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CHEMICAL THERMODYNAMICS AND THERMOCHEMISTRY

Thermodynamical Characteristics of the Reaction of Pyridoxal-5'-Phosphate with L-Amino Acids in Aqueous Buffer Solution

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Abstract—The reaction of pyridoxal-5'-phosphate with L-isomers of alanine, lysine, arginine, aspartic acid, glutamic acid, and glycine in phosphate buffer solution was studied by absorption spectroscopy and the calorimetry of dissolution at physiological acidity of the medium (pH 7.35). The formation constants of Schiff bases during reactions and changes in Gibbs energy, enthalpy, and entropy were determined. It was shown that the formation constant of the Schiff base and its spectral properties depend on the nature of the bound amino acid. The progress of the reaction with a majority of amino acids is governed by the entropy factor due to the predominant role of the dehydration effect of the reaction center of amino acids during chemical reactions. The intramolecular electrostatic interaction of an ionized phosphate group with the positively charged amino group on the end of the chain of amino acid residue stabilizes the Schiff bases formed by lysine and arginine. The extinction coefficient of the base, equilibrium constant, and the exothermic effect of the reaction then increase. The excess negative charge on the end of the chain of amino acid residues of aspartic and glutamic acids destabilizes the molecule of the Schiff base. In this case, the equilibrium constant decreases and the endothermic effect of the reaction increases.

Keywords: solutions, amino acids, UV spectroscopy, calorimetry, Schiff bases.

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INTRODUCTION

In many processes of the metabolism of amino acids, pyridoxal-5'-phosphate (POP) serves as a coenzyme [1]. It is known that the product of reaction with amino acids is a Schiff base [1–3]. The effectiveness of binding amino acids by pyridoxal phosphate coenzyme depends on the structure of the amino acid molecules and their state in solution. The chemical processes with the participation of amino acids proceeds under the strong influence of the acid–base equilibrium in the system. The purpose of this study was to obtain the thermodynamical characteristics of the reaction of pyridoxal-5'-phosphate with various amino acids in aqueous buffer solution at physiological acidity of the medium pH 7.35. Under these conditions, the predominant ionic forms of the existence of the studied amino acids differ substantially: glycine and alanine are zwitterions, aspartic and glutamic acid are anions, and lysine and arginine are cations [4]. Therefore, at constant acidity of the medium, the contribution from the electrostatic interactions of a receptor with various amino acid ions should differ substantially in the order of amino acids, determining the hierarchy of the "recognition" of the given substrates in accordance with their charge distribution.

EXPERIMENTAL

Electronic absorption spectra were obtained on a Specord M-40 two-beam spectrophotometer in the range of wavelengths from 337 to 600 nm. The studied solutions were placed in quartz cells 1×1 cm in size. All measurements were performed at 293 ± 0.3 K. The reaction between amino acids and coenzyme took place in a buffer solution containing monobasic sodium phosphate NaH_2PO_4 (0.04165 M) and dibasic sodium phosphate Na_2HPO_4 (0.20492 M), pH 7.35. The use of buffer solution maintained the constant acidity of the medium and the stability of the ionic forms of reagents during reaction. The measurements of dissolution enthalpies of amino acids in buffer solution and analogous solution with the addition of POP were performed on a calorimeter with an isothermal shell at 293 ± 0.005 K. The technical characteristics of calorimeter and the technique of measurements were described in [5]. The error in measuring the heat effect was 1×10^{-2} J.

During spectral studies, the concentrations of reagents were 4×10^{-5} mol/l for POP and up to 1.8×10^{-3} mol/l for amino acids; during calorimetric investigations, they were 1×10^{-3} and 1×10^{-2} mol/kg, respectively. The mean values of dissolution enthalpy

$\Delta_{sol}H$ from 4–5 measurements were used as the standard dissolution enthalpy$\Delta_{sol}H°$. L-isomers of amino acids (all Sigma–Aldrich, 98–99% purity) and pyridoxal-5'-phosphate (Sigma–Aldrich, 98%) were dried prior to the experiments for 48 h under vacuum and used without further purification. Concentrations of substances in the solution were calculated by test portions.

