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Thermodynamics of phenylacetamides synthesis: Linear free energy relationship with the pK of amine

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ABSTRACT

The effective equilibrium constants K'_C expressed through the total concentrations of the reagents for the synthesis of N-phenylacetyl-derivatives in aqueous medium from phenylacetic acid and various primary amino compounds have been determined with penicillin acylase as a catalyst. Broad specificity of penicillin acylase (EC 3.5.1.11) to amino components made possible to investigate the acylation of primary amines with different structures and physicochemical properties. Analysis of different components of the effective standard Gibbs energy change $\Delta G_C^{o'}$ has revealed favorable thermodynamics for the synthesis of phenylacetamides from unionized substrates forms, however the ionization of reactants carboxy and amino groups in aqueous solutions pushes the equilibrium position to the hydrolysis especially in case of highly basic amines. A linear correlation between the standard Gibbs energy change for amide bond formation from the unionized reagents species and the basicity of amino group was observed: $\Delta G_T^o = -3.56 \cdot \mathrm{pK}_{\mathrm{amine}} + 7.71\,(\mathrm{kJ/mol})$. The established linear free energy relationship (LFER) allows to predict the thermodynamic parameters for direct condensation of phenylacetic acid with any amine of known pK. Condensation of phenylacetic acid and amines with pK value within 1.5–8.5 was shown to be thermodynamically favorable in homogeneous aqueous solution.

1. Introduction

Biochemical equilibrium of enzyme-catalyzed transformations is one of the major factors which should be considered at rational bioprocess design. Nevertheless the state of the art in the area of thermodynamics of amide and peptide bond formation is rather limited and controversial [1,2]. The causes of this are mainly related to experimental problems. Slow attainment of equilibrium, difficulties to determine low equilibrium concentrations of products at some syntheses as well as side reactions stipulate application of indirect methods and non-standard experimental conditions. As a result the experimentally determined equilibrium constants are effective (apparent), and in many cases reliable only for given conditions.

The ionization of reagents in aqueous medium affects the equilibrium of condensation reactions more than other physical factors. In the early 60s Carpenter had introduced a "nonionized compound convention" by expressing the reactants concentrations in terms of all existing forms including their uncharged species [3]. This allowed to consider the contribution of the ionization of reagents and to compare the equilibrium constants of a wide range of reactions carried out in aqueous solutions at different pH. As a consequence it was found that the Gibbs energy components responsible for conversion of the nonionized reactants forms differ substantially in case of amide and ester hydrolysis, but vary within a narrow range for peptide and amide hydrolysis [3]. The following studies showed this convention to be insensitive to the nature of acyl moiety [4–6], except for formyl amides [7]. Other authors have revealed that the equilibrium constants referred to the neutral reactants species differ largely, and, moreover, with some exceptions correlate with the basicity of amino compounds [7–9]. However, recently most of the earlier published experimental data have been recalculated in terms of the "non-ionized compound convention", and a common, independent on the nature of reagents equilibrium constant K_T^o, $10^{3.6}$ M (ΔG_T^o, -20.5 kJ/mol) for hydrolysis of amide and peptide bonds has been established [10]. This discrepancy is quite a principal question as there is a need to set up a common model for quantitative estimation of the equilibrium parameters for amide bond hydrolysis/synthesis based on the physicochemical properties of reagents.

Abbreviations: IS, ionic strength; LFER, linear free energy relationship; PA, penicillin acylase.

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$$SH + Nu \underset{}{\overset{K_T}{\rightleftharpoons}} P + H_2O \qquad \text{(I)}$$

$$SH \overset{K_{SH}}{\rightleftharpoons} H^+ + S^- \qquad \text{(I-a)}$$

$$HNu^+ \overset{K_{HNu+}}{\rightleftharpoons} H^+ + Nu \qquad \text{(I-b)}$$

Scheme 1. Direct condensation of an amine (Nu) with a carboxylic acid (SH) is characterized by the thermodynamic equilibrium constant K_T (I). The reaction (I) is complicated by ionization of the reagents (I-a and I-b); K_{SH} and K_{HNu^+} are the dissociation constants for reacting carboxylic and amino groups.

In this paper the thermodynamics of amide bond formation between phenylacetic acid and various primary amino compounds has been studied. The effective equilibrium constants for the synthesis of N-phenylacetyl-derivatives in aqueous medium have been determined with penicillin acylase EC 3.5.1.11 (PA) as a catalyst. PA-catalyzed acylations in aqueous medium are widely used for antibiotic synthesis [11] as well as for chiral resolutions [12–14], therefore thermodynamic characterization of these biocatalytic conversions is very important for process optimization.

2. Equations

The thermodynamic equilibrium constant for direct condensation of unionized forms of a carboxylic acid and an amine (reaction I, Scheme 1) is expressed in terms of the activities of reaction components:

$$K_T^o = \frac{a_P \cdot a_W}{a_{SH} \cdot a_{Nu}} \qquad (1)$$

If we take into account the ionization reactions I-a and I-b (Scheme 1) as well as the water activity (a_W) being equal to 1, the equilibrium constant can be represented as:

$$K_T^o = \frac{K_{SH}^o}{K_{HNu^+}^o} \cdot \frac{a_P}{a_{S^-} \cdot a_{HNu^+}}$$

Here we consider the ideal-dilute systems only:

$$K_T^o = \frac{K_{SH}^o}{K_{HNu^+}^o} \cdot \frac{1}{\alpha_{S^-} \cdot \alpha_{HNu^+}} \cdot \frac{c_P}{c_S \cdot c_{Nu}} \qquad (2)$$

