EFFECT OF ORNITHINE, IMP, AND UMP ON CARBAMYL PHOSPHATE SYNTHETASE FROM ESCHERICHIA COLI[1,2]

Paul M. Anderson and Sally V. Marvin

Department of Chemistry
Southern Illinois University
Carbondale, Illinois 62901

Received July 29, 1968

Carbamyl phosphate synthetase from Escherichia coli catalyzes the following reaction (Anderson and Meister, 1965):

$$2\ ATP + HCO_3^- + \text{L-glutamine} + H_2O \xrightarrow{K^+,\ Mg^{++}} 2\ ADP + \text{L-glutamate} + P_i + \text{carbamyl phosphate}$$

Carbamyl phosphate is a precursor for the two metabolic pathways leading to pyrimidine nucleotides and to arginine, respectively. The enzyme is subject to feedback inhibition by UMP, a pyrimidine nucleotide, and is activated by IMP and other end products of purine nucleotide biosynthesis; these interactions provide a mechanism for maintaining a relationship between the rates of purine and pyrimidine nucleotide biosynthesis (Anderson and Meister, 1966). Pierard (1966) has shown that ornithine, which reacts with carbamyl phosphate to give citrulline in the arginine pathway, is able to reverse the inhibition by UMP; this property of the enzyme assures a continued supply of carbamyl phosphate when it is needed for arginine biosynthesis (and, hence, protein biosynthesis) under conditions where excess UMP is present. The effect of ornithine alone on

---

[1] Supported by Public Health Service Grant AM 11443

[2] Presented at the Great Lakes Regional Meeting of the American Chemical Society, Milwaukee, 1968.

the activity of the enzyme has not been reported and the mechanism whereby ornithine, IMP, and UMP exert their respective allosteric effects on carbamyl phosphate synthetase is not known.

We have found that ornithine alone activates the enzyme and that the properties of this activation are similar to those observed with IMP. Studies employing gel filtration on Sephadex G-200 and sucrose density gradient centrifugation have shown that the molecular size of the enzyme is increased in the presence of ornithine or IMP (positive allosteric effectors) and decreased in the presence of UMP (a negative allosteric effector). These observations are the subject of this communication.

METHODS AND MATERIALS

Carbamyl phosphate synthetase was isolated from Escherichia coli B by a modification of the procedure described by Anderson and Meister (1965); DEAE-sephadex was used in place of DEAE-cellulose and the step involving adsorption on calcium phosphate gel was eliminated. The enzyme activity was determined by measuring the rate of ADP or ^{14}C-carbamyl phosphate formation as previously described (Anderson and Meister, 1966). Catalase was assayed by measuring the decrease in absorbance of H_2O_2 at 240 mμ as described by Luck (1965). Sucrose density gradient centrifugation was carried out in a SW 50L rotor at 4° for 6 hours at 48,000 RPM with a Spinco Model L2-65B centrifuge, essentially as described by Martin and Ames (1961). Catalase, which has a molecular weight of 250,000 and a $s_{20,w}$ of 11.3, was used as a standard for calculating the sedimentation constants by the procedure of Martin and Ames (1961).

Glutamine, ATP, UMP, IMP, catalase (beef liver), and ornithine were obtained from Sigma Chemical Co. ^{14}C-sodium bicarbonate was obtained from New England Nuclear Corp. Escherichia coli B (washed, 3/4 log phase, grown on enriched medium) was purchased from Grain Processing Corp. Sephadex G-200 and Blue Dextran 2000 were obtained from Pharmacia Fine Chemicals, Inc.

RESULTS AND DISCUSSION

As shown in Figure 1, ornithine alone activates carbamyl phosphate synthetase. Although the maximum effect of ornithine at low ATP concentration is greater than that observed with IMP, the effects are apparently analogous. The activating effect of ornithine is not dependent on the presence of UMP, suggesting that the observed reversal of UMP inhibition by ornithine might be the result of the activating effect of ornithine competing with the inhibitory effect of UMP. The finding that ornithine is a positive allosteric effector is consistent with the results reported below.

