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Effect of cystathionase on isovalthine

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Abstract

In the course of studies on the cleavage reaction of S-(isopropylcarboxymethyl) glutathione (GSIV) into isovalthine in kidney homogenate or glutathionase preparation, it has sometimes been observed that the amount of isovalthine formed is far le than that of GSIV decomposed[1]. Furthermore, when such reaction mixture is analyzed on an automatic amino acid analyzer, prominent peak corresponding to the reasonable amount of S-(isopropy1carboxymethyl)cysteinylglycine which is an expected intermediate of the GSIV cleavage reaction cannot be found up to 400 effluent ml. Though several reasons may be considered for the explanation of the above curious phenomenon, the effect of cystathionase on isovalthine is at first examined here. But the result was negative. L- and L-Alloisovalthineused as substrate were prepared by the method of OHMORI[2]. Homoserine and purified cystathionase in ammonium sulfate solution prepared according to the method of GREENBERGB[3] were kindly furnished by Prof. M. Suda of Osaka University. Incubation mixture contains 0.1 ml of enzyme solution, 1.0 ml of 0.2 M borate buffer (pH 8.0) containing 2×10-^{3}M cysteine, 0.lml of 0.1 M substrate, and 0.8ml of deionized water containing 5×10-4M EDTA. The mixture was shaken at 37°C for 30 minutes in the air. The reaction was terminated by adding 2ml of 10% trichloroacetic acid and the α-keto acids formed were determined by the method of FRIEDEMANN and HAUGEN4 with a following modification: toluene extract was washed once with 8 ml of 10% sodium sulfate. The results obtained are summarized in Table l. When the reaction mixtures are analyzed before or after incubation on an automatic amino acid analyzer, the amount of L- or L-Alloisovalthine is found to be unchanged. Furthermore, as indicated in Table 1, L-isovalthine showed no inhibitory effect on the homoserine cleavage by cystathionase. Since amino acid oxidases have already been reported to have no effect on isovalthine[3], the curious phenomenon above cited may have to be explained by other reaction mechanism such as transpeptipation reaction.

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BRIEF NOTES

EFFECT OF CYSTATHIONASE ON ISOVALTHINE*

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In the course of studies on the cleavage reaction of S-(isopropylcarboxymethyl)glutathione (GSIV) into isovalthine in kidney homogenate or glutathionase preparation, it has sometimes been observed that the amount of isovalthine formed is far less than that of GSIV decomposed[1]. Furthermore, when such reaction mixture is analyzed on an automatic amino acid analyzer, prominent peak corresponding to the reasonable amount of S-(isopropylcarboxymethyl)-cysteinylglycine which is an expected intermediate of the GSIV cleavage reaction cannot be found up to 400 effluent ml.

Though several reasons may be considered for the explanation of the above curious phenomenon, the effect of cystathionase on isovalthine is at first examined here. But the result was negative.

L- and L-Alloisovalthineused as substrate were prepared by the method of OHMORI[2]. Homoserine and purified cystathionase in ammonium sulfate solution prepared according to the method of GREENBERG[3] were kindly furnished by Prof. M. Suda of Osaka University.

Incubation mixture contains 0.1 ml of enzyme solution, 1.0 ml of 0.2 M borate buffer (pH 8.0) containing 2×10^{-3}M cysteine, 0.1 ml of 0.1 M substrate, and 0.8 ml of deionized water containing 5×10^{-4}M EDTA. The mixture was shaken at 37°C for 30 minutes in the air. The reaction was terminated by adding 2 ml of 10% trichloroacetic acid and the α-keto acids formed were determined by the method of FRIEDEMANN and HAUGEN[4] with a following modification: toluene extract was washed once with 8 ml of 10% sodium sulfate.

The results obtained are summarized in Table 1.

When the reaction mixtures are analyzed before or after incubation on an automatic amino acid analyzer, the amount of L- or L-Alloisovalthine is found

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Table 1 Effect of Cystathionase on Isovalthine T. AZUMI

Substrates	μmoles of α-keto acid formed	
	Experiment I	Experiment II
Homoserine	0.72	0.64
Homoserine + L-Isovalthine		0.64
L-Isovalthine	0.00	0.00
L-Alloisovalthine		0.00

Reactian mixture contains 0.1 ml of cystathionase solution, 1.0 ml of 0.2 M borate buffer (pH 8.0) containing 2×10^{-3} M cysteine, 0.1 ml of 0.1 M substrate, and 0.8 ml of water containing 5×10^{-4}M EDTA. Shaken at 37°C, 30 min. in air.

to be unchanged. Furthermore, as indicated in Table 1, L-isovalthine showed no inhibitory effect on the homoserine cleavage by cystathionase.

Since amino acid oxidases have already been reported to have no effect on isovalthine[3], the curious phenomenon above cited may have to be explained by other reaction mechanism such as transpeptipation reaction.

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