ADENYLOSUCCINATE SYNTHETASE IN RAT LIVER: THE EXISTENCE OF TWO TYPES AND THEIR REGULATORY ROLES

Yoshihiro Matsuda, Hirofumi Ogawa, Sachiko Fukutome*, Hiroshi Shiraki and Hachiro Nakagawa

Division of Protein Metabolism, Institute for Protein Research
Osaka University, Suita, Osaka, Japan 565

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SUMMARY: Rat liver contains two types of adenylosuccinate synthetase which can be distinguished by isoelectroforcusing or immunochemical analysis. One type is identical with the enzyme in rat skeletal muscle (Type M) and the other is specific for the liver (Type L). Type L was more susceptible to nucleotide inhibition, but less susceptible to inhibition by fructose-1,6-diphosphate than Type M. These differences suggest that these isozymes play different regulatory roles in the liver.

INTRODUCTION

The reaction catalyzed by adenylosuccinate synthetase [IMP: L-asparate ligase (GDP), EC 6.3.4.4] is a branch point of the main pathway of purine nucleotide biosynthesis and also one reaction on the purine nucleotide cycle (1). Thus the regulation of this enzyme activity may be very important. However, except in skeletal muscle and placenta (2-4), nothing is known about regulatory and kinetic properties of this enzyme in mammalian tissues. Therefore, we attempted to purity this enzyme from rat liver to examine its function or role. During this investigation, we found that rat liver contains two isozymes. This paper reports the characteristics of these isozymes.

MATERIALS AND METHODS

Male Wister strain rats were used throughout. Adenylosuccinate synthetase was assayed by the method of Ogawa et al. (4). Protein concentration was determined by the method of Lowry et al. (6) with bovine serum albumin as a standard. One unit of enzyme activity was defined as the amount catalyzing the formation of 1 μmole of adenylosuccinate per min. Adenylosuccinate synthetase from rat sheletal muscle was purified by the method of Ogawa et al. (4). Rabbit antiserum against adenylosuccinate synthetase from rat skeletal muscle was obtained by injecting the purified enzyme into the gluteal muscle.

*Present address; School of Social Science, Kansai University, Suita, Osaka, Japan 565

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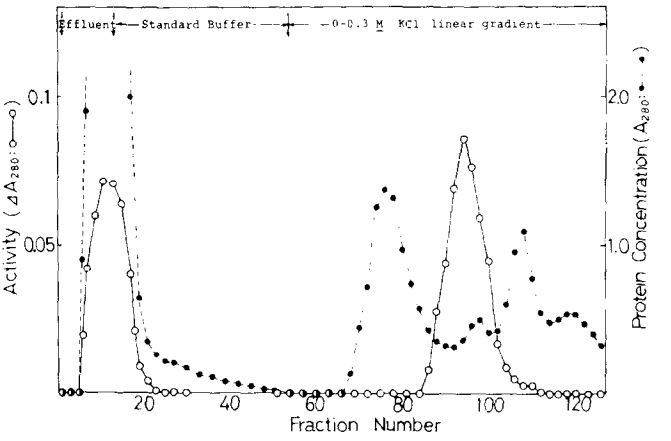

Fig. 1. Elution profile on DEAE-cellulose (DE-52) column chromatography of adenylosuccinate synthetase of rat liver. The eluate (40 ml) from a Blue-Sepharose CL 6B column was applied to a DE-52 column as described in the text. Enzyme activity is expressed as increase in optical density at 280nm under the standard assay conditions. Protein concentration is expressed as optical density at 280nm.

