Control of *Escherichia coli* Carbamyl Phosphate Synthetase by Purine and Pyrimidine Nucleotides*

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ABSTRACT: Carbamyl phosphate synthetase activity is significantly affected by a number of purine and pyrimidine nucleotides. Purine nucleotides (*e.g.*, inosine monophosphate) stimulated activity and pyrimidine nucleotides were inhibitory (uridine nucleotides) or had no effect (cytidine nucleotides); these effects are exerted maximally by the first nucleotides synthesized in each pathway and decrease as the number of steps required to synthesize the various nucleotides from inosine 5′-monophosphate or uridine 5′-monophosphate, respectively, increases. The nucleotides apparently affect activity by altering the affinity of the enzyme for adenosine triphosphate (ATP). The relationship of these findings to the regulation of pyrimidine and arginine biosynthesis and of purine nucleotide biosynthesis is considered.

The effects of the various nucleotides on the several additional reactions catalyzed by carbamyl phosphate synthetase (bicarbonate-dependent adenosine triphosphatase, hydrolysis of γ-glutamyl hydroxamate, synthesis of ATP from carbamyl phosphate and adenosine diphosphate) were also examined; only the last 2 of these reactions were significantly affected, suggesting that the first step in carbamyl phosphate synthesis (activation of carbon dioxide) is not directly involved in this type of control.

Carbamyl phosphate synthetase from *E. coli* catalyzes the following over-all reaction (Anderson and Meister, 1965a,b)[1]

$$2\text{ATP} + \text{glutamine} + HCO_3^- + H_2O \xrightarrow[Mg^{2+}]{K^+} 2\text{ADP} + \text{carbamyl phosphate} + P_i + \text{glutamate} \quad (1)$$

Studies carried out in this laboratory with the purified enzyme have shown that the rate of carbamyl phosphate synthesis as a function of ATP concentration is described by a sigmoidal curve and that a relatively high concentration of ATP is required to attain half-maximal velocity. In addition to carbamyl phosphate synthesis, the purified enzyme catalyzes the following reactions (Anderson and Meister, 1966)

$$\text{ATP} + H_2O \xrightarrow[Mg^{2+},\ K^+]{HCO_3^-} \text{ADP} + P_i \quad (2)$$

$$\gamma\text{-glutamyl hydroxamate} + H_2O \xrightarrow[K^+,\ HCO_3^-]{\text{ATP},\ Mg^{2+}} \text{glutamate} + NH_2OH \quad (3)$$

$$\text{ADP} + \text{carbamyl phosphate} + H_2O \xrightarrow{Mg^{2+},\ K^+} NH_4^+ + HCO_3^- + \text{ATP} \quad (4)$$

The effect of nucleotide concentration on reactions 3 (P. M. Anderson and A. Meister, 1966, unpublished data) and 4 is qualitatively similar to the effect of ATP on the over-all synthesis of carbamyl phosphate (reaction 1). However, much lower concentrations of ATP are required for half-maximal velocity of the ATPase activity (reaction 2), and the curve describing its initial rate as a function of ATP concentration is not sigmoidal.

Carbamyl phosphate is a common precursor for the biosynthesis of arginine and the pyrimidines. The dual function of carbamyl phosphate as a key intermediate in two major biosynthetic pathways (which provide building blocks for synthesis of both proteins and nucleic acids) suggests that regulatory mechanisms exist for controlling its rate of synthesis. Since the shape of the curve describing the effect of ATP concentration on the over-all rate of carbamyl phosphate synthesis is similar to those for the effect of substrate concentration on many enzymes in which the affinity of the enzyme for its substrate is altered by specific regulatory metabolites (Atkinson, 1965), we decided to investigate the possibility that the affinity of carbamyl phosphate synthetase for ATP might be modified by

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[1] Abbreviations used in this work: ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; ATPase, adenosine 5′-triphosphatase; TPN, triphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; UMP, uridine 5′-monophosphate; UDP, uridine 5′-diphosphate; UTP, uridine 5′-triphosphate; CTP, cytidine 5′-triphosphate; CMP, cytidine 5′-monophosphate; IMP, inosine 5′-monophosphate; XMP, xanthosine 5′-monophosphate; GMP, guanosine 5′-monophosphate; GDP, guanosine 5′-diphosphate; GTP, guanosine 5′-triphosphate; AMP, adenosine 5′-monophosphate; IDP, inosine 5′-diphosphate; ITP, inosine 5′-triphosphate.

products of the pyrimidine pathway. The results of these studies, which are described here, show that relatively low concentrations of purine and pyrimidine nucleotides can serve as activators or inhibitors of carbamyl phosphate synthetase. The effects of these nucleotides on the three additional reactions catalyzed by carbamyl phosphate synthetase have also been studied.

