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Influence of binding polynomials on feasibility of biochemical reactions

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Abstract-Each metabolite in a specific biochemical reaction at specified pH and pMg is available as an equilibrated mixture of different charged ions and it is named as "pseudo-isomers or metabolite species". In this study, the establishment of stoichiometry was carried out by considering the sum of the metabolite species taking part in a biochemical reaction at specified pH, pMg, ionic strength I, T and P. The transformed Gibbs free energy change of reaction $(\Delta_r G'^0)$ for the biochemical conversion of fructose 6-phosphate to fructose 1,6 phosphate in glycolysis pathway was calculated and compared with their corresponding standard Gibbs free energy change of reaction $(\Delta_r G^0)$. The results revealed that that there is significant difference in values of $(\Delta_r G'^0)$ and $(\Delta_r G^0)$. Thus, it is inferred that the thermodynamic property $(\Delta_r G^0)$ is not sufficient to provide a criterion for the spontaneity of biochemical reaction. Transformed Gibbs free energy change must be minimized rather than standard Gibbs free energy change of reaction at the state of equilibrium in the case of biochemical reactions at constant pH and pMg.

Keywords-Transformed and standard Gibbs free energy change of reaction; pH; pMg

1. INTRODUCTION

Biological cell metabolism is described by the framework of metabolic network made of steps of biochemical reactions leading to particular product and are catalyzed by different enzymes. Unlike chemical reactions, all biochemical reactions are mass balanced and no charge balancing equations. In biochemical reactions, the sum of species of a biochemical reactant and the elemental balances such as hydrogen and magnesium are fixed at constant pH and pMg in the state of equilibrium [1]. Based on the reaction types, there are two different types of application of thermodynamic concepts to chemical and biochemical systems such as conventional thermodynamic properties and transformed or unconventional thermodynamic properties respectively [2,3,4]. Hence, the equilibrium constant for biochemical reactions is represented as *K'* (conditional or apparent equilibrium constant) which is written as the sum of the equilibrium constants of metabolite species [5]. The advantage of defining transformed thermodynamic properties of biochemical species is that it provides the overall view about a particular reactant when pH of the reaction is specified in the pH range 5-9 [6]. The calculation of standard transformed thermodynamic properties $(\Delta_f G_i'^0, \Delta_f H_i'^0, ...)$ of the biochemical reactant '*i'* from standard thermodynamic properties $(\Delta_f G_j^0, \Delta_f H_j^0, ...)$ of metabolite species of a particular biochemical reactant is relatively straight forward whereas the calculation of thermodynamic properties of metabolite species from the standard transformed enthalpies $(\Delta_r H'^0)$ of enzyme catalyzed reactions and experimental values of apparent equilibrium constants K' is more difficult. The main reason is that the transformed thermodynamic properties of a particular biochemical reactant are the composites of individual species properties [6].

IUBMB-IUPAC (International Union of Biochemistry and Molecular Biology-International Union of pure and applied chemistry) Joint Commission on Biochemical nomenclature (JCBN) confirmed that transformed Gibbs free energy (*G'*) is preferred during the consideration of change in the binding of hydrogen and magnesium ions [1].

This study is about the calculation of transformed Gibbs free energy of reaction ($\Delta_r \boldsymbol{G'^0}$) by considering the change in the binding of hydrogen and magnesium ions in a specific biochemical reactant based on conventional thermodynamic properties at specific ionic strength (*I*), pH and pMg.

2. MATERIALS AND METHODS

2.1. Thermodynamic analysis of a biochemical network

At specific pH, each metabolite in a particular biochemical reaction may available as an equilibrated mixture of various charged ions and it is named as "metabolite species". At the state of equilibrium, each metabolite is represented in the form of pseudoisomer group of metabolite species [6].

For example, consider the hydrolysis reaction of fructose 6-phosphate (F6P). The biochemical reaction is given in Eq. 1

F6P + ATP ↔ F16P +ADP (1)

The mass and charge balanced chemical equation is given in Eq. (2)

$$F6P^{2-} + ATP^{4-} \leftrightarrow F16P^{4-} + ADP^{3-} + H^{+} \quad (2)$$

where,

F6P : Fructose 6-phosphate includes both free species $F6P^{2-}$ and all the complex species such as $HF6P^{1-}$ and $MgF6P$

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.