The effect of ornithine, IMP, and UMP on the elution of carbamyl phosphate synthetase from Sephadex G-200 at 4° is shown in Figure 2. The

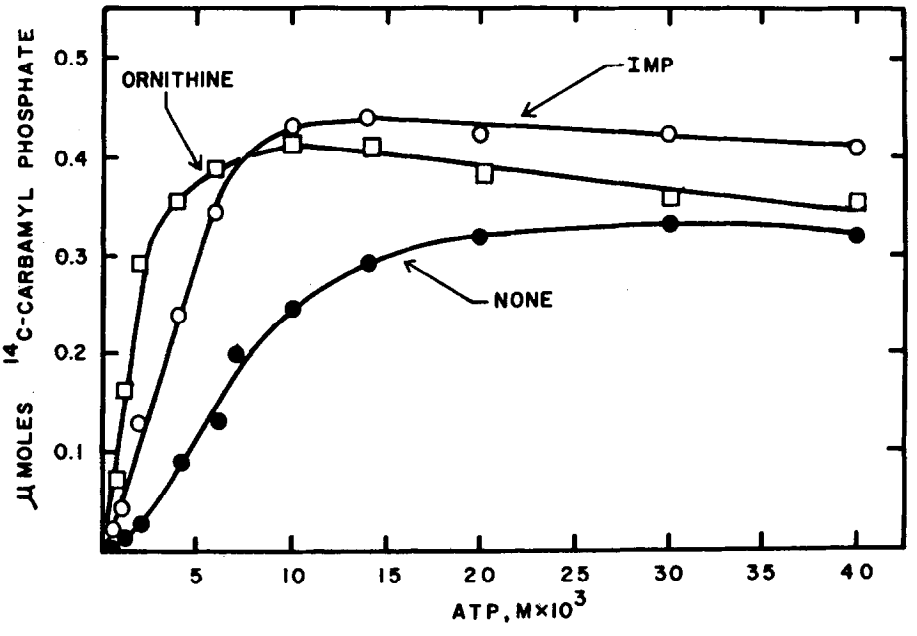

Figure 1. Effect of ornithine and IMP on carbamyl phosphate synthetase activity as a function of ATP concentration. The reaction mixtures contained $MgCl_2$ in concentrations that were equimolar with ATP, ^{14}C-$NaHCO_3$ (10 mM, 300,000 CPM), KCl (100 mM), Tris-HCl buffer (100 mM, pH 8.0), L-glutamine (10 mM), potassium phosphate (0.1 mM), and enzyme (0.01 mg) in a final volume of 1.0 ml. Ornithine (10 mM) and IMP (2 mM) were included in the reaction mixture as indicated. The ^{14}C-carbamyl phosphate synthesized after incubation for 10 minutes at 37° was determined.

