-}][Mg^{2+}][H^+] + K_{MgADP^{-}}[ADP^{3-}][Mg^{2+}]$$

$$= [ADP^{3-}]\{1 + K_{HADP^{2-}}[H^+] + K_{H_2ADP^{-}}[2H^+] + K_{MgHADP}[Mg^{2+}][H^+] + K_{MgADP^{-}}[Mg^{2+}]\}$$

$$= B_{ADP}[ADP^{3-}] \quad (6)$$

whereas, the binding polynomials of each of the biochemical reactants are given by,

$$B_{ATP} = \{1 + K_{HATP^{3-}}[H^+] + K_{H_2ATP^{2-}}[2H^+] + K_{MgHATP^{1-}}[Mg^{2+}][H^+] + K_{MgATP^{2-}}[Mg^{2+}] + K_{Mg_2ATP}[2Mg^{2+}]\} \quad (7)$$

$$B_{G6P} = \{1 + K_{HG6P^{1-}}[H^+] + K_{MgG6P}[Mg^{2+}]\} \quad (8)$$

$$B_{ADP} = \{1 + K_{HADP^{2-}}[H^+] + K_{H_2ADP^{-}}[2H^+] + K_{MgHADP}[Mg^{2+}][H^+] + K_{MgADP^{-}}[Mg^{2+}]\} \quad (9)$$

These binding polynomials depend on pH and pMg. Gibbs free energy of formation of metabolite species at T = 298.15 K, I = 0.28 M and P = 1 atm are calculated using Eq. (3) and the results are given in Table . Using transformed Gibbs free energy of formation, the Gibbs free energy of reaction of the complex metabolite species, $HG6P^{1-}$, $MgG6P$, $HATP^{3-}$, $H_2ATP^{2-}$, $MgHATP^{1-}$, $MgATP^{2-}$, $Mg_2ATP$, $HADP^{2-}$, $H_2ADP^{1-}$, $MgHADP$ and $MgADP^{1-}$were calculated (Table 1). The equilibrium constant for each of the complex species was calculated using Eq. (10) and is mentioned below.

$$K = \exp\left(\frac{-\Delta G_f'^{o}}{RT}\right) \quad (10)$$

Binding polynomials (B) given in Eq. 7, 8 and 9 were calculated at pH = 7 and pMg = 3.

$$B_{ATP} = 1.0877$$

$$B_{ADP} = 1.1009$$

$$B_{G6P} = 1.3098 \quad (11)$$

The sum of the concentration of all species in a biochemical reaction Eq. (1) is $c^0$. The concentrations of [G6P], [ATP], [Glu] and [ADP] are considered as 1M and hence the concentration of the reference species were calculated by applying the values of binding polynomials (Eq. 11) in Eq. 4, 5 and 6.

$$[Glu] = 1\ \text{M}$$
$$[ATP^{4-}] = 0.9134\ \text{M}$$
$$[G6P^{2-}] = 0.9083\ \text{M}$$
$$[ADP^{3-}] = 0.7635\ \text{M} \quad (12)$$

Step 2: The ratio of the concentration $c_i$ of a particular reactant and the overall concentration of all species $c^0$ is referred as fractional population $f_i$.

$$f_i = \frac{c_i}{C^0} \quad (13)$$

Using the concentration of reference species, the concentration of complex metabolite species were calculated and is shown below.

$$[HG6P^{1-}] = K_{HG6P^{1-}}[G6P^{2-}]\,[H^+] = 0.066$$
$$[MgG6P] = K_{MgG6P}[G6P^{2-}][Mg^{2+}] = 0.027$$
$$[HATP^{3-}] = K_{HATP^{3-}}[ATP^{4-}]\,[H^+] = 0.066$$
$$[H_2ATP^{2-}] = K_{H_2ATP^{2-}}[ATP^{4-}][2H^+] = 0.0095$$
$$[MgHATP^{1-}] = K_{MgHATP^{1-}}[ATP^{4-}][Mg^{2+}][H^+] = 0.0010$$
$$[MgATP^{2-}] = K_{MgATP^{2-}}[ATP^{4-}][Mg^{2+}] = 0.00367$$
$$[Mg_2ATP] = K_{Mg_{2ATP}}[ATP^{4-}][2Mg^{2+}] = 2.09 \times 10^{-4}$$
$$[HADP^{2-}] = K_{HADP^{2-}}[ADP^{3-}]\,[H^+] = 0.110$$
$$[H_2ADP^{-}] = K_{H_2ADP^{-}}[ADP^{3-}][2H^+] = 0.109$$
$$[MgHADP] = K_{MgHADP}[ADP^{3-}][Mg^{2+}][H^+] = 6.03 \times 10^{-3}$$
$$[MgADP^{1-}] = K_{MgADP^{1-}}[ADP^{3-}][Mg^{2+}] = 1.18 \times 10^{-2}$$

