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The role of activity coefficients in bioreaction equilibria: Thermodynamics of methyl ferulate hydrolysis

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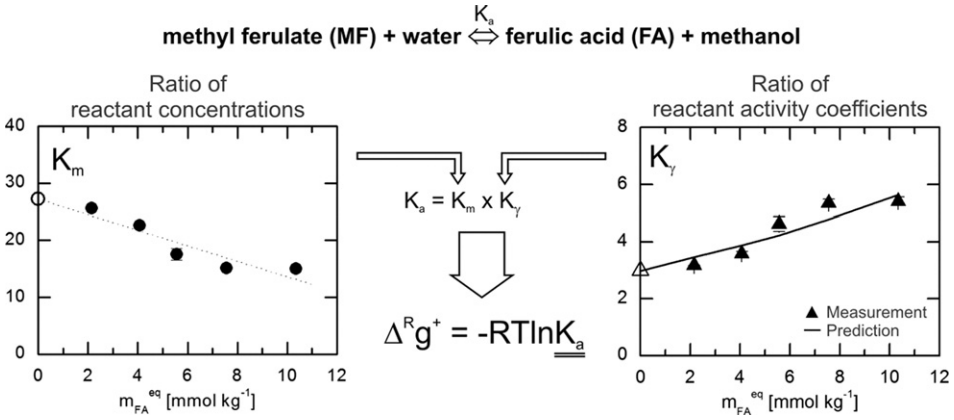
HIGHLIGHTS

- Concentration-dependent K_m values were converted into concentration-independent K_a.
- K_γ values strongly differ from unity ($3<K_\gamma<6$), depending on the reactant concentration.
- Using K_a instead of K_m causes a deviation of 40% in $\Delta^R g^+$.
- Reactant activity coefficients (causing the deviation) cannot be assumed to be unity.
- Reactant activity coeff. can be predicted with ePC-SAFT, even in the presence of salt.

GRAPHICAL ABSTRACT



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ABSTRACT

The Gibbs energy of reaction ($\Delta^R g$) is the key quantity in the thermodynamic characterization of biological reactions. Its calculation requires precise standard Gibbs energy of reaction ($\Delta^R g^+$) values. The value of $\Delta^R g^+$ is usually determined by measuring the apparent (concentration-dependent) equilibrium constants K, e.g., the molality-based K_m. However, the thermodynamically consistent determination of $\Delta^R g^+$ requires the thermodynamic (activity-based) equilibrium constant K_a. These values (K_m and K_a) are equal only if the ratio of the activity coefficients of the reactants to the activity coefficients of the products (K_γ) is equal to unity.

In this work, the impact of K_γ on the estimation of K_a for biological reactions was investigated using methyl ferulate (MF) hydrolysis as a model reaction. The value of K_γ was experimentally determined from K_m values that were measured at different reactant concentrations. Moreover, K_γ was independently predicted using the thermodynamic model ePC-SAFT. Both the experimentally determined and the predicted K_γ values indicate that this value cannot be assumed to be unity in the considered reaction. In fact, in the reaction conditions considered in this work, K_γ was shown to be in the range of $3<K_\gamma<6$ for different reactant molalities ($2<$mmol MF $kg^{-1}<10$). The inclusion of K_γ and thus the use of the thermodynamically correct K_a value instead of K_m lead to remarkable differences (almost 40%) in the determination of $\Delta^R g^+$. Moreover, the new value for $\Delta^R g^+$ increases the concentration window at which the reaction can thermodynamically occur.

The influence of additives was also investigated both experimentally and theoretically. Both procedures consistently indicated that the addition of NaCl (0 to 1 mol kg^{-1} water) moderately decreased the value of K_γ, which means that the values of K_m increase and that a higher amount of products is obtained as a result of the addition

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of salt. Additionally, K_m was found to strongly depend on pH. A ten-fold increase in the K_m values was observed in the pH range of 6 to 7; this increase corresponds to a change of more than 100% in the value of $\Delta^R g^+$.

1. Introduction

Thermodynamics has emerged as a promising tool for the refinement of metabolic network models that are developed in the scope of systems biology and for a thorough understanding of the biological reactions that comprise cellular metabolism. Although several methods exist for the thermodynamic characterization of biological reactions [1–10], these methods neglect an important thermodynamic property, namely, the activity coefficients of the reactants and the products. This quantity describes the deviation of the components from their standard state (e.g., pure component or hypothetical ideal solution) that is caused by the interactions between *all* of the present components. These interactions include molecular interactions among the reacting agents, as well as interactions of the reactants and the products with system components that do not directly participate in the reaction. Accordingly, the reactant and product activity coefficients strongly depend not only on their own concentrations but also on the nature of all of the system components. However, data on these activity coefficients are scarce, and their influence on biological and thermodynamic properties is thus largely unknown. These values are usually assumed to be unity [11], which means that these coefficients are neglected in the characterization of biological reactions.

