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A thermodynamic investigation of the glucose-6-phosphate isomerization

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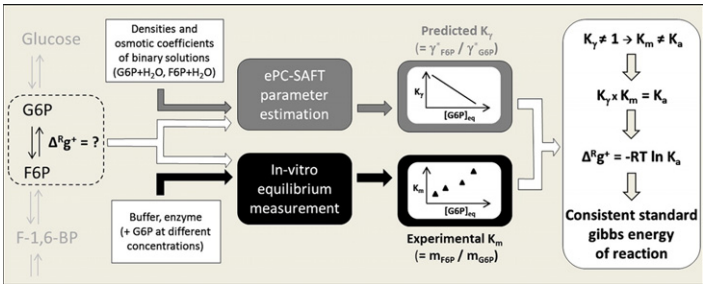
HIGHLIGHTS

- Equilibrium constant K strongly depends on G6P concentration for G6P ↔ F6P reaction.
- $\Delta^R g^+$ upon using either K_a or K_m differs by up to 30%.
- Activity-coefficient ratio of G6P and F6P strongly deviates from unity.
- Activity-coefficient ratio K_γ could be predicted with ePC-SAFT accurately.
- Influence of buffer and glutamate on K_γ could be predicted with ePC-SAFT accurately.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, $\Delta^R g^+$ values for the enzymatic G6P isomerization were determined as a function of the G6P equilibrium molality between 25 °C and 37 °C. The reaction mixtures were buffered at pH = 8.5. In contrast to standard literature work, $\Delta^R g^+$ values were determined from activity-based equilibrium constants instead of molality-based apparent values. This yielded a $\Delta^R g^+$ value of 2.55 ± 0.05 kJ mol^{-1} at 37 °C, independent of the solution pH between 7.5 and 8.5. Furthermore, $\Delta^R h^+$ was measured at pH = 8.5 and 25 °C yielding 12.05 ± 0.2 kJ mol^{-1}.
Accounting for activity coefficients turned out to influence $\Delta^R g^+$ up to 30% upon increasing the G6P molality. This result was confirmed by predictions using the thermodynamic model ePC-SAFT.
Finally, the influence of the buffer and of potassium glutamate as an additive on the reaction equilibrium was measured and predicted with ePC-SAFT in good agreement.

1. Introduction

One of the most obvious characteristics that distinguishes chemical from biological systems is the diversity of the species involved. A biological process often involves parts of a metabolic network inside a complex organism with numerous reactants, products, enzymes, and other biological compounds that are often largely undefined with respect to concentration and their physical properties. Even more complex, some of these compounds are present as different species, depending on solution conditions such as pH (degree of protonation) and Mg concentrations (degree of complex formation) [1]. While sequences of chemical reactions are already predictable, the thermodynamics of biological-reaction sequences is still in its infancy [1]. The species diversity and, even more important, the lack of knowledge of the physical properties of biological compounds and mixtures have

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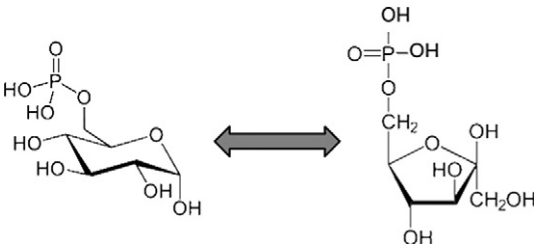
long been a drawback in the rational and efficient design of biological processes.

Several methods have recently been developed for the thermodynamic characterization of biological processes [2–9]. A thermodynamic key quantity applied in these methods, some of which are referred to as feasibility studies [10–13], is the standard Gibbs energy of reaction $\Delta^R g^+$. Thermodynamically correct, this $\Delta^R g^+$ has to be calculated from the activity-based (thermodynamic) equilibrium constant of a reaction, K_a. In the standard literature however, $\Delta^R g^+$ is usually calculated from the molality-based (apparent) equilibrium constant K_m (also referred to as K′), which is thermodynamically incorrect.

K_m can be converted into K_a as long as the activity coefficients of the reactants and products are known at reaction conditions. The activity coefficient describes the deviations of the reactants and products from their standard state (e.g., infinite dilution), caused by interactions between all present compounds in the reaction mixture at finite concentrations. These interactions include molecular interactions of the reactants and products with system compounds that do not directly take part in the reaction and interactions among the reacting compounds themselves. Therefore, the activity coefficients do not only depend on the presence and the nature of the system compounds but also directly on the reactant and product concentration. Furthermore, the system compounds may build complexes or may protonate/deprotonate depending on solution conditions such as pH, which additionally influences activity coefficients.

Unfortunately, data on species activity coefficients in biological systems is scarce and their influence on thermodynamic properties such as $\Delta^R g^+$ is largely unknown. Accordingly, the influence of activity coefficients is usually neglected for the characterization of biological reactions [14]. In our previous work, the importance to account for activity coefficients in order to characterize biological reactions was soundly demonstrated [15]. For the considered reaction in that work (hydrolysis of methyl ferulate), it was shown that the activity coefficients of the reacting agents strongly deviate from unity and thus have a large impact on $\Delta^R g^+$ values.

