Vol. 108, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 16, 1982 Pages 286-291

ROLE OF GTP IN CTP SYNTHETASE FROM EHRLICH ASCITES TUMOR CELLS

Harutoshi Kizaki, Fumihiro Ohsaka and Tomomi Sakurada

Department of Biochemistry, School of Medicine, Keio University

35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan

Received July 14, 1982

GTP was nearly essential as an activator for the CTP synthetase from Ehrlich ascites tumor cells when glutamine was the nitrogen source. GTP accelerated the glutaminase activity of the enzyme about 2.5-fold in the absence of the other substrates and about 45-fold in the presence of UTP and ATP. UTP and adenylylimidodiphosphate, a competitive inhibitor for ATP (Ki=1.1 mM), did not affect the glutaminase activity. In addition to the activating effect, GTP could replace ATP as a substrate with a Km of 1.7 mM in the glutamine reaction and 2.2 mM in the ammonia reaction.

INTRODUCTION

CTP synthetase (UTP:ammonia ligase, EC 6.3.4.2) which catalyzes the formation of CTP from UTP in the presence of ATP, glutamine and magnesium has been highly purified from E. coli (1-3) and partially from calf liver (4,5) and Ehrlich ascites tumor cells (6). Ammonia can replace glutamine as amino donor for all enzymes (1,6,7). GTP is a positive allosteric effector for the bacterial and liver enzymes when glutamine is utilized as amino donor (4,8). We reported that for the enzyme from Ehrlich ascites tumor cells, GTP activated the reaction with glutamine, but it had almost no effect on the reaction with ammonia (6).

The activity of the bacterial and liver enzymes depends absolutely upon the presence of ATP, (1,4), whereas the activity of the enzyme from Ehrlich ascites tumor cells was observed in the absence of ATP when GTP was present. This communication showns that in the enzyme from Ehrlich ascites tumor cells, GTP

Abbreviation used: ADPNP, Adenylylimidodiphosphate.

0006-291X/82/170286-06$01.00/0

acted not only as an activator of the glutaminase activity of the enzyme, but also as a substitute for ATP.

MATERIALS AND METHODS

All reagents and materials were described in the preceding paper (6). CTP synthetase activity and protein were determined as described previously (6). [U-^{14}C] glutamine (267 mCi/mmol) was purchased from New England Nuclear, Matrex Gel Green A from Amicon Corp.

Enzyme purification: The purification procedure was the same as described previously (6) except additional chromatography on Matrex Gel Green A column. The fraction VII obtained by Sephacryl S300 Gel filtration was applied on a Matrex Gel Green A column (2 X 8 cm) which had been previously equilibrated with a buffer, 35 mM Tris-HCl, pH 7.4/100 mM 2-mercaptoethanol/20% glycerol. The column was washed with the equilibration buffer and eluted with 140 ml linear gradient of KCl, 0-1.0 M, in the same buffer. The active fractions were pooled and concentrated by 50% saturating ammonium sulfate. The pellet collected was dissolved and dialyzed against the buffer, 35 mM Tris-HCl, pH 7.4/100 mM 2-mercaptoethanol/30% glycerol.

Determination of glutaminase activity. Glutaminase activity was measured under standard conditions for CTP synthetase assay except that 1 mM glutamine and [^{14}C] glutamine were used instead of 10 mM glutamine and [^{14}C] UTP. After termination of the reaction by boiling, an aliquot of the reaction mixture was applied on a thin layer cellulose plate together with carrier solution containing glutamine and glutamate. Chromatography was performed in a solvent containing isopropanol, formic acid and water (40:2:10 v/v) (9) and the marker spots were identified by lightly spraying the dried plate with a 0.2% ninhydrin in n-butanol, cut out and counted.

RESULTS AND DISCUSSION

The specific activity of the most purified enzyme was 7.62 μmol/h/mg protein, which represented about 450-fold purification. Polyacrylamide gel electrophoresis in the presence of SDS showed one major band and a few minor bands which accounted about 5-8% of the total protein.

In the absence of GTP, the activity with glutamine was about 4% of that obtained in the complete system, whereas the activity with ammonia was not activated by GTP, rather slightly inhibited by GTP (Table 1). This result suggested that GTP might stimulate the hydrolysis of glutamine by activating glutaminase activity of the enzyme. Under the conditions in which ATP was omitted and GTP was present at 1 mM the activity with glutamine or ammonia

TABLE I

EFFECT OF GTP AND ATP ON THE CTP SYNTHETASE ACTIVITY

	Activity (μmol/h/mg protein)	
	Glutamine	Ammonia
Complete	7.62	7.38
Omit GTP	0.28	7.76
Omit ATP	1.02	0.78
Omit ATP and GTP	0	0

Reaction mixture in the complete system contained 35 mM Tris-HCl buffer, pH 8.6, 1 mM UTP, 12 mM ATP, 1 mM GTP, 18 mM $MgCl_2$, 50 mM 2-mercaptoethanol, 0.0625 μCi [^{14}C] UTP and 10 mM glutamine or 10 mM ammonium sulfate.

was about 12% of that obtained in the complete system (Table 1). It was verified by high performance liquid chromatography that GTP and UTP solutions did not contain ATP as impurity. GTP might substitute for ATP as a substrate. Indeed, the activity was not detected in the absence of both ATP and GTP.