F16P : Fructose 1,6 bisphosphate includes both free species $F16P^{4-}$ and all the complex species such as $HF16P^{3-}$, H_2F16P^{2-}, $MgF16P^{2-}$ and Mg_2F16P

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 $MgHADP$.

Depending on pH and pKa, the possibility of deprotonation of each metabolite and their species occurs inside the cell.

2.2. Effect of ionic strength, I on $\Delta G'^{0}_{f,i}$

Literature studies are available on the effect of ionic strength, pH, and the change in binding of hydrogen and magnesium ions on apparent equilibrium constants of biochemical reactions. During bacterial metabolism, C6 sugar is utilized as the substrate and is metabolized mostly into organic acids and alcohols. The organic acids obtained through fermentation have weak acidic functional groups and pK_a values of these functional groups nearer to physiological pH dissociate resulting in the formation of protons and deprotonated metabolite species. Such interactions are specifically due to two reasons and are as follows. (i) Based on ionic force in the biochemical media, the different solutes interact resulting in the formation of non-ideal behavior and hence the solution happens to be electrostatic in nature; (ii) In most of the enzyme catalyzed biochemical reactions, one of the metabolite species is highly active in forming enzyme-substrate complex that is stabilized mostly by electrostatic interactions. Hence, one of the factors that highly influence each reaction feasibility in a biochemical network is based on ionic strength inside the cell. Literature reports have shown that most of the biochemical reactions occurs in the ionic force ranging from 0.1 M to 0.3 M. Thus, the effect of solution ionic force must be incorporated during the calculation of $\Delta G'^{0}_{f,i}$. $\Delta G'^{0}_{f,i}$ of metabolite species *i* at specific ionic strength *I* is calculated using Eq. (3) [6].

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

where,

$\Delta G'^{0}_{f,i}$: Standard Gibbs free energy of formation of metabolite species *i* at specific 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 $L^{1/2}mol^{1/2}$

2.3. Effect of pH and pMg on $\Delta G'^{0}_{f,i}$

An another important factor that affect formation energies of metabolite species, *i* is pH. At alkaline pH condition, the concentration of highly protonated metabolite species among the pseudo-isomers is more. Highly protonated metabolite species have higher formation energies. Literature reports have shown that most of the biochemical reactions occurs at neutral pH, 7.

In addition to ionic strength and pH, an another significant factor that affect the formation of specific metabolite species is pMg. In bacteria, the total intracellular Mg concentration is ranging from 0.25 to 0.5M [7]. Thus, the effect of pH and pMg must be incorporated during the calculation of $\Delta_f G^{0}_{j}$. $\Delta_f G'^{0}_{j}$ at specific pH and pMg is calculated using Eq. (4) [3]. The steps followed for the calculation of Gibbs free energy of formation is given in Fig. 1.

$$\begin{aligned}\Delta G''^{0}_{f,i} = \Delta G'^{0}_{f,i} &- N_i(H)\{\Delta G^{0}_{f} - 2.303RT \times pH\} \\ &- N_i(Mg)\{\Delta G^{0}_{f} - 2.303RT \times pMg\}\end{aligned} \quad (4)$$

where,

$\Delta G''^{0}_{f,i}$: Standard Gibbs free energy of formation of metabolite species *i* at specific ionic strength, pH and pMg (KJ mol^{-1})

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

$N_i(H)$ $N_i(Mg)$: No. of hydrogen and magnesium atoms in specific metabolite species

R : Universal Gas constant (8.314 KJ/mol.K)

