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Stereochemistry of Condensation and Transaldimination of Amino Acids by Pyridoxal

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Abstract—The stereochemistry of the products of condensation and transaldimination of L-α- and D-α-alanines with pyridoxal has been studied. Structure of intermediate amino alcohols, aminals, and of the final Schiff's bases depends on their fragments location with respect to the pyridine ring plane. Basing on the collected data, we have proposed the reaction mechanism involving the in-plane attack of the carbonyl group, in contrast to commonly accepted out-of-plane attack.

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Pyridoxal (PL) and pyridoxal-5'-phosphate (PLP) act as coenzymes in a variety of catalytic processes [1, 2]. In particular, they affect many transformations of amino acids, including elimination, racemization, transamination, decarboxylation, transaldimination, side groups lysis, etc.

The action of PLP-dependent enzymes has been thoroughly studied. However, the study of enzymatic reactions is often complicated as they are usually fast and are not always unambiguous. Due to this, the biochemical transformations of amines and amino acids are often elucidated by using model systems including coenzymes or structurally similar compounds.

Previously, we have studied PL and PLP condensation with various amino acids at different pH, temperature, and in different solvents [3–6]. It has been shown that the reaction proceeds via the three kinetically different stages: (1) amino acid NH_2 group addition to the pyridoxal carbonyl group with the formation of the amino alcohol (fast stage); (2) amino alcohol dehydration with the formation of the Schiff's base (slower stage); (3) the Schiff's base rearrangement into a quinoid structure with subsequent hydrolysis or decarboxylation to give pyridoxamine, keto acids, or aldehydes (very slow stage). The pathway and rate of the discussed process depends on the coenzyme and the amino acid structure as well as on the reaction conditions.

In [5] we have found that L-α-alanine is more reactive at the stages (1) and (2) as compared with D-α-alanine. At the final stage, the L-α-alanine Schiff's base gives pyridoxamine and pyruvic acid via the α-hydrogen elimination and subsequent hydrolysis, whereas the D-α-alanine Schiff's base transformations lead to pyridoxamine and ethanal. In this work, we aimed to explain that difference in the reaction route by kinetic and structural studies of the amino alcohol intermediates by means of polarimetry.

In Fig. 1, kinetics of the specific rotation changes of 0.04 mol/L pyridoxal solution in the presence of L- or D-α-alanines is illustrated.

The kinetic results could hardly be explained with the commonly accepted mechanism of the aldehydes interaction with amines and amino acids. Taking 4-nitrosalicylic aldehyde as an example, in view of the classical theory, the carbonyl group is located in plane of the aromatic ring (as confirmed by HyperChem modeling). If so, the nucleophilic attack of NH_2 group should equally occur from either side of the plane, forming racemic product.

However, polarimetry study of pyridoxal interaction with α-alanines revealed that in the course of the reaction additional chiral center appeared in the amino alcohol: "+" in the case of PL + L-α-alanine and "–" in the case of PL + D-α-alanine, and the absolute value of the optical rotation increased with time (Fig. 1).

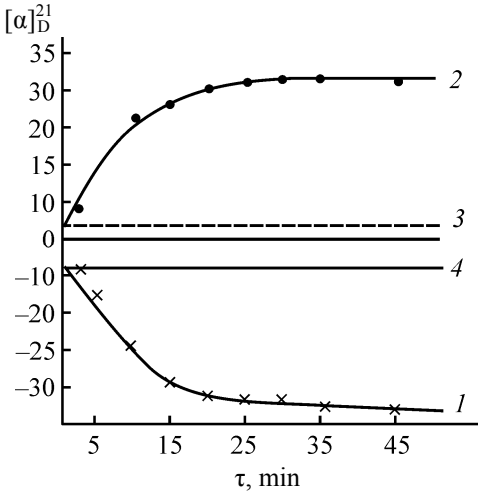


Fig. 1. Kinetics of the optical rotation change in the interaction of 0.04 mol/L pyridoxal with (*1*) D-α-alanine and (*2*) L-α-alanine (90% aqueous ethanol buffer, pH 6.7, T = 20°C). Curves for (*3*) L-α-alanine and (*4*) D-α-alanine are for comparison purpose.

Seemingly, the NH_2 addition occurred in plane of the pyridoxal carbonyl group, not perpendicularly. Thus, the chiral amino alcohol was formed; subsequently, the energy and geometry of the amino alcohol and the Schiff's base were optimized via conformational changes, and finally the *R*(D) or *S*(L) isomer was obtained.

