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Kinetic and Mechanism of Reactions of L-α-Glutamic Acid and L-Glutamine with Pyridoxal

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Abstract—The kinetics and mechanisms of condensation of pyridoxal with L-α-glutamic acid and L-glutamine were studied by UV spectroscopy and polarimetry. L-α-Glutamic acid reacts with pyridoxal to form a Schiff base whose subsequent hydrolysis gives rise to pyridoxamine and α-ketoglutaric acid. The reaction of L-glutamine with pyridoxal involves the γ-NH_2 group and affords a Schiff base whose subsequent hydrolysis gives rise to pyridoxamine and L-α-glutamic acid.

Keywords: pyridoxal, amino acids, Schiff bases, glutamine, kinetics

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Glutamic acid and glutamine (2,5-diamino-5-oxopentanoic acid) are actively involved in nitrogen transfer reactions. The amide nitrogen of glutamine takes part in many biochemical processes leading to formation of glucosamine [1], purines, *p*-amino-benzoate, and hystidine. Glutamic acid and glutamine play an important role in the elimination of nitrogen from organic compounds. The transamination reaction is the initial step in the catabolism of excess amino acids.

In our previous works [2–7] we described chemical transformations of a series of amino acids and amines under the action of pyridoxal and pyridoxal-5'-phosphate. Kinetic study and isolation of intermediate and fina reaction products showed that the formation of Schiff bases and their subsequent transformations occur in three stages. The rate of each of these stages depends on the structure of initial, intermediate, and final products, pH of the medium, solvent, temperature, and steric and thermodynamic factors.

Kinetic and mechanistic study of the reactions of pyridoxal with L-α-glutamic acid and glutamine, analysis of kinetic curves, and calculation of rate constants gave unexpected results. The rates of both reversible and irreversible pyridoxal reactions leading to Schiff base formation in the case of L-glutamine [pK_a (α-NH_2) = 9.13; k = 0.0537 min^{-1}] is ~4 times higher than in the case of L-α-glutamic acid [pK_a (α-NH_2) = 9.67; k = 0.0142 min^{-1}], even though the α-NH_2 group in glutamine is less basic. Apparently, the rate of condensation of pyridoxal with L-α-glutamic acid is much affected, along with the basicity of its α-NH_2 group at the stages of addition to aldehyde and dehydration of amino alcohol, by steric factors (two COO^- groups) which hinder addition to alcohol and formation of amino alcohol. Evidence for this sugges-tion is provided by the condensation of L-2-amino-5-methoxy-5-oxopentanoic acid with pyridoxal (Fig. 1, curve *3*). Methylation of the γ-COOH groups in glutamic acid accelerates the reaction.

Glutamine has two nitrogen atoms which, depending on conditions (pH of the medium, solvent, temperature) can act as two competing nucleophilic centers. The γ-NH_2 group in glutamine (pK_a = 5.65) is less basic than the α-NH_2 group (pK_a = 9.13). Therefore, at first glance, at the stage of amino alcohol formation pyridoxal should prefer to react by the α-NH_2 group of glutamine. However, at the dehydration stage, the reverse situation takes place: Water is preferably eliminated from amino alcohol which is formed by the reaction of pyridoxal by the γ-NH_2 group of the amide fragment. Since the dehydration stage is a limiting stage, the contribution of the reaction of pyridoxal by the γ-NH_2 group of glutamine will exceed that of the

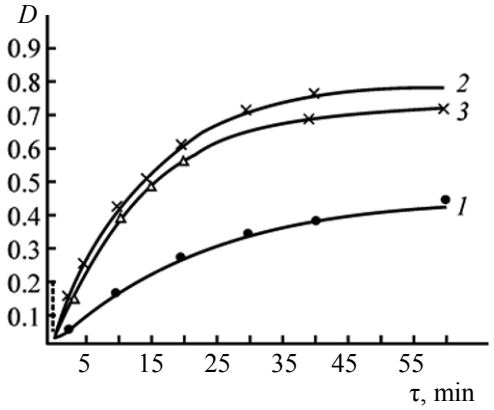


Fig. 1. Change of the optical densities of mixtures of 0.01 M solutions of pyridoxal hydrochloride and (*1*) L-α-glutamic acid, (*2*) L-glutamine, and (*3*) γ-methyl ester of L-α-glutamic acid with time at the stage of amino acid formation and dehydration (70% alcoholic aqueous buffer solutions, pH = 6.5, T = 20°C).