In this work, the reaction equilibrium of the enzymatic isomerization of glucose-6-phosphate (G6P) to fructose-6-phosphate (F6P) (Eq. (1)) was investigated.



(1)

The isomerization is catalyzed by the enzyme phosphoglucose isomerase (PGI). The G6P isomerization is the second step of glycolysis, the central carbon degradation pathway in any organism. A thorough understanding of this reaction is thus required for many biotechnological processes. Following the procedure in our previous study [15], the (thermodynamic) activity-based equilibrium constant K_a was determined by measuring the molality-based (apparent) equilibrium constant K_m at different G6P molalities and extrapolating K_m to zero G6P molality. In addition, the influence of reaction additives on the G6P isomerization equilibrium was investigated.

Next to K_a (and $\Delta^R g^+$ values calculated thereof), also the standard enthalpy of reaction ($\Delta^R h^+$) is a fundamental thermodynamic quantity required for the characterization of reactions and for the operation, design, and optimization of biochemical processes. ITC (isothermal titration calorimetry) has already proven its capability to determine physicochemical properties (molecular interactions) and reaction enthalpies for the characterization of biochemical processes [16–18]. In this work, $\Delta^R h^+$ was measured using ITC. In addition, these $\Delta^R h^+_{ITC}$ values were compared to $\Delta^R h^+$ values obtained by applying the van't Hoff equation to the temperature-dependent K_a values determined from equilibrium measurements in this work. This way, $\Delta^R h^+_{ITC}$ can be used to verify the accuracy of the K_a values (and thus of $\Delta^R g^+$) obtained from K_m measurements.

Another evidence for the quality of the experimentally-determined K_a values can be obtained by comparing with those predicted via K_m and activity coefficients obtained from thermodynamic models. In the past decades, researchers started to develop thermodynamic models in order to describe activity coefficients in biological solutions. The Pitzer equation is one of the most famous correlative models [19]. The availability of those models allows calculating activity coefficients depending on temperature, solutes, and solute molalities. One disadvantage of models like the Pitzer equation is the need for solute–solute parameters. Setting them to zero (as for predictions) means that solute–solute interactions are completely neglected, which leads to inaccurate modeling results [20]. Thus, such models are usually not able to quantitatively predict activity coefficients in multi-solute solutions.

However, there are advanced thermodynamic models that allow for quantitative predictions of activity coefficients in biological systems. One example is the Statistical Associating Fluid Theory (SAFT) and models based on SAFT. They are able to account for specific interactions between biological compounds caused by hydrogen bonding or charges. In this work, the activity coefficients were estimated by the electrolyte Perturbed-Chain SAFT (ePC-SAFT) [21]. This model is especially suitable for aqueous solutions containing biomolecules and electrolytes [21–29]. It has already successfully been used for predictions of activity coefficients in multi-solute solutions based only on model parameters fitted to properties of pure compounds and binary solute + solvent solutions.

2. Thermodynamic formalism for the G6P isomerization

This section describes the formalism for a thermodynamically consistent description of the isomerization of G6P to F6P (Eq (1)). Both compounds were used as dipotassium salts $G6PK_2$ and $F6PK_2$ in this study. The reacting agents of Eq. (1) are thus not G6P and F6P, but rather the twofold deprotonated species, denoted with $G6P^{2-}$ and $F6P^{2-}$ in the following. These species were exclusively present at reaction conditions as the chosen pH of 8.5 is significantly above the highest pK_a of G6P and F6P [30], i.e. the compounds were completely dissociated. Moreover, ion pairing between $G6P^{2-}$ and K^+ as well as between $F6P^{2-}$ and K^+ was not assumed to occur as the considered concentrations of $G6PK_2$ and $F6PK_2$ were very small (in the mmolal range). The thermodynamic equilibrium constant K_a of the G6P isomerization at these conditions is defined as

$$K_a = \frac{a^{eq}_{F6P^{2-}}}{a^{eq}_{G6P^{2-}}} \tag{2}$$

where a^{eq} are the equilibrium activities of the reacting species. The thermodynamic activity is defined as the product of concentration and respective activity coefficient, which itself depends on the standard state and on the concentration unit used:

$$a_i = m_i \cdot \gamma_i^{*,m}. \tag{3}$$

In this work, the concentration is reported as molality m_i (moles of compound i per kg water). The use of molality is recommended for thermodynamic considerations, as the reference (kg of pure water) is a temperature-independent property, which is not true for molarity (mol L^{-1}) or concentration (g L^{-1}). The standard state for the molality-based activity coefficient $\gamma_i^{*,m}$ is a hypothetical solution of compound i in water, which is defined as a one molal solution that exhibits the same interactions as at infinite dilution. The activity coefficient $\gamma_i^{*,m}$ is preferably used for solutes that are present at very low molalities in the reaction mixture (dilute solution). As $G6PK_2$ and