RESULTS AND DISCUSSION

In electronic absorption spectra of POP in aqueous buffer solution at pH 7.35, the absorption maximum is observed at 387 nm with an extinction coefficient of 6250 $cm^{-1}\,l\,mol^{-1}$. This band could be related to the absorption of a bipolar ion or anion of POP [3]. With an addition of an amino acid, the band widens and undergoes a bathochromic shift. Changes in the spectrum indicate the formation of a Schiff base [2]. The progress of the reaction between the amino acid and POP was observed by changes in the differential electronic absorption spectrum of POP upon various additions of amino acid, recorded relative to the solution of a single POP with a same concentration. A reduction in the intensity of absorption at 375 nm and a rise in the intensity of absorption of the Schiff base at 430–436 nm are observed in the spectrum upon the binding of the amino acid (Fig. 1). The molar absorption coefficient in the band maximum of the Schiff base was found from the linear correlation of changes in optical densities and the molar extinction coefficients for two bands of the differential spectrum: the absorption of POP at 375 nm (ΔD^{375}, ε^{375}) and the absorption of the Schiff base at 430–436 nm (ΔD^{ShB}, ε^{ShB}).

$$\Delta D^{ShB}/\Delta D^{375} = \varepsilon^{ShB}/\varepsilon^{375}. \quad (1)$$

The change in values ΔD^{ShB} and ΔD^{375} at various concentrations of amino acid are related by a linear correlation, as is shown in Fig. 2 for solutions with the addition of arginine. For the system under consideration, the ratio $\Delta D^{430.5}/\Delta D^{375}$ was 2.46 ± 0.15. The extinction coefficient in the band maximum of the Schiff base formed by arginine was found using Eq. (1) and approximation $\varepsilon^{375} \approx \varepsilon^{387} = 6250\ cm^{-1}\,l\,mol^{-1}$: $\varepsilon^{430.5} = 17500\ cm^{-1}\,l\,mol^{-1}$. The resulting $\varepsilon^{430.5}$ was used in the spectrophotometric determination of the equilibrium concentration of the Schiff base [ShB] by the Buger–Lambert–Beer equation

$$\Delta D^{430.5} = \varepsilon^{430.5} l [ShB]. \quad (2)$$

As follows from the obtained spectrophotometrical data, the equilibrium concentration of the Schiff base increases from 0.8×10^{-6} to 4.3×10^{-6} mol l^{-1} when the initial concentration of arginine is increased from 2.0×10^{-4} to 18.2×10^{-4} mol l^{-1}. The method for determining the equilibrium concentrations of the Schiff bases with other amino acids is analogous to the procedures described above. The formation constants of the Schiff bases were calculated on the basis of equilibrium concentrations of Schiff bases found from spectrophotometric data:

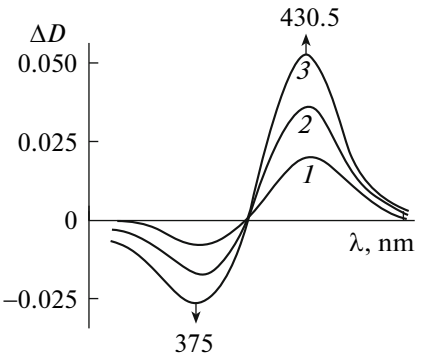


Fig. 1. Differential electronic absorption spectra of 3.99×10^{-5} M aqueous solution of pyridoxal-5'-phosphate in phosphate buffer at pH 7.35 and 293 K in the presence of various additives of L-arginine: (*1*) 4.0×10^{-4}, (*2*) 10.1×10^{-4}, and (*3*) 18.2×10^{-4} mol l^{-1}.

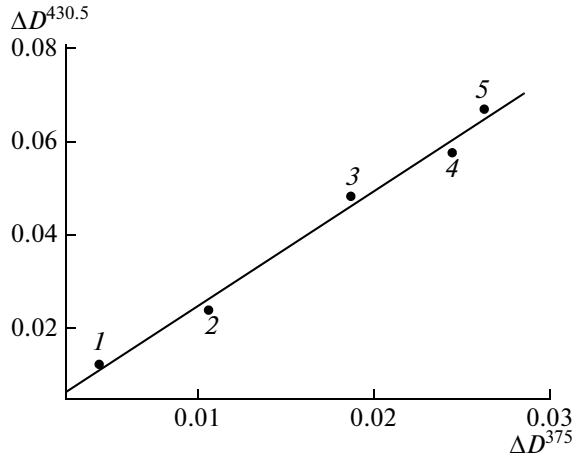


Fig. 2. Interdependence of changes in optical density at wavelengths of 430.5 and 375 nm in the differential spectrum of pyridoxal-5'-phosphate in phosphate buffer at pH 7.35 and 293 K in the presence of various additives of L-arginine: (*1*) 0.0002, (*2*) 0.0004, (*3*) 0.001, (*4*) 0.0014, and (*5*) 0.0018 mol l^{-1}.

$$R'CH{=}O + NH_2R'' \leftrightarrow R'CH{=}NR'' + H_2O. \quad (3)$$

The total equilibrium concentrations for all forms and ionic states of the reagents and products of the reaction were used in the expression for formation constant:

$$K = K'[H_2O] = [ShB]/[POP][AA], \quad (4)$$

where [ShB], [POP], and [AA] are the equilibrium concentrations of the Schiff base, pyridoxal-5'-phosphate, and amino acid, respectively. Equilibrium concentrations [ShB] were determined from spectrophotometric data by Eqs. (1) and (2). Equilibrium con-