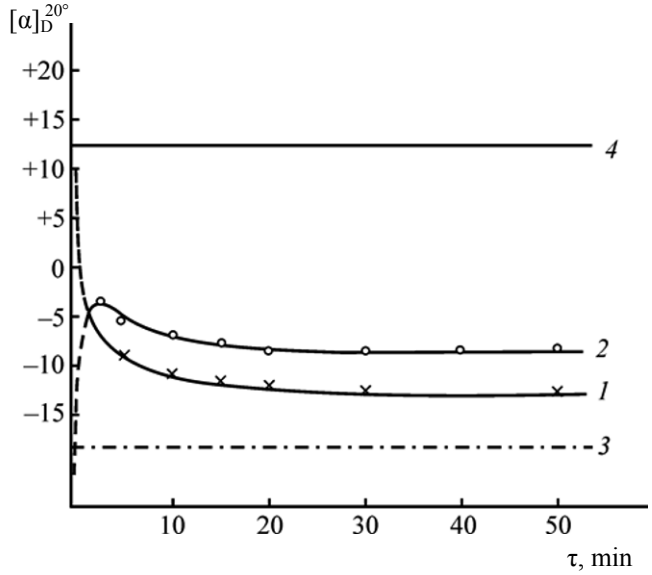


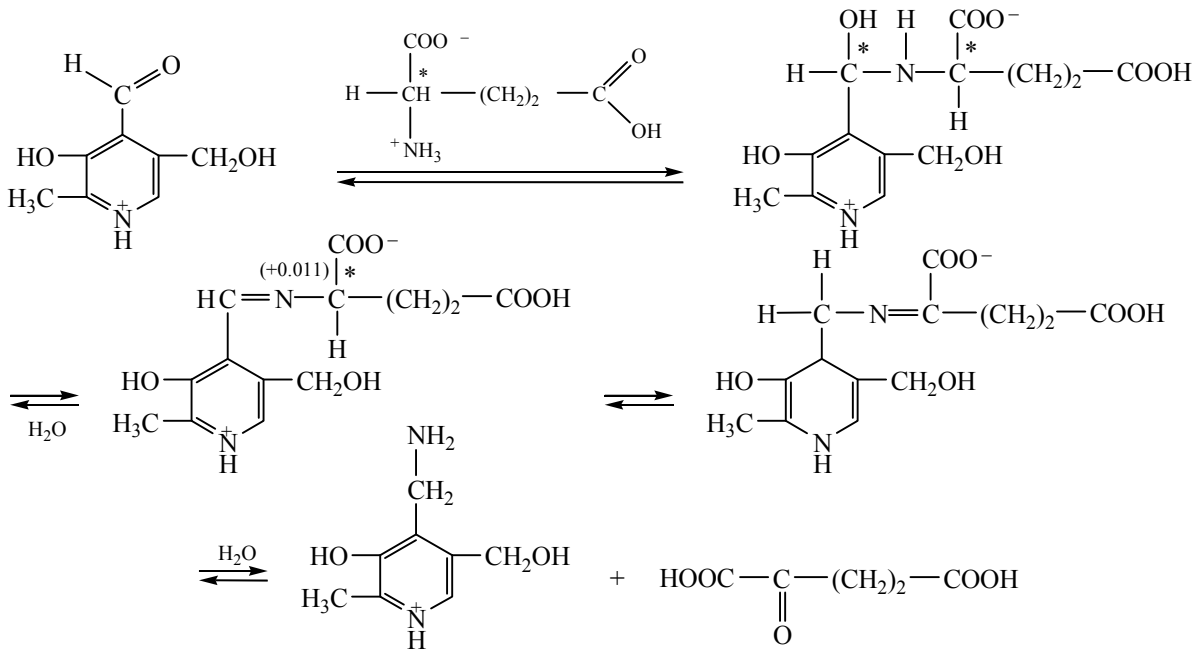
Fig. 2. Change of the specific rotation angles of 0.04 M solutions of pyridoxal hydrochloride with (*1*) L-α-glutamine, (*2*) L-α-glutamic acid with time at the stage of formation and dehydration of (*3*) amino alcohols, L-α-glutamic acid and (*4*) glutamine (70% alcoholic aqueous buffer solutions, pH = 6.35, T = 20°C).