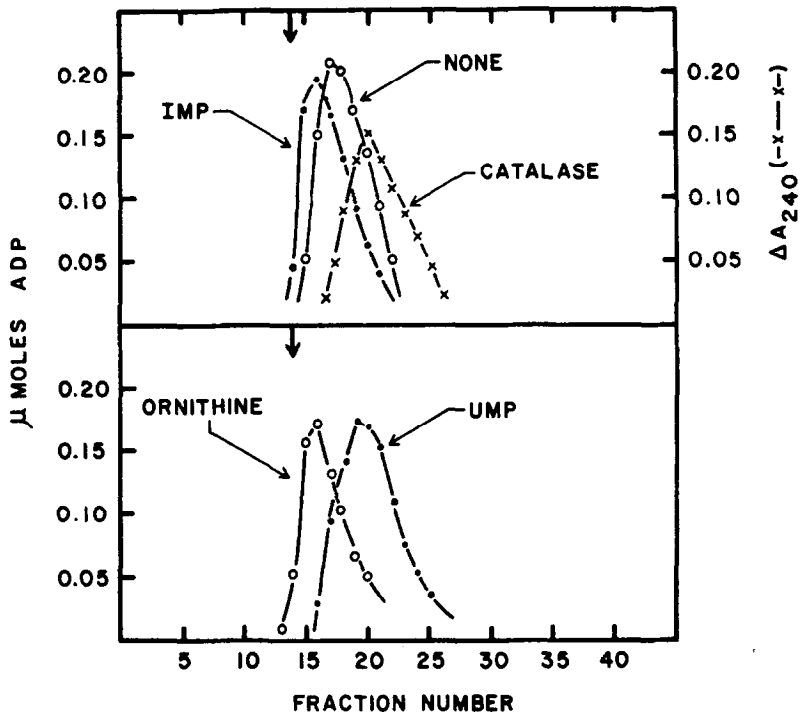

Figure 2. Effect of ornithine, IMP, and UMP on the elution of carbamyl phosphate synthetase from Sephadex G-200. The column (0.9 x 29 cm) was equilibrated immediately before each experiment with 0.1 M potassium phosphate buffer, pH 7.8, at 5°. Where indicated the buffer also contained IMP (2 mM), UMP (1 mM), or ornithine (10 mM). The sample (0.2 ml) which was applied to the top of the gel bed contained enzyme (0.2 mg), potassium phosphate buffer (0.1 M), sucrose (0.25 M), and Blue Dextran 2000 (0.1%). Elution was initiated after potassium phosphate buffer (0.1 M, pH 7.8) was layered over the top of the sample. Fractions containing 12 drops (0.44 ml) were collected at a rate of 3.5 ml per hour. The Blue Dextran was located by measuring the absorbance at 320 mμ; the void volume of the gel was considered to be identical with the elution volume of the Blue Dextran and is indicated in the figure by the vertical arrows. The enzyme was located by measuring the μmoles of ADP formed after 10 minutes when an aliquot of appropriate volume from each fraction was incubated with ATP (20 mM), glutamine (10 mM), $NaHCO_3$ (10 mM), Tris-HCl (100 mM, pH 8.0), and KCl (100 mM) in a final volume of 0.3 ml, 37°; the reaction mixtures also contained ornithine (20 mM) when UMP was present in the fractions. Catalase (0.02 mg) was applied to and eluted from the gel in the same way, except carbamyl phosphate synthetase was absent; the activity of catalase was determined by measuring the decrease in absorbance at 240 mμ when an aliquot (0.1 ml) of each fraction was incubated at 26° with 3 ml of a solution containing 0.01 M potassium phosphate buffer, pH 7.0, and 0.01 M H_2O_2.

volume of eluant required to elute the enzyme was significantly less in the presence of ornithine and IMP, and greater in the presence of UMP, than in the absence of allosteric effectors. Similar results were obtained when the elution was carried out at 26°.

As shown in Figure 3, the rate of sedimentation of carbamyl phosphate synthetase in sucrose density gradients is also markedly affected by the presence of allosteric effectors. The $s_{20,w}$ calculated from this data by the method of Martin and Ames (1961) for the enzyme is 15.2, 15.7,

