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CHEMICAL KINETICS AND CATALYSIS

Kinetics and Mechanism of the Condensation of Pyridoxal Hydrochloride with L-Tryptophan and D-Tryptophan, and the Chemical Transformation of Their Products

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Abstract—The kinetics and mechanism of interaction between pyridoxal and L-tryptophan, D-tryptophan, and their derivatives are studied. It is found that condensation reactions proceed via three kinetically distinguishable stages: (1) the rapid intraplanar addition of the NH_2 groups of the amino acids to pyridoxal with the formation of amino alcohols; (2) the rotational isomerism of amino alcohol fragments with their subsequent dehydration and the formation of a Schiff base with a specific configuration; (3) the abstraction of α-hydrogen in the product of condensation of pyridoxal with L-tryptophan, or the abstraction of CO_2 in the product of condensation of pyridoxal with D-tryptophan with the formation of quinoid structures, hydrolysis of which results in the preparation of pyridoxamine and keto acid or pyridoxal and tryptamine, respectively. Schiff bases resistant to further chemical transformations are formed in the reaction with tryptophan methyl ester.

Keywords: kinetics, mechanism, pyridoxal hydrochloride, L- and D-tryptophan, UV spectrometry and polarimetry

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INTRODUCTION

The stereochemistry of amino acids plays a key role in the structure of human and animal proteins, peptides, and enzymes. L-amino acids are components of these biological objects. The stereospecificity of the between enzymes and substrates of specific configurations is associated with this. D-Amino acids are present in many peptides produced by microorganisms and form part of the biopolymers of their cell tissues. The inclusion of D-α-amino acids in the structures of human proteins and enzymes has a fatal outcome.

Tryptophan forms part of the polypeptides of plant and animal organisms. The hydroxylation of tryptophan with subsequent decarboxylation results in the formation of serotonin, which plays an important role as a brain neurotransmitter; the failure of its normal metabolism in the body results in schizophrenia. The decarboxylation of tryptophan leads to the formation of tryptamine, an intermediate product in the formation of lysergic acid [1].

Our earlier works found that chemical transformations of amino acids and bioamines under the action of pyridoxal and pyridoxal-5'-phosphate [2–7] depend on their structure, the pH of the medium, the temperature, and the solvent. It was found by measuring the kinetics of chemical processes and isolating their intermediate and final products that the formation of Schiff bases and their chemical transformations proceed in three stages. The first stage is the addition of the amino group of an amino acid to the carbonyl group of pyridoxal to form the intermediate product, an optically active amino alcohol (a sharp drop in absorbance). This stage proceeds very rapidly, and the rates of interaction between the two tryptophan isomers are apparently close. The second stage is the dehydration of the amino alcohol to form a Schiff base (a slow drop in absorbance); and the third stage is the elimination of α-hydrogen or CO_2, followed by their hydrolysis to form the final products (the slowest stage). The rates of these limiting states depend on factors of stereochemical and energy. It is of definite interest to investigate the kinetics and mechanism of interaction between pyridoxal and L-tryptophan, D-tryptophan, and their derivatives (Fig. 1), depending on the structure of the initial, intermediate, and final products.

EXPERIMENTAL

Chemically pure pyridoxal hydrochloride (Ferak Berlin), amino acids, and amides of amino acids

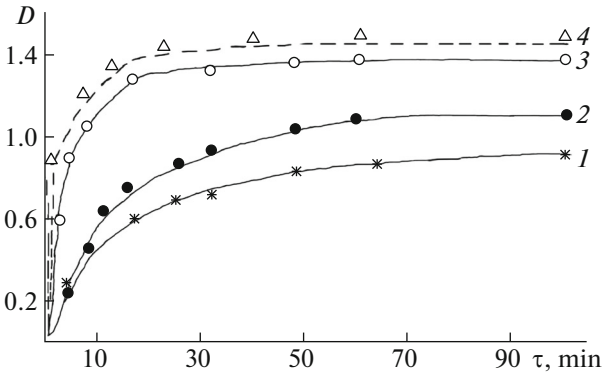


Fig. 1. Kinetics of the interactions between mixtures of 0.01 M solutions of pyridoxal hydrochloride with (*1*) L-tryptophan methyl ester λ_{max} 355 nm, (*2*) tryptamine, (*3*) L-tryptophan, and (*4*) D-tryptophan at the stage of amino alcohol dehydration (430 nm, 90% aqueous–alcoholic buffer solution, pH 7.2, 15°C).