reaction by the α-NH_2 group. To provide evidence for the suggested routes and schemes of the condensation of pyridoxal with glutamic acid and its amide, we performed a kinetic study of these reactions by means of polarimetry (Fig. 2). In the course of pyridoxal reaction with L-α-glutamic acid, the negative specific rotation angles were first decreased and then increased. Prolonged standing of a mixture of pyridoxal and L-α-glutamic acid in a solution gave pyridoxamine and α-ketoglutaric acid. The latter product was isolated and identified [elemental analysis, 2,4-dinitrophenylhydrazone (mp 200°C), negative ninhydrin test]. A different picture was observed in the reaction of pyridoxal with L-glutamine. At the addition stage, the positive specific rotation angle rapidly decreased until it became negative. At the second stage (dehydration), the negative specific rotation angle increased to a certain constant value. The fact that the specific rotation angle changed sign from positive to negative can be explained by the formation of a new chiral center at the stage of amino alcohol formation by the reaction of pyridoxal with the γ-NH_2 group of L-glutamine. The slow increase of the negative specific rotation angle can be explained in terms of hydrolysis of Schiff base and formation of pyridoxamine and L-α-glutamine acid which precipitated and was identified (elemental analysis, IR spectroscopy, and value of negative specific rotation).

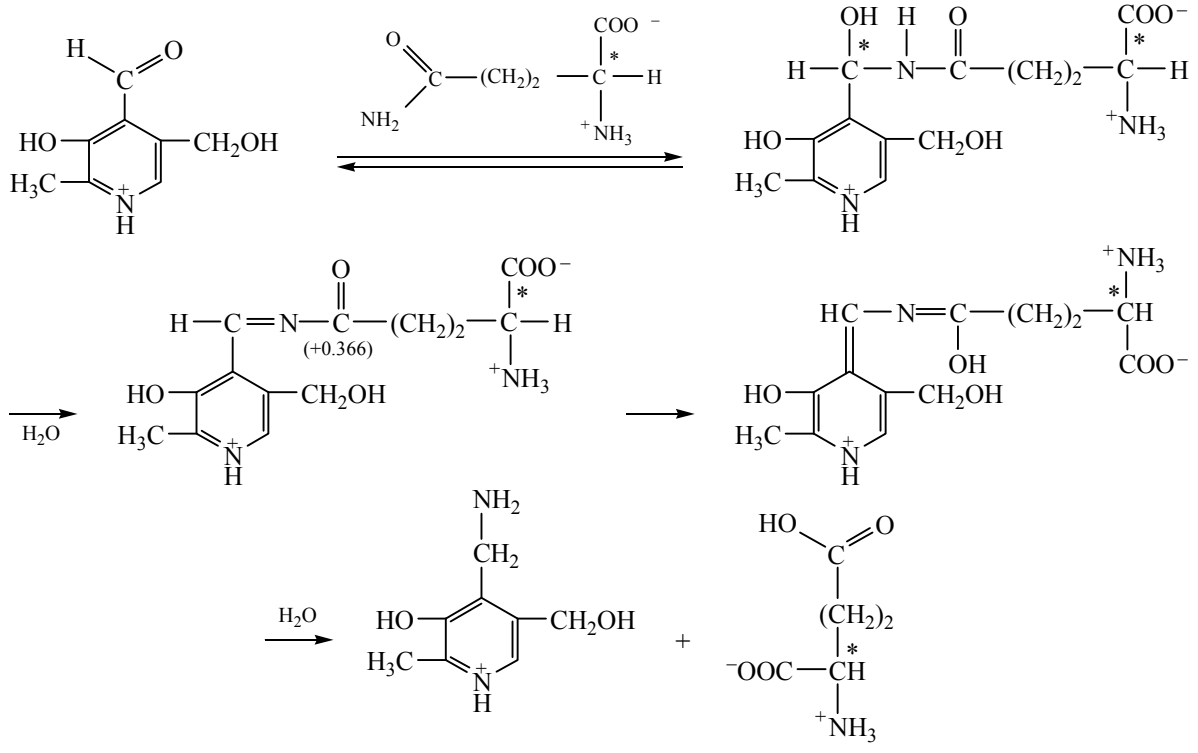
To find explanation for the resulting data, we have explored the possible structures of and bond lengths and atomic charges in the products of the reactions of pyridoxal with L-α-glutamic acid and L-glutamine by the α-NH_2 groups and with L-glutamine by the γ-NH_2 group, obtained by HyperChem [8] with energy and geometry optimization. It was shown that the reaction products of pyridoxal with L-α-glutamic acid and L-glutamine by the α-NH_2 group have similar structures and charges on nodal atoms. The reaction product of pyridoxal with L-glutamine by the γ-NH_2 group has quite different parameters.

The pyridine fragment in the Schiff bases and intermediate reaction products (amino alcohols) is planar. The OH group in the pyridine fragment is virtually coplanar to the pyridine ring, and the CH_2OH group deviates, in force of its nonlinearity, from the pyridine ring plane, i.e. this group can serve as a "probe" for the stereochemistry of the reaction products. The results of HyperChem calculations including geometry and energy optimization showed that the carbonyl group and azomethine fragments in pyridoxal and Schiff bases formed by condensation by

Scheme 1.



