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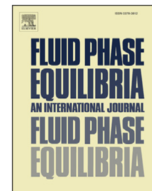

Thermodynamics of the alanine aminotransferase reaction

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ABSTRACT

The thermodynamic equilibrium of the aminotransferase reaction from L-alanine and 2-oxoglutarate to L-glutamate and pyruvate in aqueous solution was investigated in a temperature range between 25 and 37 °C and pH between at 5 and 9.

Prior to considering the reaction equilibria, measurements were carried out to ensure the enzyme activity in the aqueous reaction media. After that, equilibrium concentrations of reacting agents were measured by HPLC-analysis. At constant temperature and pH, reaction equilibrium was shown to depend on the absolute molalities (0.005–0.130 mol kg^{-1}) as well as on the ratio of initial molalities of the reactants. It could be concluded that reaction equilibrium was shifted towards the product site upon increasing reactant molalities, increasing temperature, and increasing pH. Further, yields of pyruvate were increased upon excess initial molality of L-alanine compared to 2-oxoglutarate.

The thermodynamic equilibrium constant K_{a^*} was determined by extrapolating the ratio of product equilibrium molalites and reactant equilibrium molalites to infinite dilution of all reacting agents. The activity-coefficient ratio of products and reactants in the reaction media was predicted with ePC-SAFT. Combining K_{a^*} and the activity-coefficient ratio allowed quantitatively predicting the influence of temperature, pH, and reacting-agent molalities on the reaction equilibrium.

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1. Introduction

Aminotransferase reactions are enzymatic reactions in which the amino group of one of the reactants is transferred to the other reactant, e.g. from an amino acid to a keto acid. By using different reactants and an appropriate enzyme, various amino acids and keto acids can be synthesized by aminotransferase reactions [1,2]. As aminotransferases often appear in metabolic pathways, understanding the thermodynamics of aminotransferase reactions is important for biochemists and bioengineers. Further, these reactions are of particular interest for biotechnological applications in industry as they present new synthetic routes for the production of amino acids, like aromatic amino acids or β-amino acids [3–5]. β-amino acids can be used as building blocks for drug production (e.g. antibiotics), enzyme inhibitors, or peptide mimetics with pharmacological properties [6]. Furthermore, α-amino acids are important precursors for food, animal feed, pharmaceutics or cosmetics [7].

In this work, the alanine aminotransferase reaction is investigated (Fig. 1). The alanine aminotransferase reaction is an equilibrium reaction catalyzed by the enzyme alanine aminotransferase (ALAT) in the presence of the coenzyme pyridoxal 5′-phosphate (P5P).

The reactants L-alanine and 2-oxoglutarate are converted to the products pyruvate and L-glutamate until the reaction equilibrium is reached. In general, aminotransferase reactions are thermodynamically limited; that is, yield is limited by thermodynamic reaction equilibrium due to near-unity equilibrium constants [1]. The application of thermodynamics to equilibrium-limited biological reactions can crucially contribute to the description and understanding of metabolic networks, to the ability to calculate yield limitations, and to the characterization of biological reactions [8–17].

If all reacting agents are diluted in a reaction medium, the thermodynamic reaction equilibrium is characterized by the (activity-based) equilibrium constant K_{a^*}. K_{a^*} of the ALAT-reaction is obtained by the activities a_i^* of the reactants and products (Eq. (1)) and is constant for constant temperature and pressure.

$$K_{a^*} = \frac{a^*_{pyruvate} \cdot a^*_{L-glutamate}}{a^*_{L-alanine} \cdot a^*_{2-oxoglutarate}} \quad (1)$$

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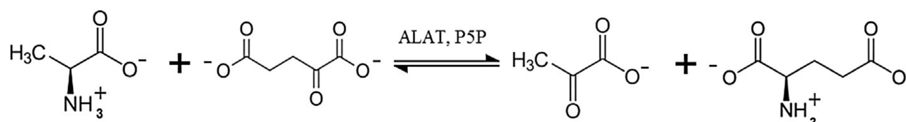

Fig. 1. ALAT-reaction of L-alanine and 2-oxoglutarate to L-glutamate and pyruvate, shown as their ionic species at pH 7 and in the presence of coenzyme P5P.

The activity a_i^* can be written as the product of the molality and the activity coefficient $\gamma_i^{*,m}$ of reacting agent i (Eq. (2)).

$$a_i^* = m_i \cdot \gamma_i^{*,m} \tag{2}$$

The standard state for $\gamma_i^{*,m}$ is the hypothetical ideal solution of reacting agent i which is defined as a one molal solution of the reacting agent i exhibiting the same interactions as at infinite dilution in the same medium [18]. In this work, the molality-based activity-coefficient $\gamma_i^{*,m}$ is used together with equilibrium molality m_i (moles of component i per kg water) as concentration unit to obtain the activity a_i^* of reactants and products of the ALAT-reaction.

