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Kinetics and Mechanism of Transaldimination of Amino Acids and Aromatic Amines with Pyridoxal

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Abstract—Kinetics and mechanism of transaldimination of amino acids and aromatic amines with pyridoxal have been studied by means of UV spectroscopy and polarimetry. It has been shown that aminal intermediates are formed in reaction of the Schiff's bases with *p*-aminobenzoic acid and β-alanine. The structure of aminal and Schiff's base is determined by the spatial arrangement of the amino acid and aromatic fragments with respect to the pyridine ring plane. The presence of OH and CH_2–OH groups in the *o*-positions in pyridoxal structure turns amino groups by 90° with respect to the pyridine ring. The scheme of Schiff's bases transaldimination by amino acids and biological amines has been developed according to stereospecific, energy, and geometric factors.

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In our previous studies [1–4] it was demonstrated that the reaction of pyridoxal with amino acids proceeded through 3 kinetically different stages: (1) NH_2 group addition to the pyridoxal carbonyl group with the formation of the amino alcohol (very fast stage); (2) amino alcohol dehydration with the formation of the Schiff's base (slow stage); (3) Schiff's base rearrangement into a quinoid structure by α-hydrogen elimination, with subsequent hydrolysis giving pyridoxamine and keto acids.

It is known that new Schiff's bases formation is likely to proceed via amino group addition to the $C=N^+$ group (via transaldimination reaction), but not via addition to the C=O group of PLP-dependent enzymes [5, 6]. The stage of addition to the *L*-lysine condensation product is followed by lysine ε-amino group elimination. The indirect proof of this mechanism is the assumption suggested in [5] that the addition to the $HC=NH^+$ bond proceeds faster than the addition to the HC=O group. According to [5], this agrees with the disappearance of the positive circular dichroism upon addition of the substrate to the coenzyme. After having the substrate reacted, the circular dichroism reappeared.

However, the discussed mechanism has been still an open question. Seemingly, it can be hardly solved by studies of the enzyme reactions, as they proceed fast and are not always unambiguous. Due to this, the biochemical processes mechanisms are often proved by studies of interaction between model enzyme and substrate fragments under the controlled conditions.

In order to fill in the gap in our knowledge on transaldimination mechanism, in this work we studied kinetics and mechanism of the Schiff's bases interaction with amino acids and aromatic amines under varied conditions. To do so, we chose the following objects: *p*-aminobenzoic acid, β-alanine, and the corresponding Schiff's bases, products of *p*-aminobenzoic acid and β-alanine condensation with pyridoxal. There are no chiral centers in these compounds, and their optical absorbance bands do not overlap [λ_{max} (pyridoxalidene–*p*-aminobenzoic acid) = 370 nm, and λ_{max} (pyridoxalidene-β-alanine) = 340 and 430 nm].

Kinetics and mechanism of pyridoxylidene-β-alanine interaction with *p*-aminobenzoic acid were studied following the absorbance at λ_{max} = 430 nm. In the initial stage the absorbance decreased sharply, this was followed by the second stage accompanied by slow continuous decrease in the absorbance. On the contrary, during the reaction of pyridoxalidene-*p*-aminobenzoic acid with β-alanine, the absorbance at λ_{max} = 430 nm initially sharply increased and then continued to grow slowly (Figs. 1, 2).

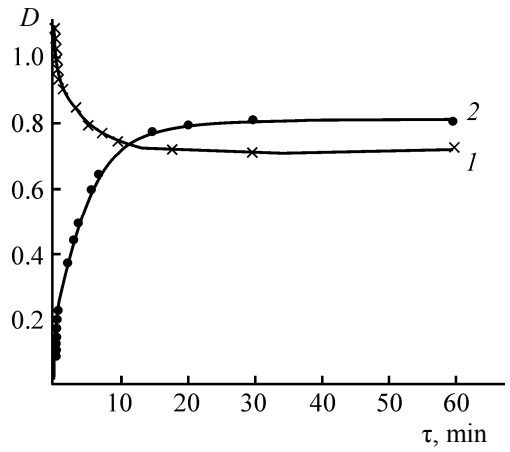


Fig. 1. Kinetics of the absorbance (λ_{max} = 430 nm) change upon interaction of pyridoxaliden-β-alanine with *p*-aminobenzoic acid (*1*) and that of pyridoxaliden-*p*-aminobenzoic acid with β-alanine (*2*), in aqueous ethanol (90 vol % of EtOH) buffer solution (pH 7.0, 20°C). (*1*) addition stage, (*2*) the stage of amino acid or *p*-aminobenzoic acid elimination.

