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PREPARATION OF α-DEUTERATED L-AMINO ACIDS USING E.coli B/It7-A CELLS CONTAINING TRYPTOPHANASE

N.G.Faleev[X], S.B.Ruvinov, M.B.Saporovskaya, V.M.Belikov, L.N.Zakomyrdina[I], I.S.Sakharova[I], Yu.M.Torchinsky[I]

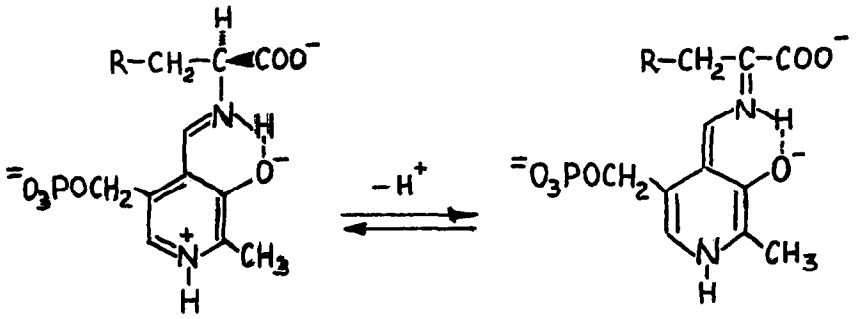
A.N.Nesmeyanov Institute of Organo-Element Compounds, Academy of Sciences of the USSR, Vavilov Str. 28, Moscow II78I3, USSR
[I]V.A.Engelgardt Institute of Molecular Biology, Academy of Sciences of the USSR, Vavilov Str. 32, Moscow II7984, USSR

ABSTRACT. A convenient method of preparation of α-deuterated L-amino acids via stereospecific isotope exchange in D_2O catalyzed by lyophilized E. coli B/It7-A cells abundant in tryptophanase is reported

Enzymes are becoming a convenient tool in organic chemistry [1]. The stereoselective hydrolysis [2] and reduction [3] to produce practically valuable synthons are well known examples of the application of enzyme technology. Now we report a practical method for preparation of α-deuterated L-amino acids with the use of lyophilized microbial cells abundant in tryptophanase.

Isotopically labelled natural and synthetic amino acids and in particular α-deuterated amino acids are widely used in mechanistic studies of many enzymes and various biochemical processes. Some pyridoxal-5'-phosphate (PLP)-dependent enzymes are known to catalyse a stereospecific isotope exchange of L-amino acids in hydrogen isotope enriched water [4,5]. These reactions are characterized by mild conditions, minimum of auxilliary stages and absolute stereoselectivity. The main disadvantage of such methods is the narrow substrate specificity of the enzymes. Our method allows for the preparation of a number of α-deuterated amino acids with the same biocatalyst.

It is known that PLP-dependent enzyme tryptophanase (EC 4.I.99.I) reacts with a variety of amino acids and that such a reaction is accompanied by appearance of an intense narrow absorption band at 500 nm which is ascribed to a quinonoid intermediate formed in the active site by abstraction of the α-proton from aldimine of the amino acid with the cofactor [6] (Figure I). The ability of tryptophanase to labilize the α-proton in a large number of amino acids allowed us to develop a new method of introducing hydrogen isotopes into the α-position of amino acids with retention of their configuration.



PLP-amino acid Schiff base Quinonoid intermediate

Figure I

The isotope exchange of L-amino acids in D_2O catalyzed by lyophilized E. coli B/It7-A cells has been studied. The cells of this strain are known to contain a significant amount of tryptophanase [7,8]. The exchange was controlled by PMR; the results are presented in Table I. It is seen that valine, norvalin leucine, isoleucine, norleucine, methionine, phenylalanine and histidine undergo enantioselective α-proton exchange. The relative intensities of β-CH_2 signals did not change significantly while α-proton signals disappeared. So, unlike methionine γ-lyase, tryptophanase does not labilize β-protons which reflects the principal difference between the mechanisms of the two enzymes. In PMR spectra of the reaction solution no noticeable signals of compounds different from the substrate were observed. So the nonquantitative yields in these cases are due to metabolism to some products which are not detectable by PMR, or to losses during isolation. L-Lysine does not exchange its α-proton which is consistent with the finding that lysine does not induce the 500 nm band in the absorption spectrum of tryptophanase [6]. In the cases of L-aspartic acid, L-glutamic acid, L-threonine, L-serine and L-cysteine PMR signals typical for these amino acids diappeared after their incubation with the cells L-Serine and L-cysteine are substrates of tryptophanase [6] and therefore are degraded by the enzyme. L-Aspartic acid, L-glutamic acid and L-threonine under the experimental conditions are completely transformed to unidentified products. In these cases the substrate signals disappeared and numerous different signals appeared in the PMR spectra. This fact limits the application of our method.

Table I. Stereospecific α-hydrogen exchange of L-amino acids in D_2O catalyzed by lyophilized E. coli cells containing tryptophanase [x]

L-amino acid	Concentration (mM)	Weight of lyophilized cells (mg)	Units of enzyme	e.e. (%)	Yield (%)
valine	50	50	I4	95	59
leucine	"	"	"	"	64
norvaline	"	"	"	"	63
norleucine	"	"	"	"	58
isoleucine	"	"	"	"	60
methionine	I00	"	"	"	76
phenylalanine	"	"	"	"	60
histidine	50	I00	28	"	55
arginine	"	250	70	"	45
3-nitrotyrosine	I00	50	I4	"	6I
3-aminotyrosine	50	I00	28	"	63
3-fluorotyrosine	I00	50	I4	"	60
3-chlorotyrosine	"	I00	28	"	5I
2-chlorotyrosine	"	"	"	"	54
2-methyltyrosine	"	I50	42	"	43

[x] The reaction mixture contained 0.I M potassium-phosphate buffer in D_2O (pH 8.I7 corresponds pD 8.57 [9]), 0.4 μmole of pyridoxal-5'-phosphate; indicated quantity of the amino acid and E. coli cells in a total volume of I0 ml. This mixture was incubated anaerobically under stirring at 30°C for 24 hours (only for L-arginine the incubation time was 48 hours). The cells were separated by centrifugation, discarded and the solution was analyzed by PMR method. According to PMR spectra the degree of deuteration was at least 95%.The solution was applied on e column of Amberlite CG-I20 (in H^+-form), washed with distilled water until neutral pH and the amino acid was eluted by 5% solution of NH_3. The eluate was then evaporated to dryness and weighted. The purity of respective amino acid was controlled by quantitative amino acid analysis and was found at least 96%. The enantiomeric purity was determined by GLC [I0] which implied transformation of amino acid into N-trifluoroacetyl-O-isopropyl derivatives and subsequent separation of enantiomers on a chiral

phase. The yields were determined by direct weighting. The optical purity of the α-deuterated amino acids obtained did not differ from the optical purity of the initial nondeuterated amino acids.

Thus, the use of the lyophilized E. coli cells abundant in tryptophanase makes it possible to prepare quite a number of α-deuterated L-amino acids in a rather simple way and to avoid a time-consuming procedure of isolation of the purified enzyme. The cells used in our experiments had been stored at -4°C for several months without significant loss of activity.

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