SYNTHESIS OF L-THREONINE BY A SHEEP LIVER ENZYME

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An enzyme which will catalyze the synthesis of L-threonine from glycine and acetaldehyde has been purified from sheep liver. The enzyme is probably related to the aldolase from sheep liver (1) and rat liver (2). The product of the reaction has been isolated and shown to be L-threonine by a number of methods. A rapid assay has been developed using purified threonine dehydrase to convert the threonine formed to α-ketobutyric acid, which is determined by 2,4-dinitrophenylhydrazine.

Standard assays were carried out in 6 ml serum bottles fitted with rubber seals to produce air tight closure. The enzyme was added to 40 μmoles of glycine and 40 μmoles of acetaldehyde plus sufficient 0.1 M phosphate buffer at pH 7.6 to make a final volume of 6 ml.

The reaction mixtures were placed in a Dubnoff metabolic shaking incubator for 20 minutes. The reaction was stopped by immersing the vials in boiling water and excess acetaldehyde removed by maintaining the temperature between 85° and 90° for 30 minutes. After removal of the protein by centrifugation, two ml of the sample were mixed with 48 units (μmoles of threonine converted to alpha ketobutyrate per hour) of threonine dehydrase isolated from E. coli (3) and incubated for 15 minutes at 35°C. The dehydrase reaction was stopped with 25% trichloroacetic acid.

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After centrifugation, an aliquot of the supernatant was assayed for keto acid by the 2,4-dinitrophenylhydrazine method (4). An optical density of 1.0 at 520 mμ corresponded to one μmole of L-threonine. Alpha keto acid production was linearly dependent upon the amount of L-threonine present under the assay conditions. Blanks were prepared by adding the trichloroacetic acid prior to the addition of the dehydrase. Blanks for the synthetase were prepared by heating aliquots of the enzyme for 10 minutes at 90° before addition of the other components of the assay.

L-Threonine was isolated from ten 50 ml reaction mixtures incubated for one hour at 37°. The enzymatic reaction was stopped by bringing to a boil and protein was removed by centrifugation. The solution was brought to dryness and the residue resuspended in the minimum amount of water and placed on preparative thin layer plates prepared by the method of Bekersky (5) which allowed separation of up to 150 mg of sample per plate. The plates were developed in the solvent system, tert-butyl-alcohol : methyl ethyl ketone : H_2O : NH_4OH (40:30:20:10). The threonine band on the plate was located by spraying a narrow strip with ninhydrin. The threonine was eluated with water, concentrated, and placed on a Dowex 50 column in the hydrogen form. The column was washed with several column volumes of distilled water and the threonine eluated with 50% ammonia. The ammonia was removed by evaporation and the threonine was recrystallized from a water-ethanol mixture. No evidence for allothreonine was obtained either on TLC or paper chromatography.

Good separation of mixtures of allothreonine and threonine could be achieved with this system, the Rf values being .42 and .53 respectively. The purity and optical configuration of the threonine were established by converting a weighed amount of the amino acid to alpha ketobutyrate by E. coli threonine dehydrase which has no activity towards allothreonine and is specific for the L-form of threonine. The results are summarized in Table I. Also, the rate of keto acid production, using

the sheep liver enzyme, was consistent with the rate observed with authentic threonine. The rate of the enzyme reaction would have been only 0.23 times as fast had the product been allothreonine (6). The fact that 80% conversion of the isolated threonine took place eliminated the possibility that the enzyme was producing a racemic mixture. Optical configuration and purity were established independently by microbiological assay in the Shankman Laboratories of California.

TABLE I

Purity of Synthesized L-Threonine

		Micromoles Ketoacid		
	Weight	Produced	Expected	% Conversion
Authentic L-threonine	.10 mg	.78	.84	97.5
Synthesized threonine	.10 mg	.68	.84	81

Substrate plus dehydrase (48 units) and buffer (Tris-HCl, pH 8.8) were incubated for 15 minutes at 37°. The reaction was stopped with TCA and the keto acid determined by the 2,4-dinitrophenylhydrazine method.

The data in this communication furnish evidence for the existence in sheep liver of an enzyme which catalyzes the synthesis of L-threonine. The enzyme has no allothreonine synthetase activity. This characteristic is significant in comparison with the rat liver enzyme which reportedly produced only allothreonine (2), since allothreonine has never been isolated from any mammalian system.

The effect of the presence of this enzyme on the indispensability of threonine for the sheep hinges on the availability of adequate amounts of acetaldehyde from other metabolic processes. Pterins do not stimulate the reaction, however, we have not eliminated the possibility that active acetaldehyde may not serve as the donor.

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