At first glance, that assumption contradicted the experimental results. The amino alcohol dehydration should have led to decrease of the alcohol concentration and, thus, to decrease of the optical rotation angle. However, it could be argued that the specific rotation of products of PL interaction with α-alanines was affected by the spatial arrangement of the functional groups at the chiral center in the course of conformational changes, in addition to the chiral centers concentration. Then, a question arose: why L- and D-α-alanines interacting with PL gave amino acids with opposite rotation angles, and why the absolute rotation angles increased with time?

In order to investigate those issues, we modeled the amino alcohols and the Schiff's bases formed in the interaction of PL with L- and D-α-alanines in the HyperChem software. To represent the structures, the pyridine ring was turned by ~90° with respect observer's eye so that the pairs of carbon atoms in *o*- and *m*-positions of the pyridine ring were superimposed. According to the structural data, the *o*-OH group was located approximately in the plane of the pyridine ring (conventionally left side), whereas the nonlinear *o*-CH_2OH group was out of the pyridine ring plane (conventionally "right side"). In the amino alcohols formed from PL and D-α-alanine, the CH_2OH group was located to the left of the pyridine ring plane, and the amino acid fragment was to the right of the same plane. In the case of L-a-alanine, the structure was opposite: in the amino alcohol, CH_2OH group was located to the right of the pyridine ring plane, whereas the amino acid fragment was located to the left. Similar difference was observed in the cases of the Schiff's based: in D-α-alanine fragment, the >C=N–R was located to the left, and in the L-α-alanine fragment it was located to the right. The marked structural differences led to formation of *R*(D)- or *S*(L)-isomer.

In the human and animal proteins almost exclusively L-amino acids are found, this is one of the reasons for the enzymes stereospecificity. Introduction of the D-α-amino acids to the animal proteins usually causes negative, and sometimes fatal, consequences. The studied system is important from this point of view, as transaldimination by PLP-dependent enzymes is one of the possible routes of D-amino acids incorporation into human proteins.

UV-spectroscopy studies of mechanism of L- and D-α-alanines transaldimination with PL revealed two kinetically distinct stages: (1) addition of the amino acid to the Schiff's base with formation of the aminal intermediate and (2) elimination of one of the amino acid enantiomers with formation of the new Schiff's base. That was confirmed by kinetic curves of the solution absorbance (Fig. 2): in the both cases, at the first stage of the reaction the absorbance abruptly decreased, further increasing due to the new Schiff's base accumulation at the second stage.

The proposed transaldimination mechanism suggested that an additional chiral center should have appeared at the stage of aminal formation. The complementary polarimetry studies (Fig. 3) confirmed the suggestion. Indeed, immediately after mixing the solutions of pyridoxalidene-L-α-alanine with D-α-alanine the optical rotation steeply decreased (stage 1), then, the positive rotation angle was slowly decreasing to become negative due to elimination of L-α-alanine and formation of pyridoxalidene-D-α-alanine (stage 2). The interaction of pyridoxalidene-D-α-alanine with L-α-alanine revealed the opposite trend in the kinetic profile: first, the negative rotation angle abruptly decreased and then it was slowly increasing to reach positive values.

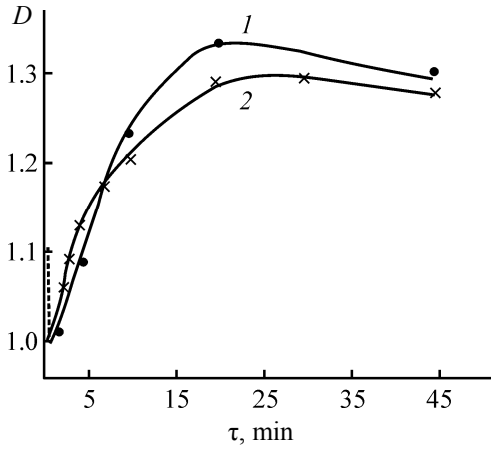


Fig. 2. (*1*) Kinetics of the absorbance in the interaction of 0.01 mol/L pyridoxalidene-L-α-alanine with D-α-alanine and (*2*) pyridoxalidene-D-α-alanine with L-α-alanine (90% aqueous ethanol buffer, pH 6.65, $T = 20°C$).

