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AN EXCHANGE OF β-HYDROGEN OF AMINO ACID WITH MEDIUM WATER BY TRANSAMINASE ACTION

It is known that the hydrogen atom attached to α-carbon of an amino acid exchanges with hydrogen atom of medium water during the course of the transaminase action (*1*, *2*). The authors found that the hydrogen atoms on β-carbon exchanged as well by the enzyme action.

42 mg. of L-alanine, 20 mg. of transaminase preparation (purified upto the stage of the third fractionation by ammonium sulphate, according to Green and others' method (*3*), dialysed and lyophilized), each trace amount of α-ketoglutaric acid and pyridoxal phosphate were dissolved in 5 ml. of 0.08 *M* phosphate buffer (pH 7.0) in 99.8 per cent deuterium oxide. The final pH was adjusted to 7.0 with concentrated sodium hydroxide in D_2O. One drop of tolune was added as anticeptic. After 38 hours' incubation at 38°, the reaction mixture was boiled and filtered. The amino acid in the filtrate was absorbed on a column (1.8 $cm.^2 \times 7$ cm.) of Amberlite IR 112, H^+ type. The column was washed with distilled water and the amino acid was eluted with 0.15 *N* ammonium hydroxide and dried under vacuum. The alanine recovered was dissolved in 10 ml. of water and passed through another column of Amberlite IR 45, acetate type, to remove acidic impurities. The solution was evaporated to dryness under vacuum. The alanine, obtained as the evaporation residue, was dissolved again in a minimum amount of water and recrystallized by the addition of ethanol, and dried under vacuum at 100°. 33 mg. of L-alanine were recovered.

The alanine preparation obtained here contained 3.76 atoms of deuterium per molecule, according to the mass spectrometric analysis (*4*). It is evident that these four deuterium atoms are attached to α- and β-carbons of the alanine, because other three hydrogen atoms in the molecule are readily exchangeable with medium water and replaced by normal hydrogen during the course of isolation.

Fig. 1 shows the infra red absorption spectrum of this α,β-tetradeuterio-L-alanine. The spectra of normal L-alanine and chemically synthesized α-deuterio-DL-alanine are also shown for comparison. The latter was synthesized by the electrolytic reduction of α-isonitrosopropionic acid in deuterium oxide. The pattern of the absorption spectrum clearly shows the disappearance of the peaks of α-CH (deformation, 1308 $cm.^{-1}$) and $-CH_3$ (degenerating deformation, 1451 $cm.^{-1}$ and symmetric deformation, 1356 $cm.^{-1}$) groups (*5*).

10 mg. of the α,β-tetradeuterio-L-alanine was treated again with transaminase in the presence of pyridoxal phosphate and α-ketoglutaric acid in 4

ml. phosphate buffer in ordinary water. 7 mg. of alanine were recovered from the reaction mixture. The infra red spectrum of the re-treated alanine agreed completely with that of normal L-alanine.

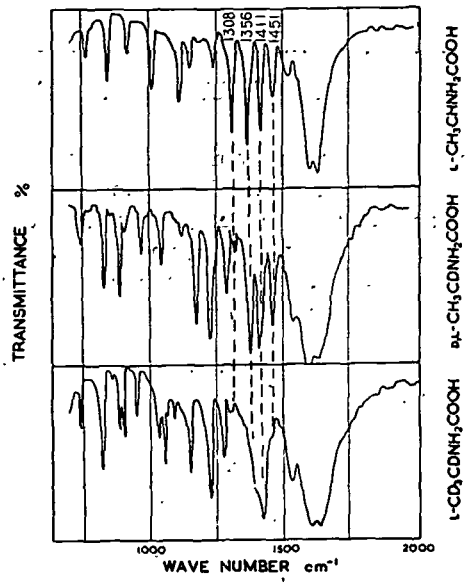

FIG. 1. Infra red absorption spectra of L-alanine, α-deuterio-DL-alanine and enzyme treated L-alanine.

TABLE I

The Incorporation of Deuterium into α and β Positions

Alanine Preparation	Percent decrease in	
	α-CH	$-CH_3$
10 minutes	13	14
25 minutes	20	29
16 hours, with boiled enzyme	2	0
16 hours, without enzyme	0	0
Chemically synthesized α-deuterio-D, L-alanine	>90	7

The rates of exchange at positions α and β were compared in D_2O by following the rates of decrease in the heights of infra red absorption peaks at 1451 cm.$^{-1}$ and 1308 cm^{-1}. The absorption at 1411 cm.$^{-1}$, which remained unchanged and was assigned to $-COO^-$ symmetric stretching, was taken as standard. The results are summarized in Table I. The values obtained with chemically synthesized α-deuterio-DL-alanine and with L-alanine recovered from the reaction mixture of control experiment with boiled enzyme or

without the enzyme, are also given in the Table. It can be seen the rate of exchange is greater at β position than at α position.

From these results, the authors propose a reaction mechanism for transaminase in which the dissociation of a proton from β-carbon is an essential and primary step of the activation of the Schiff's base which is formed from the amino acid and pyridoxal phosphate. The dissociation at β position seems to be more conceivable than that at α position (*6–8*), according to the present knowledge of electronic theory, and can explain a number of facts known for enzyme reactions, which require pyridoxal phosphate as the cofactor.

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