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STIMULATION BY ATP OF CYTOSOL-TYPE δ-AMINOLEVULINATE SYNTHASE OF RAT LIVER

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Summary

ATP stimulated the activity of δ-aminolevulinate synthase of rat liver cytosol. The enzyme exhibited negative cooperativity with respect to glycine, one of the substrates, and was inhibited by succinyl-CoA, the other substrate. ATP converted the negative cooperativity to the normal saturation kinetics and released the substrate inhibition by succinyl-CoA. These actions of ATP appear to bring about the stimulation of the enzyme.

δ-Aminolevulinate (ALA) synthase [EC2.3.1.37] which catalyzes the formation of ALA from glycine and succinyl-CoA is the first and rate-limiting enzyme in the heme biosynthesis. In the animal livers, ALA synthase can be induced by the administration of porphyrinogenic drugs such as allylisopropylacetamide or 3,5-dicarbethoxy 1,4-dihydrocollidine (1). Although ALA synthase is usually located in the mitochondrial matrix, the induction of this enzyme by the drugs results in its accumulation not only in the mitochondria but in the cytosol of various animal livers (2-6). The enzyme accumulating in the cytosol has been suggested to be a precursor of the mitochondrial enzyme (2).

Hayashi et al. (7) showed that the molecular size of the

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cytosol ALA synthase is much larger than that of the mitochondrial enzyme. Recently Ohashi and Kikuchi (8,9) have provided evidence that cytosol-type ALA synthase of rat liver is composed of three different proteins: one is mitochondrial-type ALA synthase and the other two are catalytically inactive proteins, the function of which is not yet clear.

While studying the properties of the cytosol-type ALA synthase purified to apparent homogeneity, we happened to observe that the enzyme activity is markedly stimulated by ATP. To clarify this stimulatory mechanism, some kinetic properties of the enzyme were analyzed in the presence and absence of ATP. The present communication describes the results of these studies.

Materials and Methods

The cytosol-type ALA synthase was purified to apparent homogeneity as described previously from the rat treated with allylisopropylacetamide and insulin (9). Pyridoxal phosphate, ATP, ADP, AMP, cAMP, NAD^+, $NADP^+$, NADH, HADPH, and GTP were purchased from Boehringer, Manheim. Succinyl-CoA was a product of P-L Biochemicals, Inc., Milwaukee, and assayed as the hydroxamate (10).

ALA synthase was assayed, unless otherwise indicated, in a reaction mixture containing in a final volume of 1 ml; 0.1 mM succinyl-CoA, 100 mM glycine, 0.1 mM pyridoxal phosphate, 50 mM Tris-HCl (pH 7.6) and the enzyme. Reactions were carried out for 15 min at 37°C in a shaking water bath incubator and stopped by the addition of 0.2 ml of 12.5% trichloroacetic acid. The reaction proceeded linearly with time at least up to 15 min. ALA formed was converted to the pyrrole compound, which was isolated by a Dewex-1 column, and estimated colorimetrically as described previously (5). The blank contained all the reaction mixture to which the enzyme was added just prior to the addition of trichloroacetic acid. High concentrations of glycine, however, interfered with this assay by producing a faint color. When glycine was employed as a variable substrate, the assay mixture without the enzyme was analyzed in the same manner as samples, and the activities were corrected for this nonspecific absorbance.

Inorganic phosphate was estimated by the method of Marsh (11). Protein concentrations were determined by the method of Lowry *et al.* with bovine serum albumin as a standard (12).

Results

The data in Fig.1 show that the activity of the cytosol ALA synthase increased with increasing the ATP concentration up to

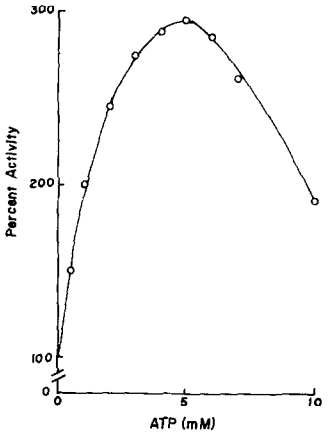

Fig.1. Effects of ATP on the activity of the cytosol ALA synthase. Enzyme activities were assayed as described in Materials and Methods except that assay mixtures contained 7 µg of the cytosol ALA synthase and varying concentrations of ATP. The value of ALA formed without ATP (100%) was 3.3 nmoles.

5 mM, but decreased at higher concentrations. Maximum stimulation occurred at about 5 mM ATP. During the reaction period, no appreciable hydrolysis of ATP could be detected and the stimulatory effect of ATP was not affected by either 5 mM $MgCl_2$ or 5 mM cAMP, suggesting that the phosphorylation of the enzyme is not involved in the stimulation. As shown in Table 1, ATP was most effective among the nucleotides tested, although ADP and AMP were slightly stimulatory. GTP, cAMP, NAD^+, $NADP^+$, NADH, and NADPH had virtually no effect on the enzyme activity.