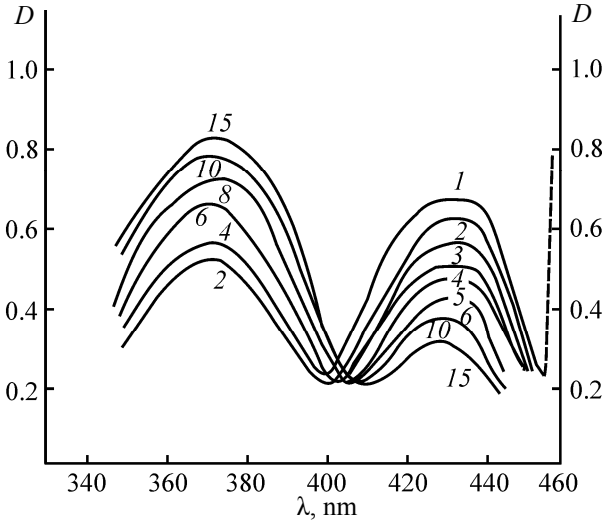


Fig. 2. Optical spectra of the reaction mixture of pyridoxaliden-β-alanine and *p*-aminobenzoic acid; in aqueous ethanol (90 vol % of EtOH) buffer solution (pH 6.9, 20°C). Numbers near the corresponding curves indicate the reaction time in min.

Analysis of the kinetic curves led to assumption that in the first stage (sharp increase or decrease of the absorbance) the addition of the NH_2 group of amino acid or *p*-aminobenzoic acid to the Schiff's base occurred resulting in the formation of an aminal intermediate. In the second stage the amino acid or *p*-aminobenzoic acid was eliminated to give the final product: pyridoxalidene-*p*-aminobenzoic or pyridoxalidene-β-alanine, respectively. The suggested transaldimination mechanism was proved by studies of the

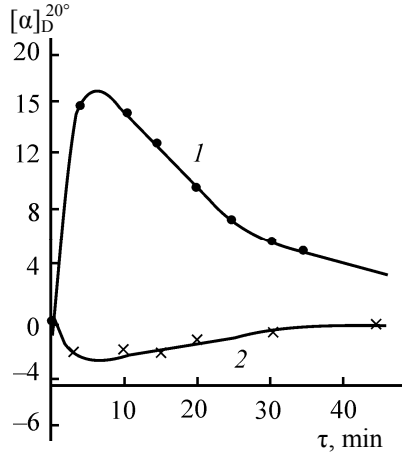
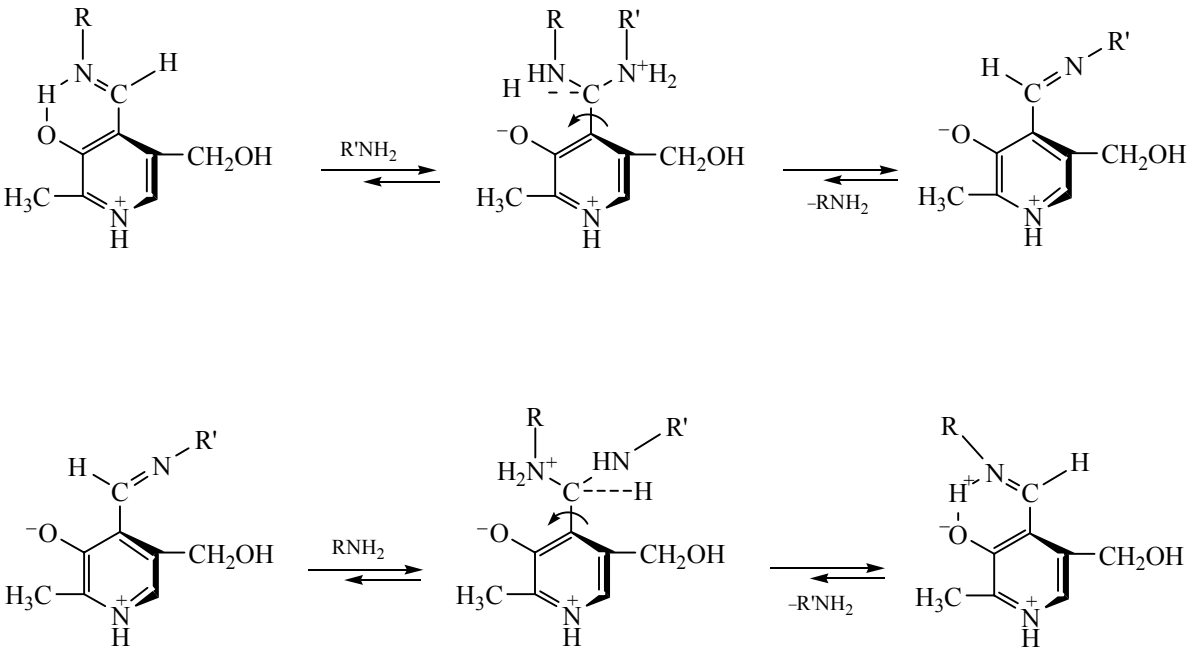