RESULTS AND DISCUSSION

Separation and Characterization of Two Types of Adenylosuccinate Synthetase from Rat Liver

Fresh rat liver (40 g) was homogenized with 3 vol. of 0.05 M Tris-HCl buffer (pH 7.4) containing 0.1 mM dithiothreitol (DTT). The homogenate was centrifuged for 60 min. at 105,000 x g. The supernatant was desalted by passage through a Sephadex G-25 column (4 x 40 cm). The protein fraction was collected and applied to a Blue-Sepharose CL 6B column (1.5 x 10 cm). The column was washed with 200 ml of the extraction buffer and then eluted with 0.5 M KCl. The fractions of eluate containing enzyme activity were pooled and dialyzed twice against 200 vol. of 10 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM dithiothreitol (standard buffer). The dialysate was then applied to a DE-52 column (2 x 16 cm) equilibrated with standard buffer. As shown in Fig. 1, the enzyme separated into two fractions of this column; one fraction was eluted in the void volume and the other was adsorbed on the column and eluted with a linear gradient of 0 - 0.3 M KCl. For further

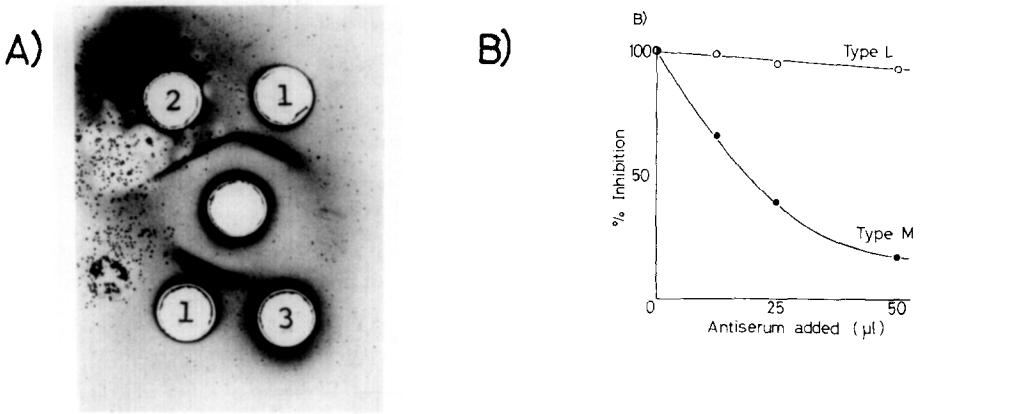

Fig. 2. Immunochemical analysis of liver adenylosuccinate synthetase with antiserum against purified adenylosuccinate synthetase from rat skeletal muscle. A) Ouchterlony gel double diffusion test. The center well contained 20 µl of antiserum and wells 1, 2 and 3 contained 20 µl of skeletal muscle enzyme (2.9 u/ml), Type M enzyme (0.55 u/ml) and Type L enzyme (0.38 u/ml), respectively. B) Inhibition of enzyme activity by antiserum. The reaction mixture contained 20 mM potassium phosphate buffer (pH 7.2), enzyme solution and various amounts of antiserum in a total volume of 0.3 ml. The reaction mixture was incubated for 30 min. at 37° and then stood for 12 hr at 4°. Then the reaction mixture was centrifuged for 20 min. at 3,000 rpm. Enzyme activity in the supernatant was assayed. As a control normal rabbit serum was added to the incubation mixture in place of the antiserum.

characterization, the two fractions were concentrated in a collodion bag, dialyzed twice against 200 vol. of standard buffer, and subjected to isoelectric forcusing (pH range, 3.5 - 10) for 48 hr at 700 V. Under these conditions the fraction not adsorbed on the DE-52 column gave one major peak of activity at pH 8.9 and several minor peaks, while the fraction adsorbed on the column gave a single peak of activity at pH 5.9. The isoelectric point of the enzyme in rat skeletal muscle is also at pH 8.9.

The two types of enzyme were examined immunochemically with antiserum against purified rat skeletal muscle enzyme. In the Ouchterlony gel double diffusion test, the antiserum gave a single precipitin line against the enzyme preparation with an isoelectric point of pH 8.9, as well as with the enzyme from skeletal muscle. Moreover, these two lines completely fused, as shown in Fig. 2. In contrast, the antiserum did not react with the fraction with

Table I. Comparison of the Michaelis constants of adenylosuccinate synthetase from liver and skeletal muscle.