Experimental Section

Materials. XMP, IDP, and CMP were obtained from Calbiochem; AMP was obtained from Schwartz BioResearch. The other nucleotides were obtained from Sigma Chemical Co. The identity of the nucleotides was checked and the concentrations of stock solutions of these were determined by measuring their ultraviolet spectra in 0.05 M potassium phosphate buffer, pH 6.8, with a Cary Model 14 spectrophotometer. All other materials were obtained from sources previously cited (Anderson and Meister, 1966). Carbamyl phosphate synthetase was isolated from *E. coli* B as previously described (Anderson and Meister, 1965b).

Methods. Carbamyl phosphate synthetase activity, enzymatic synthesis of ATP from ADP and carbamyl phosphate, bicarbonate-dependent ATPase activity, and the hydrolysis of γ-glutamyl hydroxamate were determined as described previously (Anderson and Meister, 1966).

TABLE I: Effect of Nucleotides on the Rate of Carbamyl Phosphate Synthesis.[a]

Nucleotide	[^{14}C]Carbamyl Phosphate (μmole)	Rel. Act.
None	0.120	100
Pyrimidines		
UMP	0.025	21
UDP	0.047	39
UTP	0.061	51
CTP	0.121	101
CMP	0.126	105
Purines		
IMP	0.304	254
XMP	0.278	231
GMP	0.207	173
GDP	0.182	152
GTP	0.152	127
AMP	0.160	133
IDP	0.180	150
ITP	0.142	118

[a] The reaction mixtures contained L-glutamine (10 μmoles), [^{14}C]$NaHCO_3$ (20 μmoles, 700,000 cpm), ATP (6 μmoles), $MgCl_2$ (6 μmoles), KCl (100 μmoles), Tris–HCl (100 μmoles, pH 8.1), enzyme (0.015 mg), and nucleotide (1 μmole) in a final volume of 1 ml. The solutions containing nucleoside di- and triphosphates contained equimolar concentrations of $MgCl_2$. The [^{14}C]-carbamyl phosphate synthesized after incubation for 10 min at 37° was determined as described in the text.

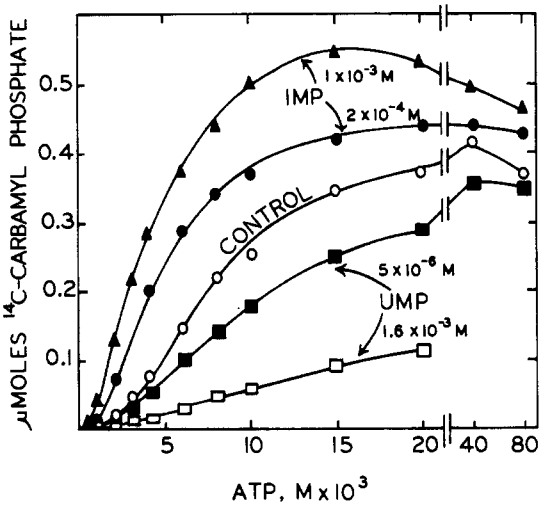

FIGURE 1: Effect of IMP and UMP on carbamyl phosphate synthetase activity as a function of ATP concentration. The reaction mixtures contained $MgCl_2$ in concentrations that were equimolar with ATP, [^{14}C]-$NaHCO_3$ (20 μmoles, 700,000 cpm), KCl (100 μmoles), L-glutamine (10 μmoles), Tris–HCl buffer (100 μmoles, pH 8.2), IMP and UMP as indicated, and enzyme (0.015 mg) in a final volume of 1 ml. The [^{14}C]carbamyl phosphate synthesized after incubation for 10 min at 37° was determined.