Activity coefficients are related to the thermodynamic (activity-based) equilibrium constant K_a. K_a is the product of two quantities, both of which are concentration-dependent: the K value, which is usually calculated from the equilibrium concentrations of the reactants and the products, and a K_γ value, which accounts for the activity coefficients of these components (Eq. (1)).

$$K_a = K \cdot K_\gamma \tag{1}$$

It needs to be noted that the K values that are published in the literature, e.g., the NIST database on 'Thermodynamics of enzyme-catalyzed reactions' [12], are usually *not* the equilibrium constants K_a. Instead, these values are the concentration-dependent K values and are often even reported without declaring the units. Molality-based (K_m) or molarity-based (K_c) constants are the most commonly reported values of K. Moreover, the reported K values were usually measured at various system conditions (e.g., T, pH, ionic strength (I), and buffer) that might differ from the tabulation conditions (25 °C, pH 7, I = 0). To indicate whether the measurements were performed at non-standard system conditions, the respective K values are usually denoted by a prime and referred to as 'apparent' equilibrium constants (K′).

The complex influence of the system conditions on the apparent K′ values has been discussed and mathematically described in an extensive work by Alberty [13]. His approach allows for the comparison of K′ values from different authors (determined at different conditions).

The present work, in contrast, focuses on the thermodynamically consistent determination of the equilibrium constant K_a by measuring the molality-based K_m value and including the reactant and product activity coefficients according to Eq. (1). For this purpose, the hydrolysis of methyl ferulate (MF) to form ferulic acid (FA) and methanol (MeOH), which is catalyzed by the feruloyl esterase enzyme (Eq. (2)), was considered as a model reaction.

$$\text{methyl ferulate} + \text{water} \rightleftarrows \text{ferulic acid} + \text{methanol} \tag{2}$$

The enzyme feruloyl esterase participates in the breakdown of hemicellulose, which forms part of the plant cell wall, and catalyzes the hydrolysis of the feruloyl group from an esterified sugar in the cell wall [14]. Because MF possesses structural similarity to the esterified sugars and because efficient biomass utilization aims to efficiently break down these substrates, thermodynamics of the reactions catalyzed by feruloyl esterases is of practical importance. Moreover, a mixture of two acids (FA and MF), an organic solvent/product (MeOH), and water is expected to exhibit substantially non-ideal behavior, which makes MF hydrolysis a meaningful model reaction to demonstrate the influence of the reactant and the product activity coefficients.

The same reaction was previously studied by Goldberg et al. [15], who focused on the determination of the apparent K′ values and the enthalpies of reaction at 25 °C using a citrate buffer (pH = 5). These researchers found that K′ strongly depends on the pH and that the equilibrium is strongly on the side of the products (FA and MeOH). Therefore, these researchers used an excess of MeOH to shift the reaction toward the reactant side and thus make the equilibrium concentrations analytically accessible. This excess of MeOH is expected to have a pronounced influence on the activity coefficients, particularly that of MeOH. However, Goldberg et al. did not address the dependence of the K′ values on the equilibrium concentrations nor the influence of the reactant and product activity coefficients on the K′ values. Both of these issues are considered in this study.

2. Thermodynamics of biological reactions using MF hydrolysis as an example

The following section describes the formalism for a thermodynamically consistent description of bioreactions using MF hydrolysis (Eq. (2)) as the model reaction.

The thermodynamic equilibrium constant K_a is defined as

$$K_a = \frac{a_{FA} \cdot a_{MeOH}}{a_{MF} \cdot a_w}, \tag{3}$$

where a is the activity of the reactants and the products in the MF hydrolysis reaction. The activity of a component can be written as the product of the component's concentration and its respective activity coefficient, which itself depends on the standard state as well as on the concentration units used:

$$a_i = x_i \cdot \gamma_i^x = m_i \cdot \gamma_i^m \text{ (refers to standard state "pure component")} \tag{4}$$

$$a_i = m_i \cdot \gamma_i^{*,m} \text{ (refers to standard state "hypothetical ideal solution")}. \tag{5}$$

The variables m_i and x_i represent the molality (moles of component i per kg water) and the mole fraction of component i, respectively. The γ_i value is the activity coefficient of component i. The standard state for the activity coefficients γ_i^x and γ_i^m in Eq. (4) is the pure component i. Thus, these activity coefficients become unity for the pure component i ($x_i = 1$). In contrast, the standard state for $\gamma_i^{*,m}$ in Eq. (5) is a hypothetical ideal solution of component i in a solvent (e.g., water), which is defined as a one molal solution that exhibits the same interactions as an infinite dilution of component i in the same solvent.

The activity coefficient γ_i is usually used for solvents (e.g., water and MeOH in this work), whereas the activity coefficient γ_i^* is usually used for solutes that are present at very low concentrations in the reaction mixture and thus exhibit infinite-dilution interactions (e.g., MF and FA in this work).

In analogy to the activity of a component, which can be expressed as the product of the molality of this component and the activity