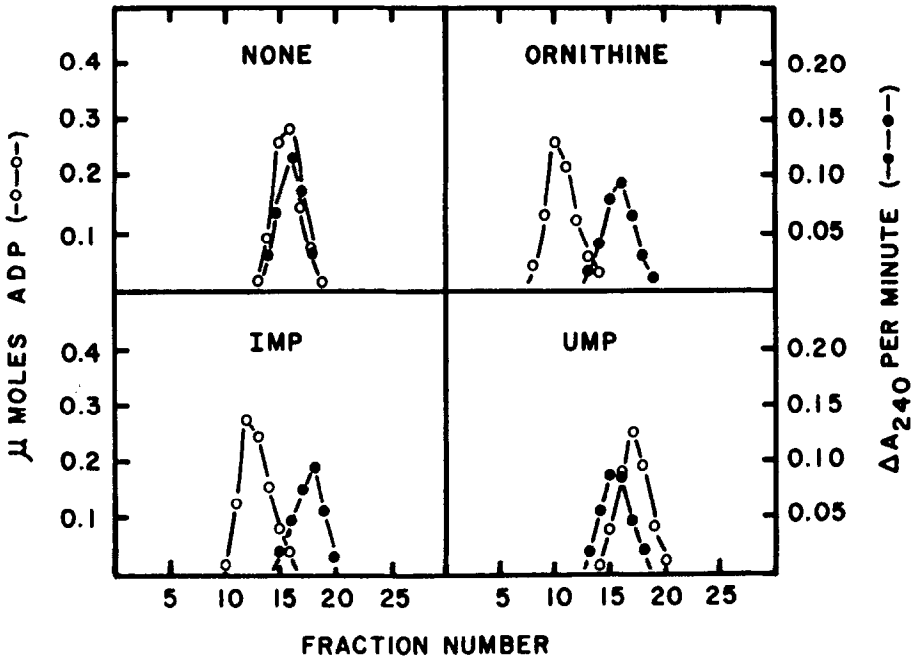

Figure 3. Effect of ornithine, IMP, and UMP on the rate of sedimentation of carbamyl phosphate sythetase in sucrose density gradients. The sucrose density gradients (5-20%) were prepared in 0.15 M potassium phosphate buffer, pH 7.8. Where indicated the gradients also contained IMP (2 mM), UMP (1 mM), or ornithine (10 mM). The sample (0.1 ml) which was layered over the top of each gradient contained 0.2 mg of carbamyl phosphate synthetase and 0.02 mg of catalase in 0.15 M potassium phosphate buffer, pH 7.8. After centrifugation 0.15 ml fractions were collected. The lowest fraction number represents the fraction from the bottom of the centrifuge tube. Catalase activity (closed circles) and carbamyl phosphate synthetase activity (open circles) were located in the fractions as described in Figure 2.

---

and 10.1 in the presence of ornithine, IMP, and UMP, respectively, and 11.7 in the absence of allosteric effectors. These results support the data obtained by gel filtration on Sephadex G-200, demonstrating that the molecular size of the enzyme is significantly greater in the presence of ornithine and IMP (positive allosteric effectors) than in the presence of UMP (a

negative allosteric effector). The enzyme apparently assumes an intermediate molecular size in the absence of allosteric effectors.

An estimation of the molecular weight of the enzyme by the method of Martin and Ames (1961) yielded values of 210,000 and 410,000 in the presence of UMP and IMP, respectively. Consideration of these approximate values together with the gel filtration data suggest that the large change in molecular size could be due to an allosteric effector-dependent equilibrium between an oligomeric and protomeric form of the enzyme; rapid equilibrium between the two forms would account for the intermediate molecular size observed in the absence of allosteric effectors (Gilbert, 1959; Whanger et al., 1968). Such a protomer $\rightleftharpoons$ dimer equilibrium could be responsible for manifesting the observed allosteric properties of the enzyme if the catalytic activity of the dimer was greater than that of the protomer (Monod et al., 1965; Klapper and Klotz, 1968).

A protomer $\rightleftharpoons$ tetramer equilibrium has been postulated to account for the range of sedimentation constants observed for AMP-activated threonine dehydrase under different conditions (Whanger et al., 1968). In the presence of AMP the equilibrium is shifted toward higher s values and an accompanying decrease in K_m for L-threonine is observed. A generalized model involving binding of small molecules to different polymeric species of a protein coexisting in equilibrium as a basis for allosteric effects has been described by Nichol et al., (1967).

Preliminary experiments involving studies on the rate of inactivation of carbamyl phosphate synthetase by reaction with N-ethyl maleimide have provided data that are consistent with the observations reported in this paper. The rate of inactivation is greatly decreased in the presence of ornithine or IMP and increased in the presence of UMP, as compared to the rate of inactivation observed in the absence of allosteric effectors. These and other studies are being carried out to establish the nature of the observed dependence of the molecular size of the enzyme on the allo-

steric effectors which are present and the role of these changes in molecular size in manifesting the allosteric properties of the enzyme.

ACKNOWLEDGEMENTS

We wish to thank Messrs. W. Light, B. Lam, J. Poon, and J. Woo for their excellent assistance in preparing the purified enzyme used in this work.

REFERENCES

Anderson, P. M., and Meister, A., Biochemistry 4, 2803 (1965).
Anderson, P. M., and Meister, A., Biochemistry 5, 3164 (1966).
Gilbert, G. A., Proc. Roy. Soc. (London), Ser. A 250, 377 (1959).
Klapper, M. H., and Klotz, I. M., Biochemistry 7, 223 (1968).
Luck, H., in Methods of Enzymatic Analysis, H. U. Bergmeyer, ed., Academic Press, 1965, p. 886.
Martin, R. G., and Ames, B. N., J. Biol. Chem. 236, 1372 (1961).
Monod, J., Wyman, J., and Changeaux, J. P., J. Mol. Biol. 12, 88 (1965).
Nichol, L. W., Jackson, W. J. H., and Winzor, D. J., Biochemistry 6, 2449 (1967).
Pierard, A., Science 154, 1572 (1966).
Whanger, P. D., Phillips, A. T. Rabinowitz, K. W., Piperno, J. R., Shada, J. D., and Wood, W. A., J. Biol. Chem. 243, 167 (1968).