TRANSAMINATION OF HYPOTAURINE BY TAURINE:α-KETOGLUTARATE AMINOTRANSFERASE

H. TANAKA*, S. TOYAMA**, H. TSUKAHARA and K. SODA†

Laboratory of Microbial Biochemistry, Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611, Japan

Received 20 May 1974

1. Introduction

Hypotaurine (2-aminoethanesulfinate) was reported to occur in certain molluscs [1] and in rat brain [2], and also to be excreted in the urine of rats fed cysteine or cystine [3]. It is an important intermediate in cysteine metabolism, and derived metabolically from L-cysteine sulfinic acid, and also from cysteamine as reviewed by Meister [4]. Although the occurrence of hypotaurine dehydrogenase which oxidizes hypotaurine to taurine in rat liver was demonstrated [5], little enzymological work on hypotaurine has been done. Recently we have found taurine: α-ketoglutarate aminotransferase in *Achromobacter superficialis* [6], purified to homogeneity, and crystallized to elucidate the physico-chemical and enzymological properties [7]. We report here evidence showing that taurine: α-ketoglutarate aminotransferase catalyzes also transamination of hypotaurine, which is the best amino donor in the enzyme reaction.

2. Materials and methods

Hypotaurine was synthesized from cystamine disulfoxide according to the procedure of Cavallini et al. [8]. α-Ketoglutarate, sodium L-glutamate and pyridoxal 5′-phosphate were products of Kyowa Hakko Kogyo Co., Tokyo Japan. Taurine:α-ketoglutarate aminotransferase was extracted from cells of *Achromobacter superficialis* ICR B-89 grown on the medium containing β-alanine, purified and crystallized as described previously [7]. The standard assay system consisted of 50 μmoles of hypotaurine, 50 μmoles of sodium α-ketoglutarate, 1 μmole of pyridoxal 5′-phosphate, 100 μmoles of potassium phosphate buffer (pH 8.0), and enzyme in a final volume of 1.0 ml. In a blank, enzyme or sodium α-ketoglutarate was replaced by water. The mixture was incubated at 30°C for 30 min. The reaction was terminated by addition of 0.1 ml of 25% trichloroacetic acid. The aminotransferase activity was assayed by measuring the amount of glutamate formed as described previously [9].

3. Results and discussion

After incubation, followed by deproteinization, aliquot samples of the supernatant solution were examined paper-chromatographically with ninhydrin. In addition to hypotaurine, glutamate was observed in a complete system, which was also identified as with L-glutamate decarboxylase of *Escherichia coli*. The concentration of hypotaurine decreased, and that of glutamate formed increased linearly within 60 min with prolonging the incubation time as shown in fig. 1. After 6 hr, hypotaurine had all disappeared, and glutamate was stoichiometrically formed from α-ketoglutarate only a trace of which was observed with 2,4-dinitrophenylhydrazine and by paper chromatography. This finding seems to be incompatible with the conception of aminotransferase reactions, because transamination is in general reversible.

The incubated reaction mixture was examined with

* Present address: Department of Pharmaceutical Biology, Kyoto College of Pharmacy, Yamashina, Kyoto 607, Japan.

** Present address: Department of Agricultural and Biological Chemistry, Ryukyu University, Naha, Okinawa 903, Japan.

† To whom correspondence should be sent.

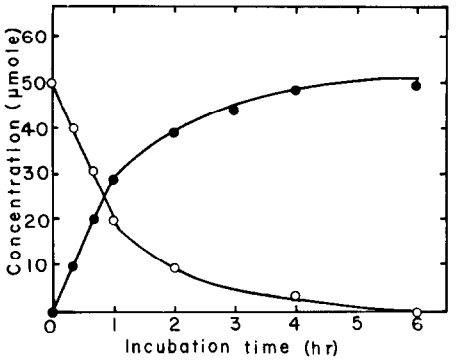


Fig. 1. Relationship between the incubation time, and the concentration of hypotaurine and L-glutamate formed. Aliquot samples of the supernatant solution obtained by centrifugation were chromatographed on Toyo filter no. 53, with phenol–ethanol–water–ammonia (150:40:1) as a solvent. Other conditions were given in the text. (○——○) Hypotaurine; (●——●) glutamate.

