CARBAMYL PHOSPHATE SYNTHETASE II IN THE MUCOSAL CELLS OF GALL BLADDERS

Beth Murphy
Department of Chemistry
Phillips University
Enid, Oklahoma 73701

Duncan W. Martin
Department of Zoology
University of Arkansas
Fayetteville, Arkansas 72701

Received May 19,1972; revised August 21,1972

SUMMARY

A glutamine dependent carbamyl phosphate synthetase has been detected in the extra mitochondrial fraction of gall bladder mucosal cells obtained from rabbits and cattle. This enzyme is inhibited by azaserine. Thus, the enzyme appears to be carbamyl phosphate synthetase II. The activity of the preparation obtained from rabbits is four to seven times that of the bovine gall bladder.

Carbamyl phosphate synthetase II, a glutamine dependent synthetase, provides for carbamyl phosphate utilized in pyrimidine biosynthesis. This enzyme occurs in mammalian tissues which exhibit continual mitotic activity. Thus, the level of carbamyl phosphate synthetase in the tissue parallels tissue growth. Tatibana and Ito (1967, 1969) studied the enzyme obtained from hematopoietic mouse spleen and they also report the occurance of the enzyme in several other mouse tissues. Fetal rat liver has been used as an enzyme source (Hager & Jones, 1967a) and the enzyme has also been described in neoplasms (Hager and Jones, 1967b, Yip and Knox, 1970). This carbamyl phosphate synthetase is distinguished from the carbamyl phosphate synthetase I of the ornithine cycle in that the latter is of mitochondrial origin, requires N-acetyl glutamate as a cofactor, and it utilizes nitrogen from transaminase reactions (Jones 1963, Cohen 1970). We present evidence which indicates that a carbamyl phosphate synthetase

occurs in the gall bladder mucosa. The origin of this enzyme is extra mitochondrial, it utilizes glutamine, and is inhibited by azaserine. This enzyme is tentatively identified as carbamyl phosphate synthetase II.

MATERIALS AND METHODS

Chemicals--Biological chemicals were purchased from Nutritional Biochemicals, except for the azaserine which was obtained from Calbiochem. All other chemicals were reagent grade and were used without further purification. $NaH\ ^{14}CO_3$ was obtained from New England Nuclear.

Ornithine carbamoyltransferase (E. C. 2. 1. 3. 3) was purified from calf liver acetone powder by the method of Caravaca and Grisolia (1960).

Preparation of tissues--Bovine gall bladders were obtained from the Mhoon Wholesale Beef Company. After the gall bladders were removed they were washed immediately with cold mammalian phosphate Ringer's solution. The mucosal cells were removed from the everted organ by scraping with a glass slide. The cells were then suspended in cold Ringer's solution in a Dewar flask for transport to the laboratory. Bovine gall bladders were handled individually and there was no pooling of cells. The method of preparation and viability of the cells was initially validated by measuring oxygen consumptions with a Clark oxygen electrode (YSI 4004), with and without added substrates. Any gall bladder with a slimy appearance was discarded since it was found that such gall bladders no longer consumed oxygen. Rabbits were sacrificed in the laboratory and mucosal cells which were scraped from the serosa were pooled and suspended in Ringer's solution.

The cell suspension was centrifuged at 600 x g for 10 minutes, the supernatant Ringer's solution was removed by suction and the packed cells

were weighed. Ten percent homogenates of these cells were made using an extraction medium similar to that of Tatibana and Ito (1969) consisting of 40 mM glycerol, 25 mM HEPES at pH 7.5, 1 mM $MgCl_2$, 2.5 mM glutathione, and 0.1 mM ATP at pH 7.0. Supernatants of a 20,000 x g or 110,000 x g were used for the enzyme assays.