T : Temperature (298 K) pMg : $-\log[Mg^{2+}]$
pH : $-\log[H^{+}]$

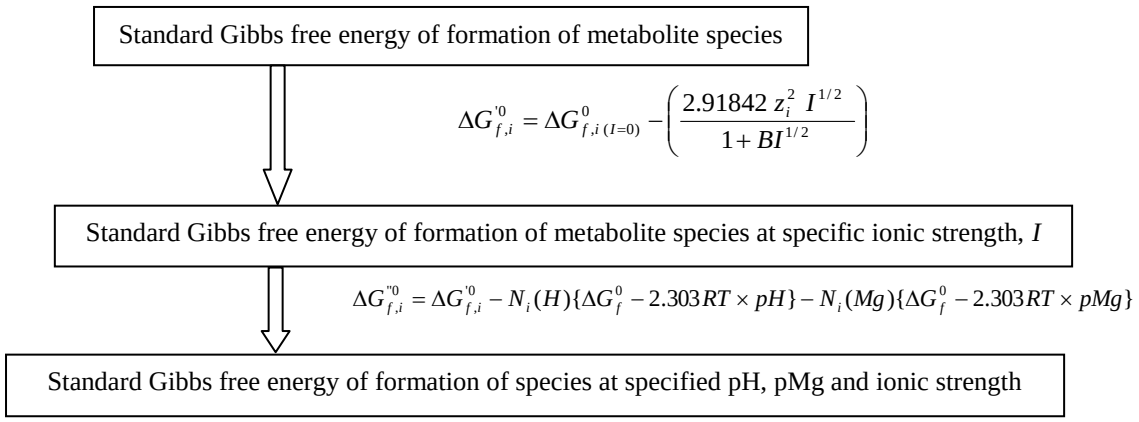

Fig. 1 Steps followed for the calculation of $\Delta G'^{0}_{f,i}$ of metabolite species of biochemical reactants at specific pH, pMg and ionic strength

2.4. Assumptions made during the construction of stoichiometry

The assumptions made during the construction of stoichiometry are as follows. (i) Water activity is held as constant, (ii) pressure P, temperature T, ionic strength I, pH and pMg are assumed as constant, (iii) standard concentration, c^0 is taken as 1M, (iv) free and complex metabolite species are at equilibrium with one another. The mass and charge balanced biochemical reaction was constructed by involving both free and complex metabolite species of all the biochemical reactants and products [8].

2.5. Calculation of $\Delta_r G'^{0}$

Gibbs free energy change of reaction, $\Delta_r G'^{0}$ is calculated using Eq. (5)

$$\Delta_r G'^{0} = \sum_i v_i \Delta G''^{0}_{f,i} \qquad (5)$$

where,

$\Delta_r G'^{0}$: Gibbs free energy change of a biochemical reaction (KJ/mol)
v_i : Stoichiometric coefficient
$\Delta G''^{0}_{f,i}$: Standard Gibbs free energy of formation of metabolite species *i* at specific ionic strength, pH and pMg (KJ mol^{-1})

Table 1 Gibbs free energy of formation of metabolite species involved in the conversion of fructose 6-phosphate to fructose 1,6 bisphosphate at T = 298.15 K, I = 0 M and P = 1 atm

Metabolite species	$\Delta G^{0}_{f,i}$ **(KJ/mol)**	**Charge**	**No. of hydrogen atoms**
$F6P^{2-}$	-1760.8	2	11
$HF6P^{1-}$	-1796.57	1	12
MgF6P	-2238.28	0	11
ATP^{4-}	-2771.1	4	12
$HATP^{3-}$	-2814.46	3	13
H_2ATP^{2-}	-2841.16	2	14
$MgHATP^{-}$	-3285.6	1	13
$MgATP^{2-}$	-3264.896	2	12
Mg_2ATP	-3738.78	0	12
$F16P^{4-}$	-2601.4	4	10
$HF16P^{3-}$	-2640	3	11
H_2F16P^{2-}	-2670	2	12
$MgFBP^{2-}$	-3080	2	10
ADP^{3-}	-1900.3	3	12
$HADP^{2-}$	-1941.26	2	13
H_2ADP^{-}	-2007.09	1	14
MgHADP	-2399.63	0	13
$MgADP^{-}$	-2385.37	1	12
Mg^{2+}	-458.54	2	0
H^{+}	0	1	1

3. RESULTS AND DISCUSSION

3.1. Construction of mass and charge balanced reaction

Each biochemical reactant dissociate at specific pH and it results in the formation of complex with Lewis acid such as H^+ and Mg^{2+}. The detailed stoichiometry construction procedure is described below. The mass balance equation for each of the biochemical reactants present in Eq. (1) are given below.