(Reanal, Great Britain) were used in this work. Buffer solutions were prepared according to the standard procedure. The reaction kinetics was measured on a Spektonom-204 spectrophotometer and a Digi Pol DS automatic saccharimeter. The reaction mixtures were thermostated using a UH-8 thermostat with an accuracy of ±0.1°C. Weighed amounts of pyridoxal hydrochloride and amino acids and their methyl esters were dissolved in equimolar quantities in aqueous–alcoholic buffer solutions and kept for 30 min at a specified temperature. The moment of mixing the thermostatted solutions of pyridoxal, amino acids, and methyl esters of amino acids was used to mark the start of the reaction. Kinetic measurements were made in thermostatted cells 1.008 mm thick and polarimetric tubes 1.9 dm long. Since the UV spectra of solutions of pyridoxal vary depending on the pH of the medium and the solvent, equimolar solutions of pyridoxal in the same solvent with the same value of the pH of the medium were placed into reference cells. The pH of the solutions was measured on an EV-74 ionometer with an accuracy of ±0.1 pH unit. The rate constants of the condensation of pyridoxal with L-tryptophan, D-tryptophan, and their esters were calculated on a computer for reversible and irreversible reactions [8]. The initial and final products were identified via elemental analysis, UV and IR spectroscopy, and TLC. The IR spectra were recorded on a Nicolet Impact 420 IR spectrophotometer. The products of interaction were analyzed on a PLC-20 liquid chromatograph (Cole Parmer) with C185 μm sorbent; the eluent was H_2O : CH_3CN = 80 : 20%. The structure and values of charges on the atoms were determined by optimizing the geometrical and thermodynamic factors using the Hyper Chem program. The Schiff bases were synthesized according to the procedures described in [2–7].

Synthesis of Tryptamine Hydrochloride

Mixtures of equimolar 0.01 M pyridoxal hydrochloride and D-tryptophan in a 90% buffer solution were kept for 18 h at T = 15°C. In preparing the solutions, the mixtures turned an intense yellow and changed their color to orange over time. The color then became less intense, and a white precipitate formed. The precipitate was filtered off, washed with alcohol, and dried to a constant weight. The yield was 0.0088 g (23.3%); T_m > 210°C (the precipitate blackened as it decomposed). The product yielded a qualitative reaction for Cl^- with a solution of $AgNO_3$ and a qualitative reaction with Br_2 (violet). The final product was poorly soluble in alcohol (IR spectrum (KBr), ν, cm^{-1}: 3150–3350 (NH_2), 2250–2700 (>NH), 1625, 1580 (C=C); UV spectrum, λ_{max}: 280 nm).

Found, %:	C 61.1,	H 6.1,	N 14.2,	Cl 18.2
Calculated, %:	C 61.2,	H 6.2,	N 14.2,	Cl 18.4

RESULTS AND DISCUSSION

The data in Fig. 1 show that at the stage of amino alcohol dehydration, D-tryptophan is more active than L-tryptophan. This is explained by the great influence of the structures of amino acids, their initial and final products, and their kinetic and thermodynamic factors. With interaction between pyridoxal and L-tryptophan, the α-hydrogen or COO^- of the amino acid fragment in a Schiff base should, according to the Danatan convention [1], be in a position favorable for their abstraction and the Schiff base's transition to a quinoid structure, the subsequent hydrolysis of which results in the formation of the final products. Upon interaction between pyridoxal and D-tryptophan, a Schiff base is formed in which the amino acid fragment is turned by 90° relative to the plane of the pyridine ring; as a result, a Schiff base with preferable abstraction of CO_2 is formed with the subsequent formation of a quinoid structure, hydrolysis of which results in the preparation of pyridoxal and tryptamine. The polarimetric data on the kinetics of the condensation of pyridoxal with L-tryptophan and D-tryptophan serve as proof of the proposed postulate (Fig. 2).