Similarly to the activity a_i^*, the thermodynamic equilibrium constant K_{a^*} is obtained as the product of two quantities, K_m and $K_{\gamma^{*,m}}$, both of which being concentration-dependent (Eq. (3)).

$$K_{a^*} = K_m \cdot K_{\gamma^{*,m}} \tag{3}$$

The apparent equilibrium constant K_m of the ALAT-reaction is defined as

$$K_m = \frac{m_{pyruvate}^{equilibrium} \cdot m_{L-glutamate}^{equilibrium}}{m_{L-alanine}^{equilibrium} \cdot m_{2-oxoglutarate}^{equilibrium}} \tag{4}$$

where $m^{equilibrium}$ are the molalities of reactants and products at reaction equilibrium. $K_{\gamma^{*,m}}$ values were calculated from the molality-based reactant and product activity coefficients (Eq. (5)).

$$K_{\gamma^{*,m}} = \frac{\gamma_{pyruvate}^{*,m} \cdot \gamma_{L-glutamate}^{*,m}}{\gamma_{L-alanine}^{*,m} \cdot \gamma_{2-oxoglutarate}^{*,m}} \tag{5}$$

Since the availability of activity-coefficient data is generally scarce, activity coefficients and $K_{\gamma^{*,m}}$ are often assumed to be unity [19–22].

In previous works, K_m values of several aminotransferase reactions have been reported. Tufvesson et al. [19] measured the K_m value for six different aminotransferase reactions. These K_m values were within a range from 1.35 to 30. Bemmelen et al. [23] determined a K_m value of 0.04 for the formation of γ-aminobutyric acid from succinic semialdehyde by aminotransferase. Goldberg et al. [1,2,24,25] reported K_m values for 22 different aminotransferase reactions, whereof a major part of these K_m values were within a range from 0.1 to 10. These works emphasize that the yields of aminotransferase reactions are strongly limited by thermodynamic equilibrium. However, in these works the activity coefficients of the reacting agents and therewith $K_{\gamma^{*,m}}$ were not taken into account.

K_m values of the ALAT-reaction have already been reported from 1940 until today. The K_m values reported in different works vary between 0.44 and 1.0, even at constant temperature [1,20,26–28]. Besides experimental difficulties in analytics and unknown substance purity in the early 1940s, one reason for non-constant K_m values is the fact that $K_{\gamma_i^{*,m}}$ is not constant for different compositions of the reaction media or different reaction conditions, e.g. pH. Since the thermodynamic equilibrium constant K_{a^*} is constant at constant temperature and pressure, K_m is increasing with decreasing $K_{\gamma_i^{*,m}}$ and vice versa according to Eq. (3).

Previous investigations of thermodynamics of biological reactions in general have shown that neglecting $K_{\gamma^{*,m}}$ may cause inconsistent statements about reaction equilibria [29–31]. Indeed, knowledge about $K_{\gamma^{*,m}}$ values is required to describe reaction equilibria and related properties, e.g. the maximum yield of a reaction.

Activity coefficients (and therewith $K_{\gamma^{*,m}}$) of biological components are accessible experimentally (e.g. osmometry [32,33]) or by thermodynamic models. Activity-coefficient models (g^E models) and equations of state have been used so far for the quantitative thermodynamic modeling of biological solutions. However, g^E models are often not reliable when applied for predicting the thermodynamic properties of biological solutions. Moreover, they require a huge number of interaction parameters [34,35]. In contrast, SAFT-based models are promising tools for modeling activity coefficients in biological solutions as they consider specific interactions due to hydrogen bonding or charges [31,38,54].

Applications of ePC-SAFT showed that thermodynamic properties (e.g. activity coefficients) and phase equilibria in multicomponent biological systems containing electrolytes can be modeled and predicted almost quantitatively [39–43]. Hoffman et al. measured and modeled the influence of the reacting agents' concentrations on $K_{\gamma^{*,m}}$ for the isomerization of glucose-6-phosphate and for the hydrolysis of methyl ferulate [29,30]. They found that $K_{\gamma^{*,m}}$ for the reaction of glucose-6-phosphate to fructose-6-phosphate decreased from unity to 0.7 upon increasing the equilibrium concentration of glucose-6-phosphate from 0 to 0.030 mol kg^{-1} [30]. Thus, despite very low concentrations, activity coefficients of reactants and products were shown to distinctly deviate from unity. A quite similar behavior was found for the enzyme-catalyzed hydrolysis of methyl ferulate [29]. The $K_{\gamma^{*,m}}$ values predicted with ePC-SAFT were found to be in very good agreement with the experimental data in both cases [29,30].

In this work, the reaction equilibrium of the ALAT-reaction is investigated from thermodynamic-point-of-view. The goal is to determine the thermodynamic equilibrium constant K_{a^*} by combining reaction-equilibrium experiments and ePC-SAFT modeled activity coefficients according to Eq. (3). The reaction is considered at different reacting-agent concentrations, pH values, and temperatures. Further, ePC-SAFT is used to model the activity coefficients of the reactants (L-alanine, 2-oxoglutarate) and the products (L-glutamate, pyruvate) to predict equilibrium molalities of all reacting agents (and therewith K_m values) in the multi-component reaction mixtures at different reaction conditions.

2. Materials and methods

2.1. Materials and solution preparation

All the substances used in this work are listed in Table 1 and were used as obtained without further purification. The equilibrium reactions were catalyzed by ALAT (EC 2.6.1.2; CAS # 9000-86-6) isolated from porcine heart (Alfa Aesar, Karlsruhe, Germany). Millipore water was used for all aqueous solutions. All solutions were prepared gravimetrically by using a Sartorius CPA324S balance (Sartorius, Göttingen, Germany) with an accuracy of $\pm 10^{-4}$ g. The pH of the samples were measured with the pH-meter GMH

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