$$\begin{aligned}
[F6P] &= [F6P^{2-}]+[HF6P^{1-}]+[MgF6P] \\
&= [F6P^{2-}]+K_{HF6P^{1-}}[F6P^{2-}][H^+]+K_{MgF6P}[F6P^{2-}][Mg^{2+}] \\
&= [F6P^{2-}]\{1+K_{HF6P^{1-}}[H^+]+K_{MgF6P}[Mg^{2+}]\} \\
[F6P] &= B_{F6P}[F6P^{2-}] \qquad (6)
\end{aligned}$$

$$\begin{aligned}
[ATP] &= [ATP^{4-}]+[HATP^{3-}]+[H_2ATP^{2-}]+[MgATP^{2-}]+[MgHATP^{1-}]+[Mg_2ATP] \\
&= [ATP^{4-}]+K_{HATP^{3-}}[ATP^{4-}][H^+]+K_{H_2ATP^{2-}}[ATP^{4-}][2H^+]+ \\
&\quad K_{MgATP^{2-}}[ATP^{4-}][Mg^{2+}]+K_{MgHATP^{1-}}[ATP^{4-}][Mg^{2+}][H^+]+ \\
&\quad K_{Mg_2ATP}[ATP^{4-}][2Mg^{2+}] \\
&= [ATP^{4-}]\{1+K_{HATP^{3-}}[H^+]+K_{H_2ATP^{2-}}[2H^+]+K_{MgATP^{2-}}[Mg^{2+}]+ \\
&\quad K_{MgHATP^{1-}}[H^+][Mg^{2+}]+K_{Mg_2ATP}[2Mg^{2+}]\} \\
[ATP] &= B_{ATP}[ATP^{4-}] \qquad (7)
\end{aligned}$$

$$\begin{aligned}
[F16P] &= [F16P^{4-}]+[HF16P^{3-}]+[H_2F16P^{2-}]+[MgF16P^{2-}] \\
&= [F16P^{4-}]+K_{HF16P^{3-}}[F16P^{4-}][H^+]+K_{H_2F16P^{2-}}[F16P^{4-}][2H^+] \\
&\quad +K_{MgF16P^{2-}}[F16P^{2-}][Mg^{2+}] \\
&= [F16P^{4-}]\{1+K_{HF16P^{3-}}[H^+]+K_{H_2F16P^{2-}}[2H^+]+K_{MgF16P^{2-}}[Mg^{2+}]\} \\
[F16P] &= B_{F16P}[F16P^{4-}] \qquad (8)
\end{aligned}$$

$$\begin{aligned}
[ADP] &= [ADP^{3-}]+[HADP^{2-}]+[H_2ADP^{1-}]+[MgADP^{1-}]+[MgHADP] \\
&= [ADP^{3-}]+K_{HADP^{2-}}[ADP^{3-}][H^+]+K_{H_2ADP^{1-}}[ADP^{3-}][2H^+] \\
&\quad +K_{MgADP^{1-}}[ADP^{3-}][Mg^{2+}]+K_{MgHADP}[ADP^{3-}][H^+][Mg^{2+}] \\
&= [ADP^{3-}]\{1+K_{HADP^{2-}}[H^+]+K_{H_2ADP^{1-}}[2H^+]+K_{MgADP^{1-}}[Mg^{2+}] \\
&\quad +K_{MgHADP}[H^+][Mg^{2+}]\} \\
[ADP] &= B_{ADP}[ADP^{3-}] \qquad (9)
\end{aligned}$$

where, the binding polynomials of each of the biochemical reactant are given below.

$$\begin{aligned}
B_{F6P} &= \{1+K_{HF6P^{1-}}[H^+]+K_{MgF6P}[Mg^{2+}]\} \\
B_{ATP} &= \{1+K_{HATP^{3-}}[H^+]+K_{H_2ATP^{2-}}[2H^+]+K_{MgATP^{2-}}[Mg^{2+}]+K_{MgHATP^{1-}}[H^+][Mg^{2+}] \\
&\quad +K_{Mg_2ATP}[2Mg^{2+}]\} \\
B_{F16P} &= \{1+K_{HF16P^{3-}}[H^+]+K_{H_2F16P^{2-}}[2H^+]+K_{MgF16P^{2-}}[Mg^{2+}]\} \\
B_{ADP} &= \{1+K_{HADP^{2-}}[H^+]+K_{H_2ADP^{1-}}[2H^+]+K_{MgADP^{1-}}[Mg^{2+}]+K_{MgHADP}[H^+][Mg^{2+}]\}
\end{aligned}$$