Table 1. Positions of the absorption maximum, extinction coefficients (ε, cm^{-1} l mol^{-1}), and formation constants of the Schiff bases (K, l/mol) with various amino acids

Amino acid	λ, nm	ε	$\log K \pm 0.05$
Glycine	430.5	8160	1.78
L-Alanine	436.5	3430	1.74
L-Lysine	430.0	17860	1.83
L-Arginine	430.5	17500	1.92
L-Glutamic acid	434.0	8590	1.61
L-Aspartic acid	434.0	6250	1.48

centrations [POP] and [AA] were calculated using the expressions

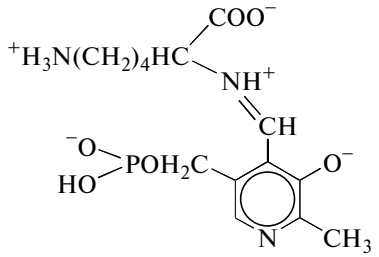
$$[POP] = c^0_{POP} - [ShB],$$

$$[AA] = c^0_{AA} - [ShB],$$

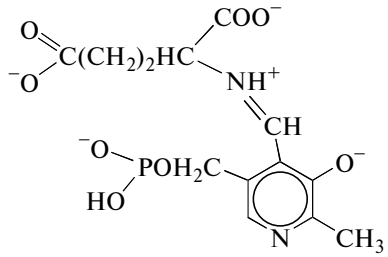
where c^0_{POP} and c^0_{AA} are the initial molar concentrations of the starting compounds. The calculated formation constants were averaged for the studied concentration range of amino acids from 2.0×10^{-4} to 1.8×10^{-3} mol l^{-1}. The resulting spectral characteristics and averaged formation constants of the Schiff bases obtained with various amino acids are given in Table 1.

The data of Table 1 confirm that the formation constant of a Schiff base and its spectral properties depend on the nature of the bound amino acid. As follows from a comparison of the data on glycine and alanine, the breaking of the conjugation of the aldimine group CH=N with aromatic oxypyridine chromophore under the effect of the more branched alanyl residue leads to a rise in wavelength in the absorption maximum and a reduction in the molar absorption coefficient of the Schiff base. The binding constant tends to decrease. Residues of aspartic and glutamic acids having even longer chains break the conjugation even more strongly. The binding constants of these amino acids are considerably lower than those for glycine and alanine. The positive inductive effect of the additional carboxylic group on the ends of the chains of amino acid residues manifests itself in an increase in the extinction coefficient of the Schiff bases with their participation relative to the base formed by alanine.

Maximal binding with the Schiff base is observed for arginine and lysine, the predominant form of which is the cation at pH 7.35. It is evident that the intramolecular electrostatic attraction between the positively charged amino group on the end of the amino acid residue and the negatively charged phosphate group stabilizes the molecules of the Schiff base, as can be seen from Scheme (I) for a Schiff base with the contribution of lysine. The degree of conjugation of the aldimine and oxypyridine group increases due to the reduction in the degree of freedom of the amino acid residue. The rapid growth of the molar absorption coefficients of the Schiff bases formed by lysine and arginine relative to other bases confirms this. The excess negative charge in the side chain of aspartic and glutamic acid reduces the yield from the reaction of the formation of the Schiff base. Electrostatic repulsion between the negatively charged carboxylic and ionized phosphate group in the molecules of the forming Schiff bases is an additional factor in the breaking of the conjugation of the aldimine group with the oxypyridine ring (Scheme (II) for a Schiff base with the participation of glutamic acid).



(I)



(II)

It is interesting to note the correlation between the logarithm of the formation constant of the Schiff base and the hydration enthalpy of the amino acids (Fig. 3). The hydration enthalpies of the studied amino acids were calculated from the values of the dissolution enthalpies [6]. The sublimation enthalpies of glycine and alanine are taken from [7]. The sublimation enthalpies of the L-isomers of lysine, arginine, aspartic, and glutamic acid were estimated on the basis of the correlation between the specific-volume sublimation enthalpy of a number of compounds (amino acids and amides) with the sum of the bond lengths in the polyatomic molecule [8].

The dissolution enthalpies of crystalline amino acids in the buffer solution upon the addition of pyridoxal-5'-phosphate were determined by calorimetry ($\Delta_{sol}H_2$). The measured value includes the contribution from amino acid dissolution, determined in a blank experiment in the absence of coenzyme

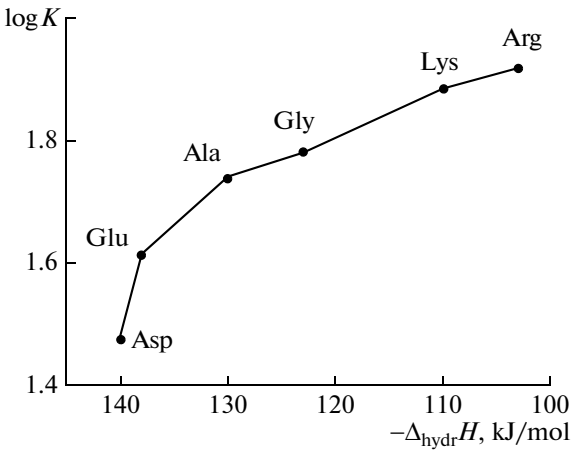


Fig. 3. Correlation of the formation constants of the Schiff bases in reaction (3) with the hydration enthalpies of L-amino acids.