Assay of the enzyme--The assay of carbamyl phosphate synthetase II was a modification of that of Tatibana and Ito (1969). ^{14}C-carbamyl phosphate formed during enzyme incubation with NaH $^{14}CO_3$ is converted to ^{14}C-citrulline in the presence of ornithine carbamyltransferase (OTC). The assay was carried out in 1 ml volume in a 6 ml centrifuge tube. The basic assay mixes for the homogenates prepared at the two different centrifuge speeds were as follows: 20,000 x g) 18.8 mM HEPES at pH 7.4, 18.8 mM $KHCO_3$, 7.5 mM ATP at ph 7.0, 11 mM $MgCl_2$, 0.75 mM ornithine, and 2.5 mM glutathione. 100,000 x g) 21 mM HEPES at pH 7.5, 21 mM $KHCO_3$, 9 mM ATP at pH 7.0, 13 mM $MgCl_2$, 0.9 mM ornithine, 2.7 mM glutathione. To each of these mixtures was added 2.5 units of OTC (a unit being defined as the production of one millimole of citrulline in 15 min at 38°) and 2.27 x 10^6cpm NaH $^{14}CO_3$. The volumes of homogenates used and the concentrations of glutamine and azaserine are given in Tables I and II. The incubation period was 25 minutes at 38°. The reaction was terminated by plunging the tubes into an ice bath and adding 1 ml of ice cold 5% Cl_3CCOOH to precipitate the proteins and drive out the $^{14}CO_2$. The latter was trapped in 20% KOH coated on a glass rod inserted into a rubber stopper which sealed the centrifuge tubes. Precipitated protein was removed by centrifugation at 4000 x g. 0.1 ml or a 0.02 M citrulline solution was added and the supernatant was run through a Dowex 50-H+, 200-4--mesh column 0.6 x 4 cm, the

TABLE I

Carbamyl phosphate synthetase activity in bovine gall bladder mucosal cells 20,000 xg homogenate. See text for assay mix. 10 mM glutamine added as substrate

Exp.	homogenate ml	citrulline formed nanomoles/g/h	
		No OTC	With OTC
1	0.3	1.7	57
2	0.3	2.6	76
3	0.3	-	40
	0.35	0	44
4	0.2	-	28
	0.35	2.1	27

TABLE II

Effect of azaserine on enzyme activity of bovine and leporine gall bladders. Homogenates prepared at 100,000 xg. Homogenate vol 0.3 ml.

Exp.	Substrate mM		citrulline formed nanomoles/g/h	
cattle	gln^2	azaserine	no azaserine	with azaserine
1*	5	5	40	32
2	0.01	11	70	19
3**	0.01	11	52	6
rabbits				
a	0	0	280	-
b	2	11	372	92

* Homogenate prepared at 20,000 x g.
** Homogenate stirred with Dowex-1-Cl, 20-40 beads for 2 min.

column was washed with 10 ml of water and the amino acids were eluted with 5 ml of 10% pyridine. The pyridine was removed by evaporation with

a blower-heater. The dried planchets were counted with a Nuclear-Chicago gas flow counter.

RESULTS

Enzyme activity in 20,000 x g homogenates-- Table I shows the results of the assay for carbamyl phosphate synthetase II in 20,000 x g ml homogenates of bovine gall bladder mucosal cells. The incorporation of ^{14}C in the absence of OTC was considered negligible so no corrections were made in subsequent experiments.

Effect of azaserine--Azaserine is a competitive inhibitor of glutamine utlizing enzymes (Mahler and Cordes, 1971). Hager and Jones (1967a) showed that this compound inhibits carbamyl phosphate synthetase II in fetal rat liver. As shown in Table II the activity both of bovine and leporine gall bladders carbamyl phosphate synthetase II is inhibited by azaserine. The inhibitor had only a modest effect when the glutamine concentration was relatively high but at low concentrations of glutamine and at much higher concentrations of azaserine (Exps. 2 and 3) the inhibition is quite pronounced. Presumably the greater inhibition shown in Exp. 3 even though added glutamine concentration is higher, is due to removal of some endogenous substrate by the Dowex-1-Cl.

Activity of the enzyme in rabbit gall bladder--Table II also shows the results of the assay of the enzyme in mucosal cells of the gall bladder of the rabbit. These results indicate that the level of endogenous substrate (glutamine) in these cells must be relatively high. In addition, the activity of the enzyme is about 4 to 7 times that obtained for the bovine gall bladder.

Acknowledgment--This work was supported by grant number NIH AM 12832 to D. W. M.

LITERATURE CITED

Caravaca, J., and Grisolia, S., J. Biol. Chem, 235, 684 (1960).
Cohen, P. P. Science, 168:533 (1970).
Hager, S. E., and Jones, M. E., J. Biol. Chem., 242, 5667 (1967).
Hager, S. E., and Jones, M. E., J. Biol. Chem., 242, 5674 (1967).
Jones, M. E. Science, 140:1373 (1963).
Mahler, H. R., and Cordes, E. R., Bioligical Chemistry, Harper and Row, New York, (1971).
Tatibana, M., and Ito, K., Biochem. Biophys. Res. Commun. 26, 221 (1967).
Tatibana, M., and Ito, K., J. Biol. Chem. 244, 5403 (1969).
Yip, M. C. M., and Knox, W. E., J. Biol. Chem. 245, 2199 (1970).