The binding polynomials of each of the biochemical reactant are dependent on pH, pMg and pKa. Gibbs free energy change of reaction was calculated for all the biochemical reactions involving complex species. The corresponding equilibrium constant was calculated using Eq. (10).

$$K_{eq} = \exp\left(\frac{-\Delta_r G_i}{RT}\right) \quad (10)$$

Table 2 $\Delta_f G_j^{'0}$ involved in the conversion of fructose 6 phosphate to fructose 1,6 bisphosphate at T = 298.15 K, I = 0.28M and P = 1 atm

Metabolite species	$\Delta_f G_j^{'0}$ **(KJ/mol)**
$F6P^{2-}$	-1764.15
$HF6P^{1-}$	-1797.41
MgF6P	-2238.28
ATP^{4-}	-2784.48
$HATP^{3-}$	-2821.99
H_2ATP^{2-}	-2844.51
$MgHATP^{-}$	-3286.44
$MgATP^{2-}$	-3268.24
Mg_2ATP	-3738.78
$F16P^{4-}$	-2614.78
$HF16P^{3-}$	-2647.53
H_2F16P^{2-}	-2673.35
$MgFBP^{2-}$	-3083.35
ADP^{3-}	-1907.83
$HADP^{2-}$	-1944.61
H_2ADP^{-}	-2007.93
MgHADP	-2399.63
$MgADP^{-}$	-2386.21
Mg^{2+}	-461.89
H^{+}	-0.836

Table 3 $\Delta_r G_i$ for all the complex species involved in the conversion of F6P to F16P at T = 298.15 K, I = 0.28 M and P = 1 atm

Biochemical reactions involving complex species	$\Delta_r G^{'0}$ **(KJ mol^{-1})**
$F6P^{2-}$ + H^{+} ⇔$HF6P^{1-}$	-32.43
$F6P^{2-}$ +Mg^{2+} ⇔MgF6P	-12.25
ATP^{4-}+ H^{+} ⇔$HATP^{3-}$	-36.67
ATP^{4-}+ $2H^{+}$ ⇔ H_2ATP^{2-}	-20.85
ATP^{4-}+ Mg^{2+} +H^{+} ⇔ $MgHATP^{-}$	-39.24
ATP^{4-}+ Mg^{2+} ⇔$MgATP^{2-}$	-21.88
ATP^{4-}+ $2Mg^{2+}$ ⇔ Mg_2ATP	-30.53
$F16P^{4-}$+ H^{+} ⇔$HF16P^{3-}$	-31.9
$F16P^{4-}$+ $2H^{+}$ ⇔H_2F16P^{2-}	-56.89
FBP^{4-} +Mg^{2+} ⇔$MgFBP^{2-}$	-6.68
ADP^{3-}+ H^{+} ⇔ $HADP^{2-}$	-35.94
ADP^{3-}+ $2H^{+}$ ⇔H_2ADP^{-}	-98.43
$HADP^{2-}$+ Mg^{2+} ⇔ MgHADP	-29.08
ADP^{3-}+ Mg^{2+} ⇔ $MgADP^{-}$	-16.49

Using $\Delta_f G^{'0}$ of complex species, [H^{+}] and [Mg^{2+}], the binding polynomials was calculated using Eq's. 6,7, 8 and 9.

$$B_{F6P} = 1.189$$
$$B_{ATP} = 8.326$$
$$B_{F16P} = 1.054$$
$$B_{ADP} = 1.794\times10^{3}$$

Fractional population of a biochemical reactant, f_i is defined as the ratio of concentration of j^{th} metabolite species to overall concentration c^0 and is given in Eq. (11).

$$f_i = \frac{c_i}{c_0} \quad (11)$$

The overall concentration (c^0) of each biochemical reactant is assumed as 1M. Using overall concentration, the concentrations of [F6P], [ATP], [F16P] and [ADP] were calculated and their values are given below.