The stoichiometric coefficient of each biochemical metabolite species was obtained by multiplying the concentration of each metabolite species indicated as $f_i$ with the stoichiometric coefficient of the reactant present in the particular biochemical reaction and is represented in Eq. (15).

$$\upsilon_{A_i} = f_{A_i}\upsilon_A, \upsilon_{B_i} = f_{B_i}\upsilon_B, \upsilon_{C_i} = f_{C_i}\upsilon_C \quad (15)$$

Based on Eq. (1),

$$\upsilon_{G6P^{2-}} + \upsilon_{HG6P^{1-}} + \upsilon_{MgG6P} = \upsilon_{G6P} = 1;$$

$$\upsilon_{ATP^{4-}} + \upsilon_{HATP^{3-}} + \upsilon_{H_2ATP^{2-}} + \upsilon_{MgHATP^{1-}} + \upsilon_{MgATP^{2-}} + \upsilon_{Mg_2ATP} = \upsilon_{ATP} = 1;$$

$$\upsilon_{ADP^{3-}} + \upsilon_{HADP^{2-}} + \upsilon_{H_2ADP^{1-}} + \upsilon_{MgHADP} + \upsilon_{MgADP^{1-}} = \upsilon_{ADP} = 1 \quad (16)$$

The stoichiometric coefficients for reactants and products are considered as negative and positive respectively. To balance [$H^+$] ion and [$Mg^{2+}$] concentration on both sides, Eq. (18) was used.

$$\upsilon_{H^+} = -\sum_i \upsilon_i N_i^H = -\Delta_r N(H^+)$$

$$\upsilon_{Mg^{2+}} = -\sum_i \upsilon_i N_i^{Mg} = -\Delta_r N(Mg^{2+}) \quad (17)$$

$$\upsilon_{H^+} = 0.820$$

$$\upsilon_{Mg^{2+}} = 0.0814$$

Hence, 0.820 [$H^+$] ion was added to right side and 0.0814 [$Mg^{2+}$] ion was added to left side to balance the biochemical Eq. (1). The biochemical reaction at T = 298.15 K, P = 1 atm, I = 0.28M, pH = 7 and pMg=3 becomes,

$$1Glu^0 + 0.9198\mathrm{ATP}^{4-} + 0.0662\ \mathrm{HATP}^{3-} + 0.0095\mathrm{H_2ATP}^{2-} + 0.0010\ \mathrm{MgHATP}^{-} + 0.0037\mathrm{MgATP}^{2-} + 0.0002\mathrm{Mg_2ATP} + 0.0814\mathrm{Mg}^{2+} \leftrightarrow 0.9083\ G6P^{2-} + 0.0652\ HG6P^{1-} + 0.0265\ MgG6P + 0.8292\ \mathrm{ADP}^{3-} + 0.1196\ \mathrm{HADP}^{2-} + 0.0319\ \mathrm{H_2ADP}^{1-} + 0.0065\ \mathrm{MgHADP} + 0.0128\mathrm{MgADP}^{-} + 0.8202\ \mathrm{H}^{+} \quad (18)$$

To check Eq. (18), charge balance between reactants and products as given by Eq. (19) is used.

$$\sum_r \upsilon_r z_r = \sum_p \upsilon_p z_p \quad (19)$$

Gibbs free energy change of reaction (Eq. 1) was calculated for the formation of glucose 6 phosphate from glucose and is shown in Table 2. The results indicated that the transformed Gibbs free energy change of reaction showed 1.42 fold higher value than standard Gibbs free energy change of reaction.

## Conclusion

The transformed Gibbs free energy change of reaction was calculated based on the change in binding of hydrogen and magnesium ions in a metabolite and it is inferred that there is difference between transformed ($\Delta_r G'$) and standard Gibbs free energy change of reaction($\Delta_r G$). These results contradict the results reported by Iotti, (2010) in which ($\Delta_r G'$) is shown to be equal to ($\Delta_r G$) in ATP hydrolysis. From the results obtained in this study, it is concluded that change in binding of hydrogen and magnesium ions resulting in the formation of different metabolite species have an effect on the Gibbs free energy change of biochemical reaction at constant pH and pMg and conclusions given by Iotti, (2010) cannot be generalized to all biochemical reactions.

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