Hence the experimentally determined and expressed through the total concentrations of the reagents effective equilibrium constant makes up

$$\begin{aligned}
K'_C &= K_T^o \cdot \frac{K_{HNu^+}^o}{K_{SH}^o} \cdot \alpha_{S^-} \cdot \alpha_{HNu^+} \\
K'_C &= K_T^o \cdot \frac{[H^+] \cdot [Nu]}{[HNu^+]} \cdot \frac{[SH]}{[H^+] \cdot [S^-]} \cdot \frac{1}{1 + ([SH]/[S^-])} \cdot \frac{1}{1 + ([Nu]/[HNu^+])} \\
K'_C &= K_T^o \cdot \frac{1}{([S^-]/[SH]) + 1} \cdot \frac{1}{([HNu^+]/[Nu]) + 1} \\
K'_C &= K_T^o \cdot \frac{1}{1 + (K_{SH}^o/[H^+])} \cdot \frac{1}{1 + ([H^+]/[K_{HNu^+}^o])} \\
K'_C &= K_T^o \cdot \frac{1}{(1 + 10^{(pH - pK_{SH})}) \cdot (1 + 10^{(pK_{HNu^+} - pH)})}
\end{aligned} \qquad (3)$$

Assuming designations

$$\Delta G_{ion}^{o'} = R \cdot T \cdot \ln\left[(1 + 10^{(pH - pK_{SH})}) \cdot (1 + 10^{(pK_{HNu^+} - pH)})\right] \qquad (4)$$

Eq. (3) can be expressed in terms of Gibbs energy change:

$$\Delta G_C^{o'} = \Delta G_T^o + \Delta G_{ion}^{o'} \qquad (5)$$

This equation combines true thermodynamic and effective equilibrium parameters of amide bond formation. Evidently, the pH-independent component of standard Gibbs potential ΔG_T^o characterizes the thermodynamic equilibrium of the reaction for unionized species; the contribution of ionization $\Delta G_{ion}^{o'}$ takes into account the acid–base properties and reflects Gibbs energy change related to the neutralization of the reagents charged functional groups at given conditions, i.e. protonation of carboxylic group and deprotonation of amino group in aqueous solution.

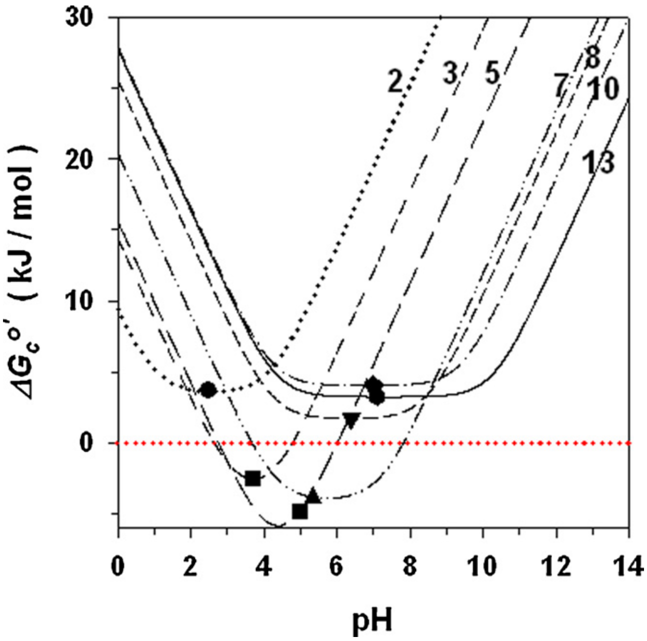

Fig. 1. Thermodynamics of direct condensation of phenylacetic acid and amines (Scheme 2, experimental conditions: 298 K, IS 0.1 M. Theoretical curves were calculated according to Eqs. (4) and (5) using the experimental data from Tables 1 and 2.

3. Results and discussion

The effective equilibrium constants K'_C for the synthesis of N-phenylacetyl-derivatives from phenylacetic acid and different primary amino compounds in aqueous medium have been determined at 298 K and ionic strength (IS) 0.1 M using PA from *Escherichia coli* as a catalyst. Broad specificity of PA to amino components made possible to investigate the equilibrium parameters of acylation of various primary amines (Scheme 2), which differ largely by structure and basicity of their amino group. The effective equilibrium constants K'_C have been determined by the most rigorous method, when the equilibrium position is attained from both sides: the side of enzymatic synthesis and the side of enzymatic hydrolysis (Table 1).

The values of the thermodynamic equilibrium constant K_T^o and the corresponding standard Gibbs energy change ΔG_T^o related to direct condensation of unionized reagents forms for each of the investigated reactions were calculated on the basis of the experimental data at given pH according to Eqs. (3)–(5) (Table 2). When considering amines containing additional ionogenic group the corresponding microconstants for their zwitterions were taken into account according to the procedure described earlier (Table 3) [10,15]. Further, based on the determined intrinsic value ΔG_T^o of each condensation reaction the pH-profile of the effective Gibbs energy change $\Delta G_C^{o'}$ has been calculated. According to the obtained profiles (Fig. 1) the condensation of phenylacetic acid with many of the amino compounds appears to be thermodynamically unfavorable.

It is interesting to consider how much each of the both components of $\Delta G_C^{o'}$ (according to Eq. (5) depends on the nature of amino