$[HF6P^{1-}]$	=	$K_{HF6P^{1-}}[F6P^{2-}][H^{+}]$	=	0.0406 M
$[MgF6P]$	=	$K_{MgF6P}[F6P^{2-}][Mg^{2+}]$	=	0.118 M
$[HATP^{3-}]$	=	$K_{HATP^{3-}}[ATP^{4-}][H^{+}]$	=	0.0322` M
$[H_2ATP^{2-}]$	=	$K_{H_2ATP^{2-}}[ATP^{4-}][2H^{+}]$	=	5.42×10^{-12} M
$[MgHATP^{1-}]$	=	$K_{MgHATP^{1-}}[ATP^{4-}][H^{+}][Mg^{2+}]$	=	9.06×10^{-5} M
$[MgATP^{2-}]$	=	$K_{MgATP^{2-}}[ATP^{4-}][Mg^{2+}]$	=	0.821 M
$[Mg_2ATP]$	=	$K_{Mg_2ATP}[ATP^{4-}][2Mg^{2+}]$	=	0.027 M
$[HF16P^{3-}]$	=	$K_{HF16P^{3-}}[F16P^{4-}][H^{+}]$	=	0.0372 M
$[H_2F16P^{2-}]$	=	$K_{H_2F16P^{2-}}[F16P^{4-}][2H^{+}]$	=	8.91×10^{-5} M

$[MgF16P^{2-}]$	=	$K_{MgF16P^{3-}}[F16P^{4-}][Mg^{2+}]$	=	0.0141 M
$[HADP^{2-}]$	=	$K_{HADP^{2-}}[ADP^{3-}][H^{+}]$	=	1.11×10^{-4} M
$[H_2ADP^{1-}]$	=	$K_{H_2ADP^{1-}}[ADP^{3-}][2H^{+}]$	=	0.999 M
$[MgHADP]$	=	$K_{MgHADP}[ADP^{3-}][H^{+}][Mg^{2+}]$	=	6.98×10^{-9} M
$[MgADP^{1-}]$	=	$K_{MgADP^{1-}}[ADP^{3-}][Mg^{2+}]$	=	4.34×10^{-4} M

Table 4 $\Delta_f G_i^{"0}$ of free species and $\Delta_r G_i^{'0}$ complex species at T = 298.15 K, I = 0.28 M, pH=7, pMg=3 and P = 1 atm

Reactants	**Biochemical reactions of free and complex species**	$\Delta_f G_i^0$ **at I=0.28M (KJ/mol)**	v_i	**Concentration (M)**	$\Delta_r G_i^0$ **at I=0.28M, pH=7, pMg=3 (KJ/mol)**
$F6P^{2-}$	NA	-1764.15	0.842	0.842	-1484.53
$HF6P^{1-}$	$F6P^{2-}+ H^+ \Leftrightarrow HF6P^{1-}$	-1797.41	0.0406	0.0406	-73.29
MgF6P	$F6P^{2-} +Mg^{2+} \Leftrightarrow MgF6P$	-2238.28	0.118	0.118	-264.74
ATP^{4-}	NA	-2784.48	0.1201	0.1201	-335.05
$HATP^{3-}$	$ATP^{4-}+ H^+ \Leftrightarrow HATP^{3-}$	-2821.99	0.0322	0.0322	-91.14
H_2ATP^{2-}	$ATP^{4-}+ 2H^+ \Leftrightarrow H_2ATP^{2-}$	-2844.51	5.42×10^{-12}	5.42×10^{-12}	-1.58×10^{-8}
$MgHATP^-$	$ATP^{4-}+ Mg^{2+} +H^+ \Leftrightarrow MgHATP^-$	-3286.44	9.06×10^{-5}	9.06×10^{-5}	-0.2998
$MgATP^{2-}$	$ATP^{4-}+ Mg^{2+} \Leftrightarrow MgATP^{2-}$	-3268.24	0.821	0.821	-2683.63
Mg_2ATP	$ATP^{4-}+ 2Mg^{2+} \Leftrightarrow Mg_2ATP$	-3738.78	0.027	0.027	-101.19
$F16P^{4-}$	NA	-2614.78	0.949	0.949	-2480.63
$HF16P^{3-}$	$F16P^{4-}+ H^+ \Leftrightarrow HF16P^{3-}$	-2647.53	0.0372	0.0372	-98.79
H_2F16P^{2-}	$F16P^{4-}+ 2H^+ \Leftrightarrow H_2F16P^{2-}$	-2673.35	8.91×10^{-5}	8.91×10^{-5}	-0.240
$MgFBP^{2-}$	$FBP^{4-} +Mg^{2+} \Leftrightarrow MgFBP^{2-}$	-3083.35	0.0141	0.0141	-43.62
ADP^{3-}	NA	-1907.83	0.00056	0.00056	-1.0787
$HADP^{2-}$	$ADP^{3-}+ H^+ \Leftrightarrow HADP^{2-}$	-1944.61	1.11×10^{-4}	1.11×10^{-4}	-0.218
H_2ADP^-	$ADP^{3-}+ 2H^+ \Leftrightarrow H_2ADP^-$	-2007.93	0.999	0.999	-2005.92
MgHADP	$HADP^{2-}+ Mg^{2+} \Leftrightarrow MgHADP$	-2399.63	6.98×10^{-9}	6.98×10^{-9}	-1.71×10^{-5}
$MgADP^-$	$ADP^{3-}+ Mg^{2+} \Leftrightarrow MgADP^-$	-2386.21	4.34×10^{-4}	4.34×10^{-4}	-1.0439
Mg^{2+}	NA	-461.885	0.978	1.0×10^{-3}	-468.46
H^+	NA	-0.83627	0.962	1.0×10^{-7}	-39.24