Scheme 2.



the α-NH_2 group are turned by ~90° and reside on the same side with respect to the "probe," whereas the OH group and amino acid fragment in amino alcohols reside on different sides from the "probe."

In the reaction products of L-glutamine and pyridoxal by the γ-NH_2 group, the OH group and amino acid fragments in amino alcohols reside on different sides with respect to the "probe." This most likely explains the differences in the trends in specific rotation angles (Fig. 2).

The probability of rapid hydrolysis of the Schiff base by the γ-NH_2 group is confirmed by the larger positive charge on the carbon atom (+0.336) in the =N–C=O group compared with that on the carbon atom (+0.011) in the Schiff base by the α-NH_2 group. Chromatography showed that the solutions contain 2 major products of the reaction of pyridoxal with L-glutamine by the γ-NH_2 group: pyridoxamine and glutamic acid. The reaction products were eluted with plates and identified by elemental analysis, UV and IR spectroscopy, and liquid chromatography. The mechanisms of the reactions of L-α-glutamic acid and L-glutamine with pyridoxal are shown in Schemes 1 and 2, respectively.

Thus, the kinetic study of the reactions of pyridoxal with L-α-glutamic acid and glutamine, as well as synthesis and identification of the reaction products showed that the reactions involves formation of a Schiff base which undergoes hydrolysis on prolonged standing of the reaction mixture to form pyridoxamine and α-ketoglutaric acid.

The reaction of glutamine with pyridoxal occurs predominantly by the γ-NH_2 group of the amide fragment to form a Schiff base; the latter rapidly rearranges into a quinoid structure whose hydrolysis forms pyridoxamine and L-α-glutamic acid.

EXPERIMENTAL

Pyridoxal hydrochloride of chemical grade obtained from Ferak Berlin and amino acids and their amides obtained from Reanal were used. Buffer solutions were prepared by conventional procedures. Kinetic measurements were performed on a Spektonom-204 spectrophotometer and a Digi Pol DS Automatic Sacharimeter. Reaction mixtures were thermostated in a UH-8 thermostat with an accuracy of ±0.1°C. Equimolar amounts of pyridoxal hydrochloride and amino acids and their amides were dissolved in aqueous alcoholic buffer solutions and kept for 30 min at a required temperature. The reaction time was measured starting from the point of mixing of thermostated solutions of pyridoxal and amino acids and their amides. Kinetic measurements were performed in thermostated cells 1.008 mm thick and polarimetric tubes 1.9 dm long. Reference cells were charged with equimolar solutions of pyridoxal in the same solvent with the same pH. pH measurements were performed on an EV-74 ionometer with an accuracy of ±0.1 units. The condensation rate constants of pyridoxal with glutamic acid and glutamine were computed for reversible and irreversible reactions [9]. The starting and final products were identified by elemental analysis, UV and IR spectroscopy, and thin-layer and liquid chromatography. The IR spectra were measured on a Nicolet Impact 420 spectrophotometer. The reaction products were analyzed on a Cole Parmer PLC-20 chromatograph, sorbent C-185μ, eluent H_2O–CH_3CN (80 : 20). The structures and atomic charges were calculated with optimization of geometric and thermodynamic parameters using HyperChem [8].

The reaction of pyridoxal with L-α-glutamic acid was performed by the procedures described in [2–7]. The Schiff base was obtained as yellow crystals melting with coaling, yield ~83%. The elemental analysis, UV and IR spectra are consistent with published data.

Reaction of pyridoxal with glutamine. To a mixture of 0.0103 g of pyridoxal hydrochloride and 0.0073 g of glutamine, 8 mL of 96% ethanol was added. The mixture was heated at 50°C until the reagents dissolved completely. The reaction progress was monitored by UV spectroscopy (λ_{max} 350 and 420 nm) and TLC. After reaction completion the solvent was let to evaporate at room temperature. Yield 0.082 g (~52%), hygroscopic dark red substance, mp >310°C. IR spectrum (KBr), ν, cm^{-1}: 3187 (NH), 1665 and 1540 (amide I, amide II). The UV maxima are stronger and shifted blue by 7–10 nm compared to the spectrum of the condensation product of pyridoxal with L-α-glutamic acid. Found, %: C 47.4; H 5.2; N 12.6. $C_{13}H_{17}N_3O_5 \cdot HCl$. Calculated, %: C 47.2; H 5.1; N 12.7.

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