Substrate	Liver		Skeletal muscle
	Type L	Type M	
	$\times 10^{-4}$ M	$\times 10^{-4}$ M	$\times 10^{-4}$ M
IMP	3.2	6.9	7.0
GTP	1.3	0.9	1.2
Aspartate	14.7	3.3	2.5

an isoelectric point of pH 5.9. Accordingly we named these two fractions Type M and Type L enzyme, respectively. In confirmation of these results, Type M enzyme and skeletal muscle enzyme were strongly inhibited by the antiserum, but Type L enzyme was not. These results suggest that there are two types of adenylosuccinate synthetase in rat liver; one is identical with skeletal muscle enzyme and the other is specific for the liver. It was calculated from the results of inhibition experiments with antiserum that normal liver contained 45% of Type M and 55% of Type L. The Type M and Type L enzymes were purified 35-fold and 100-fold, respectively, from liver extracts. These preparations contained no IMP dehydrogenase [EC 1.2.1.14], IMP cyclohydrase [EC 3.5.4.10] or nucleotidase [EC 3.1.3.31] activity.

Properties of Type M and L Enzymes

As described in the previous reports (4,5), the enzyme in rat skeletal muscle was inhibited by fructose-1,6-diphosphate (FDP) and by various nucleotides. To compare the regulatory properties of Type M and Type L enzyme in rat liver, we calculated the Km values of three substrates and the Ki values of FDP and nucleotides for each isozyme. The Km values for Type M, Type L and the enzyme from skeletal muscle are summarized in Table I. The Km values of three substrates for Type M enzyme were almost the same as those for

Table II. Comparison of the Ki values of various inhibitors for adenylosuccinate synthetase from liver and skeletal muscle.

Inhibitor	Liver		Skeletal muscle
	Type L	Type M	
	$\times 10^{-3}$ M	$\times 10^{-3}$ M	$\times 10^{-3}$ M
AMP	0.8	2.8	3.0
GMP	0.3	0.6	0.5
FDP	1.6	0.6	0.6

skeletal muscle enzyme. On the other hand, although the Km value of GTP for Type L enzyme was similar to that for Type M enzyme, the Km value of aspartate was higher for Type L than for Type M, and the Km value of IMP was higher for Type M than for Type L. These results imply that Type M enzyme, with higher affinity for aspartate, should be effective for deamination of amino acids as a member of the purine nucleotide cycle, if the cycle actually operates in the liver as reported by Moss and McGivan (7), whereas Type L enzyme, with higher affinity for IMP, should be effective in biosynthesis of purine nucleotides.

Both types of enzyme in the liver were inhibited by nucleotides: like the enzymes from rat skeletal muscle (4) and human placenta (3), they were inhibited strongly by nucleoside monophosphate and less strongly by nucleoside diphosphate, and were not inhibited by nucleoside triphosphate. However, as shown in Table II, we found that the Ki values of GMP and AMP, respectively, for Type L enzyme were 2 and 4 times lower than those for Type M enzyme. Type M and L enzymes were also inhibited by FDP, but the Ki value for Type M was 2 to 3 times lower than that for Type L enzyme. These findings indicate that Type L enzyme is the more susceptible to nucleotide inhibition, while Type M enzyme is the more sensitive to FDP inhibition. From these results it can be

concluded that Type L adenylosuccinate synthetase is important in regulation of purine nucleotide synthesis, whereas Type M enzyme contributes to regulation of glycolysis or ammoniagenesis, as the skeletal muscle enzyme does.

REFERENCES

1) Lowenstein, J. M. (1972) Physiol. Rev., 52, 382-414.
2) Muirhead, K. M. and Bishop, S. H. (1974) J. Biol. Chem., 249, 459-464.
3) Van der Weyden, M. B. and Kelley, W. N. (1974) J. Biol. Chem., 249, 7282-7289.
4) Ogawa, H., Shiraki, H., Matsuda, Y., Kakiuchi, K. and Nakagawa, H. (1977) J. Biochem., 81, 859-869.
5) Ogawa, H., Shiraki, H. and Nakagawa, H. (1976) Biochem. Biophys. Res. Commun., 68, 524-528.
6) Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem., 193, 265-275.
7) Moss, K. M. and McGivan, J. D. (1975) Biochem. J., 150, 275-283.