The stoichiometric coefficient (v_i) of each metabolite species was calculated by multiplying fractional population with stoichiometric coefficient of the biochemical reactant present in specific reaction and is given in Eq. (12).

$$v_{A_i} = f_{A_i} v_A ; v_{B_i} = f_{B_i} v_B ; v_{C_i} = f_{C_i} v_C \quad (12)$$

The overall stoichiometric coefficient of free and complex species are represented in Eq. (13). Using Eq. (13), the stoichiometric coefficients of complex species were calculated.

$$v_{F6P^{2-}} + v_{HF6P^{1-}} + v_{MgF6P} = v_{F6P} = 1;$$
$$v_{ATP^{4-}} + v_{HATP^{3-}} + v_{H_2ATP^{2-}} + v_{MgHATP^{1-}} + v_{Mg_2ATP} = v_{ATP} = 1;$$
$$v_{F16P^{4-}} + v_{HF16P^{3-}} + v_{H_2F16P^{2-}} + v_{MgF16P^{2-}} = v_{F16P} = 1;$$
$$v_{ADP^{3-}} + v_{HADP^{2-}} + v_{H_2ADP^{1-}} + v_{MgADP^{1-}} = v_{ADP} = 1 \quad (13)$$

Using Eq. (14), $[H^+]$ and $[Mg^{2+}]$ ion concentrations are balanced on both sides.

$$v_{H^+} = -\sum_i v_i N_i^H = -\Delta_r N(H^+);$$
$$v_{Mg^{2+}} = -\sum_i v_i N_i^{Mg} = -\Delta_r N(Mg^{2+}) \quad (14)$$
$$v_{H^+} = 0.9624;\ v_{Mg^{2+}} = 0.978$$

Hence, 0.9624 $[H^+]$ ion and 0.978 $[Mg^{2+}]$ ion concentrations were added to left and right terms respectively in order to balance the biochemical reaction.

The mass and charge balanced fructose 1,6 bisphosphate hydrolysis reaction from fructose 6-phosphate at 298.15 K, 1 atm, 0.28M, pH 7 and pMg 3 is given below.

$$0.842F6P^{2-} + 0.041HF6P^{1-} + 0.118MgF6P + 0.1201ATP^{4-} + 0.032HATP^{3-} + (5.42\times10^{-12})H_2ATP^{2-} + (9.06\times10^{-5})MgHATP^{1-} +$$
$$0.821MgATP^{2-} + 0.027Mg_2ATP + 0.963H^+ \leftrightarrow 0.949F16P^{4-} + 0.037HF16P^{3-} + (8.91\times10^{-5})H_2F16P^{2-} + 0.014MgFBP^{2-} +$$
$$0.0006ADP^{3-} + (1.11\times10^{-4})\,HADP^{3-} + 0.999H_2ADP^- + (6.98\times10^{-9})MgHADP + (4.34\times10^{-4})\,MgADP^{1-} + 0.978Mg^{2+} \quad (15)$$