($\Delta_{sol}H_1$), and the effect of the reaction of coenzyme with amino acid,

$$\Delta_{sol}H_2 = \Delta_{sol}H_1 + \alpha_r\Delta_rH, \quad (5)$$

where α_r is the degree of conversion of the amino acid, and Δ_rH is the change in enthalpy during the reaction, as calculated for the mole of the product, the Schiff base. The degrees of conversion of the amino acids under the given conditions were calculated from the formation constant K of the Schiff bases (Table 1). Combining the values $\Delta_{sol}H_2$, $\Delta_{sol}H_1$, and α_r allows us to calculate the change in enthalpy for the reaction of the formation of the Schiff base (Δ_rH) by Eq. (5).

The calculations for the change in Gibbs energy and entropy (in molar scale) during a reaction are performed according to the known correlations from the formation constants of the Schiff bases and the combination of the Gibbs energy and enthalpy of the reaction:

$$\Delta_rG = -2.303RT\log K, \quad (6)$$

$$\Delta_rG = \Delta_rH - T\Delta_rS. \quad (7)$$

All of the determined thermodynamic values are given in Table 2; i.e., the complete thermodynamic characteristics of the reaction between the coenzyme and various amino acids are given at physiological acidity of the medium (pH 7.35). As follows from Table 2, the progress of the interaction of the coenzyme with the majority of amino acids is governed by the entropy factor, due to the predominant role of the dehydration effect of the amino acid's reaction center during the chemical reaction. The contribution from enthalpy in the cases of alanine, glutamic, and aspartic acid prevents the interaction from progressing. Two exceptions are lysine and arginine containing positively charged amino groups in the side chain of the amino acid. In reactions with their participation, the exothermic enthalpy effect dominates over the entropy contribution to the change in Gibbs energy.

CONCLUSIONS

Thermodynamical analysis thus shows that the discrimination of amino acids of a given series in a reaction with coenzyme pyridoxal-5'-phosphate is determined by the principles of dehydration of their reaction groups. This conclusion is supported by the correlation between the formation constants of the Schiff bases and the hydration enthalpies of the amino acids, and the domination of the positive contribution from entropy to the change in Gibbs energy over the enthalpy of the reaction with the participation of most amino acids.

Intramolecular electrostatic interaction between ionized groups in the molecules of the Schiff base is an additional factor determining the stability of the products of the reaction between the coenzyme and amino acid. The reaction between an ionized phosphate group and the positively charged amino group on the end of a chain of amino acid residue stabilizes the Schiff bases formed by lysine and arginine. The increase in the extinction coefficient, equilibrium constant, and the exothermic effect of reaction confirms this. The reaction of the ionized phosphate group with the negatively charged carboxyl group on the end of a chain of amino acid residue reduces the stability of the Schiff bases formed by aspartic acid and glutamic acid. The equilibrium constant declines and the endothermic effect of the reaction increases.

Table 2. Thermodynamic characteristics of the reactions (kJ/mol) of pyridoxal-5'-phosphate with various L-amino acids in phosphate buffer at pH 7.35 and 293.15 K

Amino acid	$\Delta_{sol}H_1$	$\Delta_{sol}H_2$	α_r	$-\Delta_rG$	Δ_rH	$T\Delta_rS$
Glycine	13.89 ± 0.02	13.81 ± 0.02	0.0366	10.15 ± 0.3	−2.2 ± 0.8	7.95
L-Alanine	7.23 ± 0.02	7.77 ± 0.02	0.0346	9.9 ± 0.3	15.6 ± 0.9	25.5
L-Lysine	−28.90 ± 0.02	−29.29 ± 0.02	0.0424	10.8 ± 0.3	−9.2 ± 0.7	1.6
L-Arginine	−33.04 ± 0.02	−33.38 ± 0.02	0.0442	11.0 ± 0.3	−7.7 ± 0.7	3.3
L-Glutamic acid	24.80 ± 0.02	25.15 ± 0.02	0.0285	9.2 ± 0.3	12.3 ± 1.0	21.5
L-Aspartic acid	24.70 ± 0.02	24.90 ± 0.02	0.0227	8.4 ± 0.3	10.6 ± 1.3	19.0

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