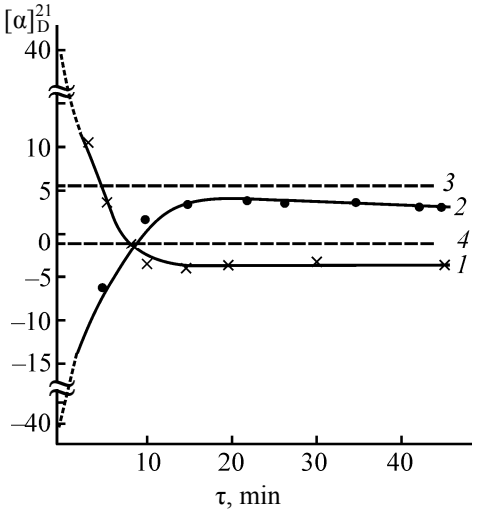
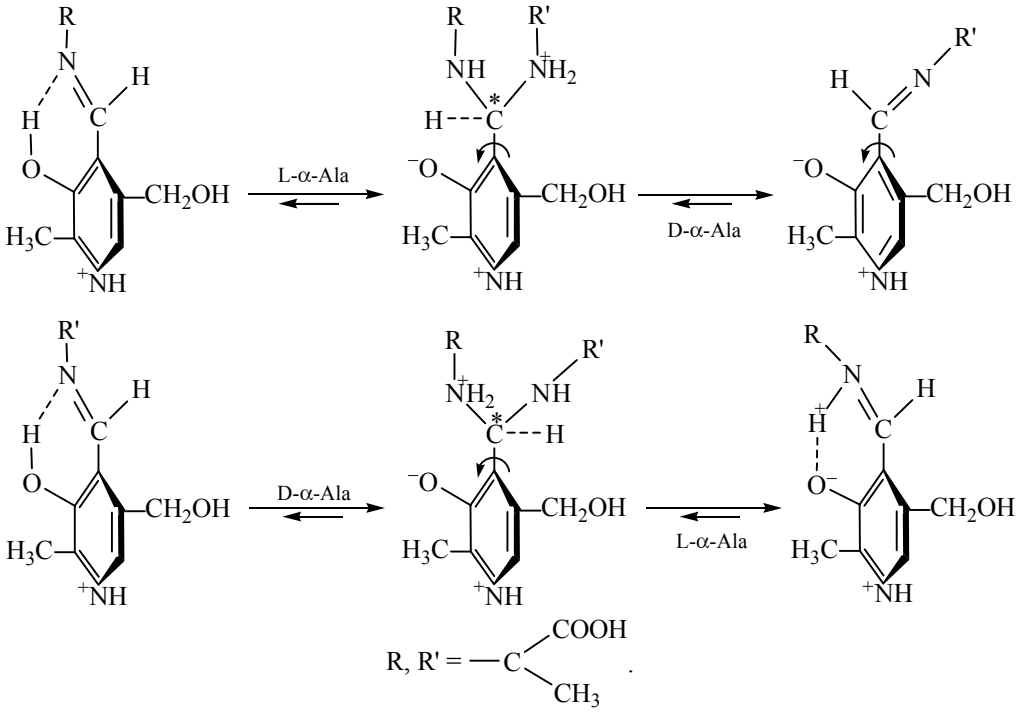


Fig. 3. (*1*) Kinetics of the optical rotation change in the interaction of 0.04 mol/L pyridoxalidene-L-α-alanine with D-α-alanine and (*2*) pyridoxalidene-D-α-alanine with L-α-alanine (90% aqueous ethanol buffer, pH 6.65, $T = 20°C$). Curves for (*3*) L-α-alanine and (*4*) D-α-alanine are for comparison purpose.

Thus, HyperChem modeling has revealed that aminal intermediates with different arrangement of amine fragment with respect to the pyridine ring are formed in the interaction of pyridoxalidene-L-α-alanine with D-α-alanine and of pyridoxalidene-D-α-alanine with L-α-alanine. The aminals this formed have different values and signs of the specific optical rotation. Trans-aldimination mechanism can be represented as follows.



Upon amino acids condensation and transaldimination with PL, the attack at the HC=O and >C=NR reaction centers occurs in plane.

In the intermediates of PL interaction with L- and D-α-amino acids and of the subsequent transaldimination, the structural fragments are turned by ~90° with respect to pyridine ring plane, thus forming new stereospecific chiral centers.

EXPERIMENTAL

L- and D-α-alanines (Reanal, Hungary) and pyridoxal hydrochloride (chemical pure grade, Ferak, Germany) were used as received. The Schiff's bases were prepared and identified (elemental analysis, UV, IR, and polarimetry) as described in [3–6]. Condensation and transaldimination kinetics was followed by measurements of the optical rotation angle (Digipol DS Automatic, Saccharimeter, USA) and of the absorbance (SpektroMoM-204, Hungary). In the course of the measurement the temperature was maintained constant at ±0.1°C with U-4 thermostat. Time of mixing was considered the reaction start time.

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