Eq. (16) is applied to check the charge balance in Eq. (15).

$$\sum_r v_r z_r = \sum_p v_p z_p = 2.98 \quad (16)$$

Table 5 Standard and transformed $\Delta_r G_i^{'0}$ for the conversion of F6P to F16P

Biochemical reactions	$\Delta_r G^{0'(a)}$	$\Delta_r G^{0'(b)}$	**pH**	**pMg**	**T**	$\Delta_r G^{0(c)}$	$\Delta_r G^{0'(d)}$	$\Delta_r G^{0'(e)}$	$\Delta_r G^{0'(f)}$
F6P + ATP <=> FDP + ADP	-14.23	-19.50	8	2.16	303.15	30.2	-14.74	-29.1162	-29.22

Gibbs free energies change of reactions are given in (KJ/mol), temperature in K and ionic strength in M.
[a]Standard Gibbs free energy change of reaction values at pH 7 and temperature 298.1 K as reported by Maskow and von Stockar (2005).
[b] Free energy change values as reported by Goldberg et al., 2004 and is available in NIST database. The experimental pH, I and pMg are provided in the subsequent columns.
[c] Calculated standard Gibbs free energy change of reaction values without considering pH, ionic strength and metabolite complex with H^+ and Mg^{2+} ions at 298 K
[d]Transformed Gibbs free energy change of reactions values with corrected pH 7 and ionic strength (0.28M) at 298K
[e]Transformed Gibbs free energy change of reactions values with corrected pH 7, ionic strength (0.28M) and the inclusion of metabolite species complex with H^+ ions at 298K;
[f]Transformed Gibbs free energy change of reaction values with corrected pH 7, pMg 3, ionic strength (0.28M) and the incorporation of metabolite species complex with H^+ and Mg^{2+} ions at 298 K.

$\Delta_r G_i^{'0}$ for the conversion of fructose 6 phosphate to fructose 1,6 bisphosphate in glycolysis is minimized after incorporating the corrections of pH, pMg, ionic strength and change in binding of H^+ and Mg^{2+} ions. There is no significant difference between $\Delta_r G_i^{'0}$ before and after including the corrections of change in binding of Mg^{2+} ions. The results indicated that $\Delta_r G^{'}$ showed 1.95 fold more than standard Gibbs free energy change of reaction.

4. CONCLUSION

$\Delta_r G^{'}$ was calculated by incorporating the effect of pH, pMg, ionic strength and change in the binding of hydrogen and magnesium ions. There is a significant difference between $\Delta_r G^{'}$ and $\Delta_r G$. These results obtained from this study is inconsistent with the results reported by Iotti, [8] in which $\Delta_r G^{'}$ is shown to be equal to $\Delta_r G$ in ATP hydrolysis. From this study, it is inferred that pH, pMg, ionic strength and change in the binding of hydrogen and magnesium ions have an effect on $\Delta_r G^{'}$. The conclusions specified by Iotti, [8] cannot be generalized to all biochemical reactions.

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REFERENCES

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

[2] R. A. Alberty. "Equilibrium calculations on systems of biochemical reactions at specified pH and pMg". Biophysics Chemistry, 42: 117-131, 1992a.
[3] R. A. Alberty. "Calculation of transformed thermodynamic properties of biochemical reactants at specified pH and pMg". Biophysical Chemistry, 43: 239-254, 1992b.
[4] A. Sabatini, A. Vacca, and S. Iotti. "Balanced biochemical reactions: A new approach to unify chemical and biochemical thermodynamics". Plos One, 7(1): 29529, 2012.
[5] G. Schwarzenbach, and H. A. Flaschka. Complexometric Titrations, London: Methuen, 1969.
[6] R. A. Alberty. "Calculation of Thermodynamic Properties of Species of Biochemical Reactants Using the Inverse Legendre Transform". Biophysical chemistry, 109: 9132-9139, 2005.
[7] E. Demedicis. "Magnesium, manganese and mutual depletion systems in halophilic bacteria". FEMS Microbiol Rev, 39(1–2): 137–143, 1986.
[8] S. Iotti, A. Sabatini, and A. Vacca. "Chemical and biochemical thermodynamics: from ATP hydrolysis to a general reassessment". Journal of Physical Chemistry, 114, 1985–1993, 2010.