o-aminobenzaldehyde and glycine by a modification of the method for the determination of glyoxylic acid [10]; a yellow color, whose absorption maximum was at 440 nm was developed. No color formation was observed in a blank. Plots of the absorbance against the amount of enzyme and the incubation time, were

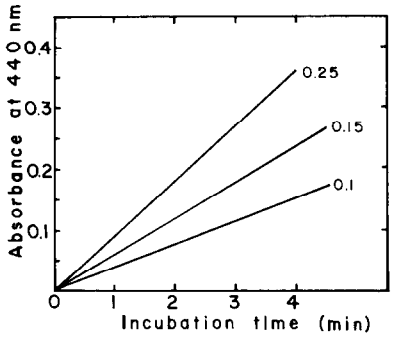
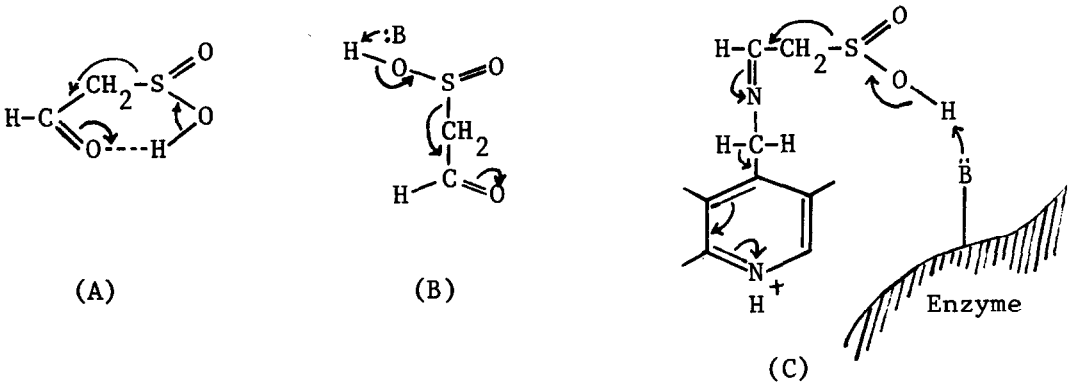


Fig. 2. Effects of the incubation time and the amount of enzyme on the hypotaurine: α-ketoglutarate aminotransferase reaction. The assay mixture consisted of 1 mmole of glycine–KCl buffer, pH 8.0, 0.5 μmole of pyridoxal 5′-phosphate, 1.25 μmoles of *o*-aminobenzaldehyde, 150 μmoles of hypotaurine, 150 μmoles of sodium α-ketoglutarate and enzyme in a final volume of 3.25 ml. The reaction was started by the addition of enzyme solution. Water was substituted for hypotaurine in a blank. Change in the absorbance at 440 nm was followed at 25°C in a cuvet with a 1-cm light path with a Shimazu MPS-50 L recording spectrophotometer. The numbers in the figure indicate the amount of enzyme protein used (mg).

linear (fig. 2). These finding suggests that hypotaurine is enzymatically transaminated with α-ketoglutarate to yield an aldehyde, probably an aldehydic acid (sulfinoacetaldehyde) or an aliphatic aldehyde, and L-glutamate. Thus, attempts were made to isolate the reaction product derived from hypotaurine.