Fig. 3. Time dependence of the change in the specific optical rotation angle at the interaction of pyridoxaliden-*p*-aminobenzoic acid with β-alanine (*1*) and that of pyridoxaliden-β-alanine with *p*-aminobenzoic acid (*2*), in aqueous ethanol (90 vol % of EtOH) buffer solution (pH 6.7, 20°C).

pyridoxalidene-β-alanine reaction with *m*-aminobenzoic acid. The final product of this reaction, pyridoxalidene-*m*-benzoic acid, was poorly soluble and thus precipitated from the reaction mixture. This final product precipitate was separated and characterized by elemental analysis and IR spectroscopy.

At this research point, a question appeared: having known from literature and our data that the intermediate (aminal) formed at the first stage was the same in both studied reactions, why during the pyridoxalidene-β-alanine interaction with *p*-aminobenzoic acid the reaction mixture absorbance initially decreased, while the reaction of pyridoxalidene-*p*-benzoic acid with β-alanine was accompanied by the absorbance increase. To clear this out, kinetics and mechanism of transaldimination were additionally studied by polarimetry. At the stage of β-alanine or pyridoxalidene-*p*-benzoic acid addition to the Schiff's base, a chiral center should have appeared. Data in Fig. 3 show that in the initial stage of pyridoxalidene-*p*-benzoic acid interaction with β-alanine, a product with positive specific angle of optical rotation was rapidly formed, and then in the course of the reaction (*p*-aminobenzoic acid elimination) the positive optical rotation angle steadily decreased. On the contrary, in the reaction of pyridoxalidene-β-alanine with *p*-aminobenzoic acid, a product with negative optical rotation angle was initially formed, with the absolute value of the rotation angle steadily decreasing as β-alanine was eliminated.

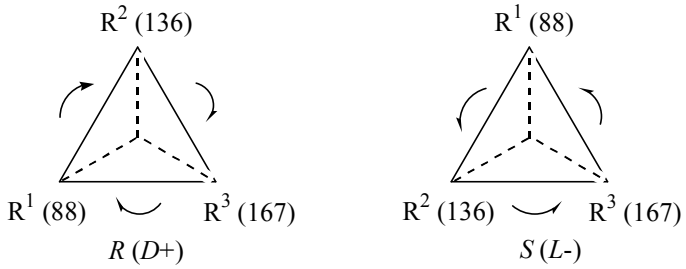
The analysis of kinetic data from UV spectroscopy and polarimetry measurements, as well as structural analysis of the intermediates (amino alcohols) and final product revealed that in the Schiff's bases of pyridoxalidene-*p*-aminobenzoic acid and pyridoxalidene-β-alanine, the aminal group was turned by 90° with respect to the pyridine ring plane. Due to sterical hindrance (presence of the OH and CH_2OH groups in *o*-positions), the addition of amino acid or aromatic amine to the Schiff's base was stereospecific along the C=NH group plane. Thus, the addition was accompanied by very fast change of the solutions absorbance (either sharp increase or sharp decrease) and appearance of the chiral centers. Then, seemingly, rotational isomer was formed due to optimization of geometric and energy parameters accompanying the elimination stage (according to HyperChem software data). The transaldimination mechanism may be presented as follows.