The results from the above measurements show that at the first stage of condensation, there is a rapid increase or decrease in the observed angles of rotation of a mixture of solutions, and these angles gradually change to zero over time. At the stage of amino alcohol formation, new chiral centers with angles of rotation different in magnitude and sign form in both cases (a fast stage). If we start from the premise that nucleophilic reagents attack the plane of the carbonyl group from both sides with equal probability, there should be no change in the magnitudes and signs of the angles of rotation, since racemates are in this case formed. These contradictions can only be explained by assum-

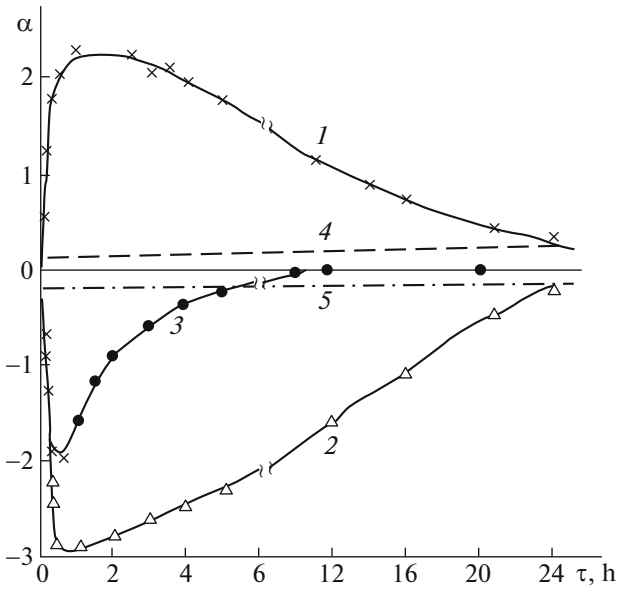
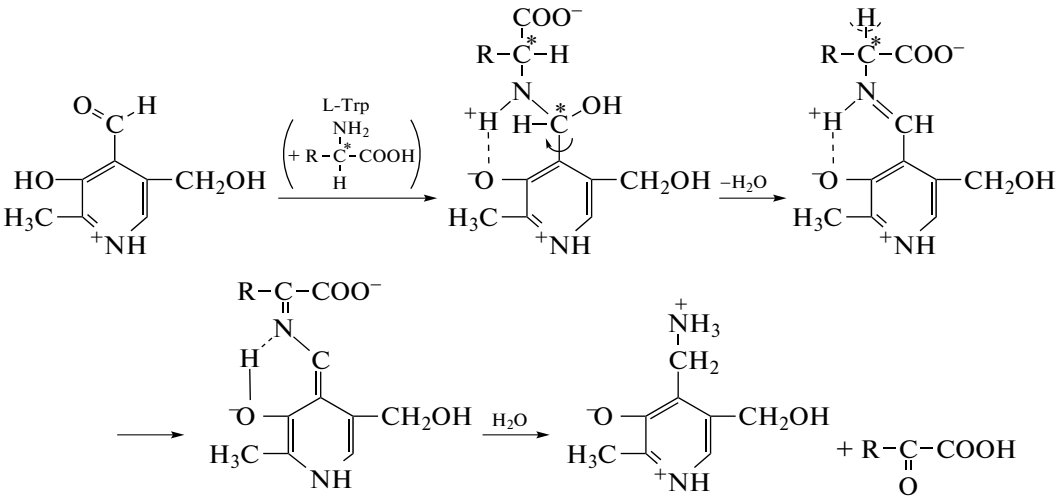


Fig. 2. Kinetics of the change in the observed angles of rotation of mixtures of 0.04M solutions of pyridoxal hydrochloride with (*1*) D-tryptophan, (*2*) L-tryptophan, (*3*) methyl ester, (*4*) D-tryptophan, and (*5*) L-tryptophan over time (70% aqueous–alcoholic buffer solution; pH 6.1; 20°C).

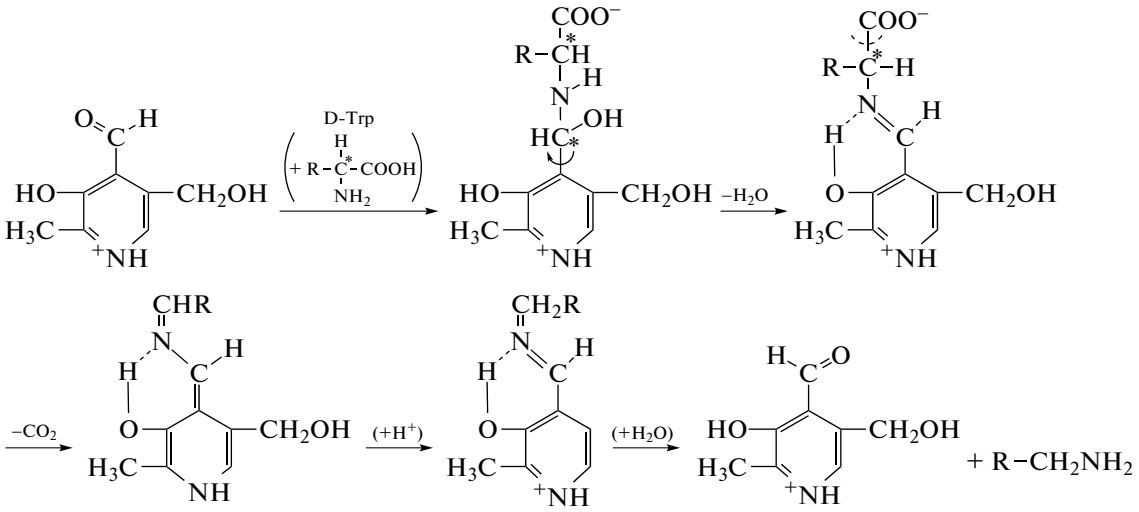
ing that the nucleophilic attack on the carbonyl group of pyridoxal occurs along its plane to form an intermediate product, optically active amino alcohol (the first stage is a rapid change in the absorbance of the mixture of solutions (Fig. 1) or their angles of rotation (Fig. 2)) with two chiral centers of a specific structure in close vicinity.