To the reaction mixture, incubated for 120 min and deproteinized, was added 2 ml of 0.5% (w/v) 2,4-dinitrophenylhydrazine solution in 2 N HCl to yield the hydrazone. After incubation at 37°C for 10 min, 10 ml of ethyl acetate was added to the solution, and air was bubbled through the mixture for 3 min. The aqueous layer was washed again with ethyl acetate in the same manner, and the washing (ethyl acetate layer) was added to the first ethyl acetate layer. Some aliquots of the combined ethyl acetate solution were chromatographed on Toyo filter paper no. 51 with butan-1-ol–ethanol–water (7:1:2) as a solvent at about 20°C. In addition to 2,4-dinitrophenylhydrazone of α-ketoglutarate (R_f 0.73), the hydrazone of an unknown compound (R_f 0.89) was observed. To separate these two 2,4-dinitrophenylhydrazones the combined ethyl acetate solution was mixed with 1.0 ml of 10% (w/v) Na_2CO_3 solution and air bubbled through the mixture for 3 min. The ethyl acetate layer and the Na_2CO_3 layer were separately examined by paper chromatography using the same solvent. The Na_2CO_3 layer was found to contain only the hydrazone of α-ketoglutarate. The ethyl acetate layer, which contained 2,4-dinitrophenylhydrazone of an unknown product and a small amount of that of α-ketoglutarate, was washed with Na_2CO_3 solution to remove the hydrazone of α-ketoglutarate. The resultant ethyl acetate fraction contained almost all of the hydrazone of unknown product. The substance was identified as 2,4-dinitrophenylhydrazone of acetaldehyde by a comparison of the R_f values with those of the authentic sample using the above mentioned and two other solvents (butan-1-ol–ethanol–ammonia 0.5 N (7:1:2); R_f 0.85, isopropanol–water–ammonia (20:2:1); R_f 0.82). The incubation mixture terminated by addition of 1 N acetic acid was also reacted with pararose-aniline-formaldehyde reagent to show the stoichiometric formation of sulfite [11].

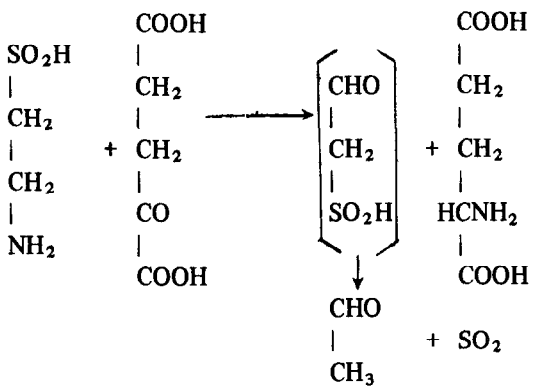
The expected product from hypotaurine by transamination, sulfinoacetaldehyde was not observed in the reaction mixture. It is likely that sulfinoacetaldehyde formed directly from hypotaurine is labile, and



Scheme 1

decomposes spontaneously (Scheme 1, A or B), or at least in part enzymatically through a ketimine intermediate of sulfinoacetaldehyde (C). The reaction is analogous to the desulfination of β-sulfinylpyruvate formed enzymatically from L-cysteinesulfinate to form pyruvate [12].

Thus, good evidence has been obtained that hypotaurine transaminates with α-ketoglutarate to yield L-glutamate, acetaldehyde and sulfite as follows.



The rate of enzymatic transamination of hypotaurine was approximately 6 times that of taurine under the standard conditions. Hypotaurine is the best amino donor in this enzyme system among the amino acids so far tested.

Acknowledgements

We thank Dr. K. Ogata, Dr. T. Fujita and Dr. T. Yamamoto for their helpful advice.

References

[1] Ouchi, S. (1959) J. Biochem. (Tokyo) 46, 765–769.
[2] Bergeret, B. and Chatagner, F. (1954) Biochim. Biophys. Acta 14, 543–550.
[3] Cavallini, D., Mondovi, B. and DeMarco, C. (1955) J. Biol. Chem. 216, 577–582.
[4] Meister, A. (1965) Biochemistry of the Amino Acids, 2nd edn, pp. 799–811, Academic Press, New York.
[5] Sumizu, K. (1962) Biochim. Biophys. Acta 63, 210–212.
[6] Toyama, S. and Soda, K. (1972) J. Bacteriol. 109, 533–538.
[7] Toyama, S., Misono, H. and Soda, K. (1972) Biochem. Biophys. Res. Commun. 46, 1374–1379.
[8] Cavallini, D., Mondovi, B. and DeMarco, C. (1965) in: Biochemical Preparations (Brown, G. B. ed), Vol. 10, pp. 72–75, John Wiley & Sons, New York.
[9] Soda, K., Tochikura, T. and Katagiri, H. (1961) Agr. Biol. Chem. 25, 811–819.
[10] Soda, K., Toyama, S., Misono, H., Hirasawa, T. and Asada, K. (1973) Agr. Biol. Chem. 37, 1393–1400.
[11] West, P. W. and Gaeke, G. C. (1956) Anal. Chem. 28, 1816–1819.
[12] Fromageot, P., Chapeville, F. and Petir, L. (1957) Biochim. Biophys. Acta 23, 12–17.