In order to elucidate the stimulatory mechanism, experiments were carried out to determine the effect of ATP on the kinetic properties of the enzyme. When the activity was assayed over a wide range of glycine concentrations (0-400 mM), the usual Michaelis-Menten hyperbola could not be obtained. As illustrated in Fig.2 the double reciprocal plot is biphasic. A concave downward appearance and a Hill coefficient of 0.6 calculated from the same data suggest

Table 1. EFFECT OF NUCLEOTIDES ON THE ACTIVITY OF THE CYTOSOL ALA SYNTHASE

Addition	% of Control
None	(100)
ATP	280
ADP	175
AMP	135
cAMP	108
GTP	118
NAD^+	109
$NADP^+$	117
NADH	115
NADPH	109

Enzyme activities were assayed as described in Materials and Methods except that assay mixtures contained 7 μg of the cytosol ALA synthase and a nucleotide at a final concentration of 5 mM. The value of ALA formed in control experiment (100%) was 3.3 nmoles.

that the enzyme exhibits negative cooperativity with respect to glycine. An apparent Km value of 12 mM was estimated by extrapolating the linear portion of the curve corresponding to the glycine concentrations over 20 mM. This value is in accord with the value of 11 mM obtained by Scholnick et al. (13) with a less purified enzyme.

Figure 2 also shows that the cooperativity of the enzyme was altered by the addition of ATP. The curved line of the control experiment was transformed by 5 mM ATP into a straight line which

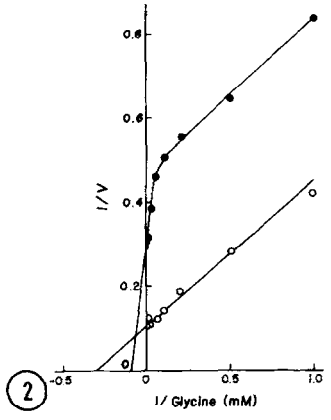

Fig.2. Relationship between the rates of ALA formation and glycine concentrations. Enzyme activities were assayed as described in Materials and Methods except that assay mixtures contained 7.5 μg of the cytosol ALA synthase and varying concentrations of glycine with (o—o) or without (●—●) 5 mM ATP. V represents nmoles of ALA formed under the assay conditions.

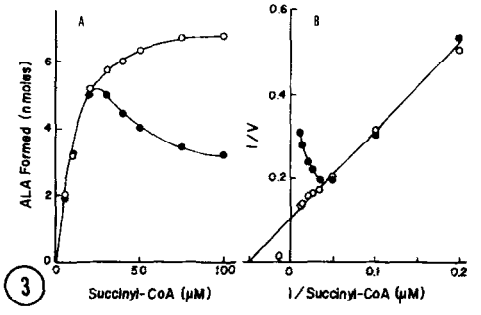

Fig.3. Relationship between the rates of ALA formation and succinyl-CoA concentrations. A: Enzyme activities were assayed as described in Materials and Methods except that assay mixtures contained 7 μg of the cytosol ALA synthase and varying concentrations of succinyl-CoA with (o—o) or without (●—●) 5 mM ATP. B: Double reciprocal plot of the same data.

was also confirmed by a Hill coefficient of 1.0 calculated from the same data. ATP also caused a two-fold increase in the maximum velocity. An apparent Km was estimated to be 3.3 mM, which corresponds well to the value reported by Hayashi et al. who estimated it in the presence of ATP with a partially purified enzyme (2).

When succinyl-CoA was used as a variable substrate, the enzyme exhibited the Michaelis-Menten kinetics up to 20 μM. At higher concentrations, however, the activity was considerably inhibited (Fig. 3). These results confirm the earlier finding obtained with a less purified enzyme from rat liver cytosol (13). From a double reciprocal plot shown in Fig. 3 B, an apparent Km value was estimated to be about 20 μM. Figure 3 also shows that the addition of ATP yielded a

Michaelis-Menten hyperbola or a straight line in a double reciprocal plot over the entire range of concentration. The Km value was not changed appreciably by the addition of ATP (Fig.3 B)

Discussion

The present study has revealed some novel properties of ALA synthase: (i) the enzyme activity is stimulated by ATP; (ii)the enzyme displays negative cooperativity with respect to glycine which is abolished by ATP; (iii) the substrate inhibition by succinyl-CoA is released by ATP. Although the exact mechanism is unknown, ATP may bind to an allosteric site(s) of the enzyme and may cause a conformational change of the substrate binding sites for both glycine and succinyl-CoA, resulting in the stimulation of enzyme activity.

The present study explains the discrepancy between the reported Km values for glycine of the ALA synthase of rat liver cytosol. Hayashi et al.(2) carried out their study using an assay mixture containing ATP as a constituent of a succinyl-CoA generating system. However, Scholnick et al. (13) employed an assay system containing no ATP but chemically synthesized succinyl-CoA. It is obvious from the data in Fig.2 that the Km value for glycine was lowered from 12 to 3.3 mM by the addition of ATP; the former value corresponds well to that reported by Scholnick et al. and the latter value to that by Hayashi et al.

The present results were obtained with the cytosol-type ALA synthase, which is a possible precursor of the enzyme in the mitochondria where it functions physiologically (2). Further studies using ALA synthase isolated from mitochondria are necessary to clarify whether ATP actually plays an important role in the regulation of ALA synthesis in the liver.

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