Role of GTP as an activator of the glutaminase activity.

The effects of GTP and the other substrates on the glutamine hydrolysis were examined (Table 2). The glutaminase activity in the absence of GTP and the other substrates was about 1% of that observed in the complete system. It was stimulated about 2.5-fold by the addition of GTP and this activation was not accompanied by GTP hydrolysis (data not shown). UTP or ATP alone did not affect the glutaminase activity. When UTP and ATP were present in the reaction mixture, CTP was formed and its amount (2.2 nmol) was nearly equivalent to that of glutamate formed. Addition of adenylylimidodiphosphate (ADPNP) in place of ATP prevented CTP formation and had no effect on the glutaminase activity. ADPNP, which is a nitrogen analogue of ATP prepared by

TABLE II

EFFECT OF GTP AND THE OTHER SUBSTRATES ON THE GLUTAMINASE ACTIVITY OF CTP SYNTHETASE

Additions	Glutamate formed (nmoles/10 min)
Mg^{2+}	0.87
Mg^{2+} + GTP	2.44
Mg^{2+} + UTP	0.88
Mg^{2+} + ATP	0.86
Mg^{2+} + UTP + ATP	2.13
Mg^{2+} + UTP + ADPNP	0.90
Mg^{2+} + UTP + ATP + GTP	95.04[a]

Reaction mixture contained 1 mM glutamine, 0.125 μCi [^{14}C] glutamine, 50 mM 2-mercaptoethanol, and 35 mM Tris-HCl buffer (pH 8.6) and 7.6 μg enzyme in a final volume of 50 μl and the substances specified in the Table at the following concentration: 18 mM Mg^{2+}, 1 mM GTP, 1 mM UTP, 12 mM ATP, 12 mM ADPNP.
a: Calculated value from the experiments with 0.38 μg enzyme

Yount et al. (10), was shown to be a competitive inhibitor for ATP with a Ki value of 1.1 mM. In the bacterial enzyme, UTP and ADPNP activate the glutaminase activity about 6-fold accompanying with polymerization of the enzyme from dimer to tetramer.

Marked acceleration of the glutamine hydrolysis by GTP was observed in the presence of UTP and ATP, and the equivalent amount of CTP (96.8 nmol) was produced.

It appears that the glutaminase activity of the enzyme may normally present in a latent form in the absence of the other substrates and be enhanced by GTP, particularly in the presence of UTP and ATP. UTP and ATP might form the true acceptor of $-NH_2$, presumably a phosphorylated intermediate of UTP (11). A latent glutaminase activity which is activated by other sub-

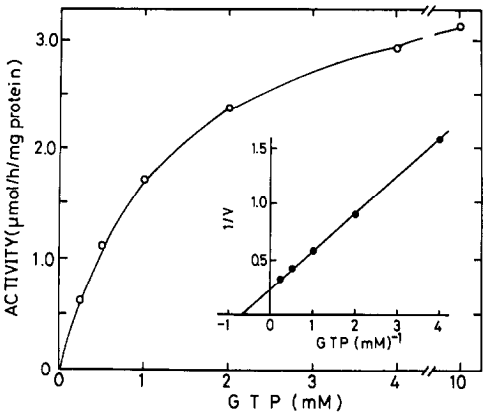

Fig. 1. Effect of GTP on CTP synthetase activity in the absence of ATP. CTP synthetase activity was determined under standard conditions except ATP was omitted and GTP concentration was varied. The insert shows the data plotted in the double reciprocal form. 1/V is expressed as $(\mu mol/h/mg\ protein)^{-1}$.

strates has been reported in other glutamine amidotransferases (8,12,13).

Role of GTP as a substrate.

CTP was formed in the absence of ATP depending on GTP concentration (Fig. 1). The saturation curve was hyperbolic over the ranges examined (0.25 - 10 mM). The double reciprocal plot was almost linear and yielded a Km = 1.7 mM. Maximum activity observed at 10 mM GTP was about 40% of that obtained under standard conditions. GTP could also substitute for ATP in the ammonia reaction in the absence of ATP with a Km of 2.2 mM. The substrate specificity for high energy source in the enzyme from Ehrlich ascites tumor cells was not strict compared to that of the bacterial and liver enzymes, whose activity depends absolutely upon the presence of ATP.

The affinity for GTP as a substrate was about 30-fold lower than that as an activator in the presence of ATP and 2-fold lower than that for ATP (6). Taking account of the concentrations of ATP (1570 nmol/g cells) and GTP (390 nmol/g cells, unpublished data), GTP may act, in vivo, mainly as an activator which in-

creases the glutaminase activity of the enzyme in concert with UTP and ATP. The present studies suggest that the enzyme from Ehrlich ascites tumor cells might have different properties and reaction mechanism from those of the bacterial and liver enzymes.

ACKNOWLEDGEMENT

The authors wish to thank Mr. Y. Tanizaki and Mrs. A. Sasaki for technical assistance.

This work was supported in part by a Grant in-Aid from the Ministry of Education, Science and Culture of Japan.

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