GLUTAMIC ACID RACEMASE FROM LACTOBACILLUS FERMENTI PURIFICATION AND PROPERTIES

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Since the discovery of alanine racemase (Wood and Gunsalus, 1951), there have been reports of a number of amino acid racemases. Some of them have been found to need pyridoxal phosphate as a cofactor. Certain other racemases were not activated by this cofactor. DPN has been reported to stimulate proline racemase (Stadman and Elliott, 1957) and ATP and AMP to stimulate threonine racemase (Amos, 1954).

Previously one of the authors reported that the enzyme preparation of Lactobacillus fermenti ATCC 9338 was able to racemize several amino acids such as glutamic acid, aspartic acid, alanine, methionine, valine, isoleucine and phenylalanine. Pyridoxal phosphate was found to have not any stimulatory effect on the rate of glutamic acid racemization by this enzyme preparation, while the racemization of some of the other amino acids mentioned above were apparently activated by this cofactor (Tanaka,1960). Evidence for the occurence of a glutamic acid racemase was described by some investigators (Ayengar and Roberts, 1952; Narrod and Wood, 1952; Itoh, 1958). Glaser (1960) has recently reported a method for purification of glutamic acid racemase from Lactobacillus arabinosus. However, few details of the properties of glutamic acid racemase have been given up to the present.

The present communication is concerned with glutamic acid racemase from L. fermenti, which was proved to be a flavoprotein.

Lactobacillus fermenti ATCC 9338 was cultivated at 37° for 16 hrs in the same medium as that used previously (Tanaka, 1960). About thirteen grams of the dried cells obtained were disrupted by using a 10 kc sonic oscillator at 0° - 3° for 15 minutes with 150 ml of 0.05 M potassium phosphate-0.005 M cysteine, pH 7.5. After centrifugation at 15,000 x g for 20 minutes, 20 ml of 2 %

protamine sulfate were added to the supernatant and the resulting precipitate was discarded. The protamine treated supernatant was brought to 30 % saturation by addition of saturated ammonium sulfate, and the pH was adjusted to 4.0 by addition of 0.5 N HCl. After centrifugation, further saturated ammonium sulfate was added to the supernatant to 70 % saturation. The resulting precipitate was dissolved in 40 ml of 0.05 M potassium phosphate-0.005 M cysteine-0.001 M DL-glutamic acid, pH 7.5, and dialyzed against 1 liter of the same buffer solution. The dialyzed solution was again brought to 20 % saturation with saturated ammonium sulfate, and its pH was adjusted to 2.9. After removing the precipitate, saturated ammonium sulfate was added to the supernatant (to 65 % saturation). The precipitate by ammonium sulfate of 20 to 65 % saturation was again dissolved in 15 ml of 0.05 M potassium phosphate-0.005 M cysteine-0.001 M DL-glutamic acid, pH 7.5, and dialyzed against 1 liter of the same buffer. To the dialyzed solution, acetone was added to 52 % and the precipitate formed was removed. The enzyme was precipitated by addition of acetone to the supernatant (to 62 %).

Table I

Purification of Glutamic Acid Racemase

Fraction	Specific activity	Total activity
	units/mg protein	units
Sonic extract	1.32	7210
Protamine treated supernatant	1.39	6630
Ammonium sulfate,(pH 4.0) ppt.	3.46	3900
Ammonium sulfate,(pH 2.9) ppt.	9.75	2060
Acetone precipitate	76.2	1097
DEAE-cellulose eluates*		
Fraction 4	703	549
Fraction 5	517	300

* 870 units of enzyme were put on the column, and volume of each fraction was 4 ml.

The precipitate thus obtained was dissolved in 5 ml of 0.05 M potassium phosphate-0.005 M cysteine-0.001 M DL-glutamic acid, pH 7.5, and was poured onto a 16 x 1.5 cm column of DEAE-cellulose equlibrated with the same buffer, and eluted with the same buffer. The enzymic activity of the preparations obtained

by these treatments are shown in table I. About a 500 fold increase in specific activity was achieved.

The purified enzyme preparation was proved to have not any racemizing activities of other amino acids than glutamic acid such as, alanine, valine and aspartic acid. The pH-optimum for the activity of glutamic acid racemase is 7.5 and the Michaelis constant for D-glutamic acid is 4.7×10^{-2} mole per liter. A boiled extract of the enzyme preparation showed yellow fluorecence under ultraviolet irradiation. The absorption spectrum of the enzyme preparation was observed to have three bands at 270, 360 and 450 mμ respectively. Neither pyridoxal phosphate nor FAD affected the activity of the enzyme.

Table II

Effect of Various Inhibitors on Glutamic Acid Racemase

Inhibitor	Concentration (M)			Percent
	Inhibitor	Cysteine	FAD	Inhibition
p-Chloromercuribenzoate	10^{-3}	-	-	98
"	"	10^{-4}	-	5
Phenylmercuric acetate	"	-	-	99
Monoiodeacetate	"	-	-	100
Riboflavin	10^{-4}	-	-	26
"	"	-	3×10^{-3}	12
"	"	-	6×10^{-3}	6
FMN	2×10^{-3}	-	-	10
Tetracycline	"	-	-	25
Acriflavine	5×10^{-4}	-	-	25
Quinine	4×10^{-4}	-	-	10
Hydroxylamine	10^{-2}	-	-	0
Semicarbazide	10^{-3}	-	-	0
Phenylhydrazine	"	-	-	0
EDTA	"	-	-	0

10 units of enzyme were used.

p-Chloromercuribenzoate and other -SH inhibitors exerted distinct inhibitory effects on the enzyme. The inhibition caused by p-chloromercuribenzoate was reversed by cysteine. Riboflavin, FMN, tetracycline and acriflavine also inhibited the enzyme activity, but the activity could be recovered by the addition of a

rather high concentration of FAD (table II). Carbonyl reagents and metal chelating agents were ineffective.

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