The second (slow) stage then proceeds. The rotational isomerism of the optically active structures of amino alcohols caused by the optimization of energy and geometrical parameters results in their dehydration with the formation of specific structures of Schiff bases that facilitate the possible abstraction of the α-hydrogen or CO_2 groups. At the third stage, the Schiff bases are rearranged with the formation of quinoid structures, the hydrolysis of which results in the formation of the final products. Data on the structures of amino alcohols and Schiff bases, obtained using the Hyper Chem program with allowance for the optimization of geometrical and energy parameters, confirm this assumption. The results from these studies showed that the products of the condensation of pyridoxal (amino alcohols and Schiff bases) with L-tryptophan and D-tryptophan differed from each other in the positions of the amino acid fragments relative to the plane of the pyridine ring of pyridoxal. To prove the proposed scheme of the mechanism, the final products were synthesized and identified via elemental analysis, qualitative reactions, UV and IR spectroscopy, and TLC.

It was noted that when mixtures of solutions of pyridoxal hydrochloride with L-tryptophan and D-tryptophan were kept, they turned an intense yellow with λ_{max} of 350 and 430 nm. When the mixtures were kept for long periods, sediments precipitated from them that were isolated and identified via elemental analysis and UV and IR spectroscopy. The procedure for synthesizing and identifying the precipitate that is the product of interaction between pyridoxal and L-tryptophan was described in [2]. The precipitate that is the product of interaction between D-tryptophan and pyridoxal (tryptamine hydrochloride) was isolated, purified, and analyzed via elemental analysis and UV and IR spectroscopy. The schemes of the interaction between pyridoxal and L-(1) and D-(2) tryptophans can be presented as



Scheme 1.



Scheme 2.

Studying the kinetics and mechanism of the interaction between L-tryptophan methyl ester and pyridoxal is of definite interest. In earlier works, we showed that the higher the basicity of the NH_2 group of the amino acid, the higher the rate at which the amino acid is added. An inverse dependence is observed at the stage of amino alcohol dehydration; i.e., the higher the basicity of the NH_2 group, the lower the rate of dehydration. The data presented in Fig. 1 show that the rate of amino alcohol dehydration in the products of interaction between pyridoxal and L-tryptophan $\kappa_2 = 0.2913$ min^{-1}, and the rate of dehydration of its methyl ester $\kappa_2 = 0.0662$ min^{-1}. Tryptamine falls in the middle, in the activity series $\kappa_2 = 0.0837$ min^{-1}.

The higher reactivity of the condensation of pyridoxal with L-tryptophan when compared to its methyl ester at the stage of amino alcohol dehydration is apparently explained by the higher accepting ability of the COOH group, relative to the $COOCH_3$ group during the abstraction of water from the amino alcohol. The lower reactivity of the interaction between pyridoxal and tryptamine when compared to L-tryptophan at the stage of amino alcohol dehydration is explained by the lahigherrger value of pK_a of the NH_2 group of the former when compared to pK_a of the latter. Tryptamine with pyridoxal forms a Schiff base that is resistant to further chemical transformation.

The products of the condensation of L-tryptophan methyl ester with pyridoxal were somewhat unexpectedly resistant to further chemical transformation. The results from our study and analysis of the kinetic measurements and structure of the final products showed that a Schiff base resistant to chemical transformations formed during the condensation of pyridoxal with tryptophan methyl ester.

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