R = 4-C_6H_5COOH; R' = CH_2CH_2COOH.

Why in reactions of pyridoxalidene-*p*-aminobenzoic acid with β-alanine and of pyridoxalidene-β-alanine with *p*-aminobenzoic acids the aminals with the opposite signs of the optical rotation angle and different kinetic profiles of their absolute values were formed? We did not succeed in the aminals isolation, even at low reaction temperature, as the intermediates were highly unstable, and their structural studies were thus impossible. To clear up this question, the structures of aminals and Schiff's bases were studied in the HyperChem software, after the optimization of their geometric and energy parameters. As it was indicated earlier, $C{=}N^+HR$ and $RH_2N^+{-}C{-}NHR'$ groups were turned by ~90° with respect to the pyridine ring plane, and it was convenient to orient the structures so that the pairs of carbon atoms in *o*- and *m*- positions of the pyridine ring were superimposed. According to the structural data, *o*-OH group was located approximately in the plane of the pyridine ring (conventionally "left side"), whereas the *o*-CH_2OH group, being non-linear, was out of the pyridine ring plane (conventionally "right side").

The aromatic fragment was located to the left in the aminals and the Schiff's bases, whereas the amino acid fragment was located to the right. Thus, upon the interaction of pyridoxalidene-*p*-aminobenzoic acid with β-alanine and that of pyridoxalidene-β-alanine with *p*-aminobenzoic acid, the aminal intermediates with different location of the aromatic and amino acid fragments were formed: *R*(*D*+) and *S*(L^-). According to the Kahn-Prelog rule, this may be represented as follows.



R^1 – amino acid fragment, R^2 – *p*-aminobenzoic acid fragment, R^3 – pyridine fragment.

Subsequently, the elimination of one of the fragments from the aminal structure occurred, accompanied with the formation of rotational isomer, the final product with optimal energy and geometric parameters. These experimental data seem to explain the relation between the optical rotation angle and its sign with the structures of intermediates and final products as well as with UV spectroscopy data.

EXPERIMENTAL

Pyridoxal hydrochloride of the chemically pure grade (Ferak Berlin) and amino acids (Reanal, Hungary, England) were used. Buffer solutions were prepared according to published procedures. Reaction kinetics were followed by measurements using SpectroMOM-204 spectrophotometer and DigiPol DS saccharimeter (USA). Temperature control of the reaction mixtures in the spectrophotometer cells and polarimeter tubes was assured with UH-8 temperature bath, temperature accuracy being of ±0.1°C. In kinetic studies, equimolar amounts of the reactants were dissolved separately in aqueous ethanol buffer solutions and incubated for 30 minutes at reaction temperature. The moment of the incubated reactants mixing was taken as the reaction start time.

Condensation and transaldimination products were prepared according to previously published procedures [1–4]. Initial and final products were characterized by elemental analysis, UV and IR spectroscopy.

Pyridoxalidene-*p*-aminobenzoic acid. Ethanol, 6 ml, (96 vol %) was added to a mixture of pyridoxal hydrochloride (0.103 g) and *p*-aminobenzoic acid (0.0685 g). The resulting mixture was heated up to 50°C during 20 min (till complete dissolution) using the water temperature bath. Reaction course was monitored with the UV spectrophotometer and by thin-layer chromatography. The reaction was run till the constant absorbance at λ_{max} = 370 nm was reached, and till the TLC spots corresponding to initial reactants disappeared. Intensive orange coloring appeared upon mixing the reactants solutions. The mixture was evaporated at room temperature till the red precipitate was formed. Product yield 0.144 g (88%), mp 280°C (decomp.). UV spectrum: λ_{max} = 370 nm. IR spectrum (KBr), ν, cm^{-1}: 1620 (C=N), 1385, 1512 (C=O, COO^-). Found, %: C 55.1; H 5.05; N 8.1. $C_{15}H_{14}N_2O_4 \cdot HCl$. Calculated, %: C 55.9; H 4.9; N 8.6.

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