Results

Effect of Various Purine and Pyrimidine Nucleotides on Carbamyl Phosphate Synthetase Activity. The data given in Table I demonstrate the inhibitory effect of various pyrimidine nucleotides on the rate of carbamyl phosphate synthesis. It is interesting to note that the effect of the nucleotides diminishes as the number of biosynthetic steps required to synthesize them from the initial intermediates of the purine and pyrimidine pathways (IMP and UMP, respectively) increases. Orotate, dihydroorotate, and uracil had little effect on the reaction.

Effect of IMP and UMP on Carbamyl Phosphate Synthetase Activity. The effect of IMP and UMP on the rate of carbamyl phosphate synthesis is described in Figure 1. These results suggest that the nucleotides act by altering the affinity of the enzyme for ATP. Although it was not practical to investigate this effect at higher concentrations of ATP, it appears that with increasing ATP concentration the rate approaches a maximum value which is not markedly affected by added UMP or IMP.

As shown in Figure 2, the effects of IMP and UMP are exerted maximally at relatively low concentrations.

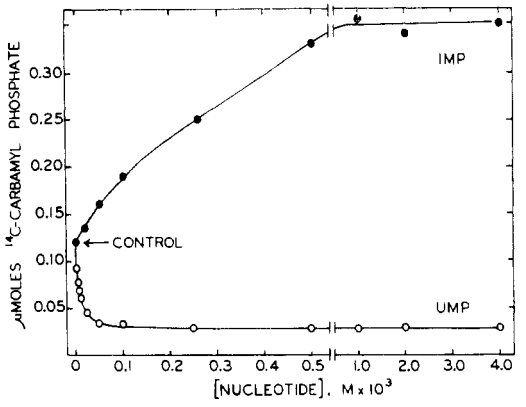

FIGURE 2: Effect of various concentrations of IMP and UMP on carbamyl phosphate synthetase activity. The reaction mixtures contained ATP (6 μmoles), $MgCl_2$ (6 μmoles), [^{14}C]$NaHCO_3$ (20 μmoles, 700,000 cpm), KCl (100 μmoles), L-glutamine (10 μmoles), Tris–HCl buffer (100 μmoles, pH 8.2), enzyme (0.015 mg), and IMP or UMP as indicated in a final volume of 1 ml. The [^{14}C]carbamyl phosphate synthesized after incubation for 10 min at 37° was determined.

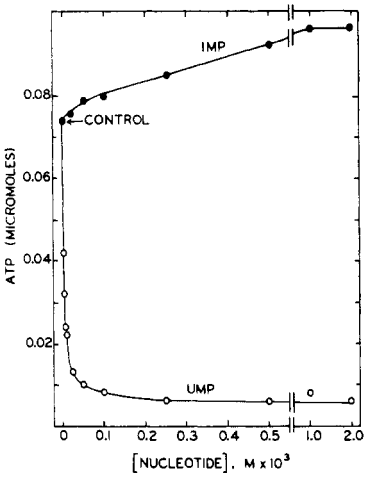

FIGURE 3: Effect of various concentrations of IMP and UMP on the rate of ATP formation from ADP and carbamyl phosphate. The reaction mixtures contained ADP (1 μmole), $MgCl_2$ (1 μmole), carbamyl phosphate (3.3 μmoles), KCl (33 μmoles), Tris–HCl buffer (33 μmoles, pH 8.1), enzyme (0.036 mg), and potassium lithium phosphate (approximately 6 μmoles) in a final volume of 0.3 ml. The amount of ATP synthesized after incubation for 10 min at 37° was determined.

The concentration of UMP required to obtain half-maximal inhibition is approximately 5×10^{-6} M; half-maximal stimulation by IMP is attained at a concentration of 2×10^{-4} M.

Effect of IMP and UMP on Other Reactions Catalyzed by Carbamyl Phosphate Synthetase.

$$\text{ADP} + \text{carbamyl phosphate} + H_2O \xrightarrow{Mg^{2+},\ K^+} \text{ATP} + NH_4^+ + HCO_3^-$$

As shown in Figure 3, the effect of various concentrations of IMP and UMP on the rate of ATP synthesis is qualitatively similar to the effects of these nucleotides on the over-all reaction rate; thus, half-maximal stimulation and inhibition are attained at about the same respective concentrations of IMP and UMP.

$$\gamma\text{-glutamyl hydroxamate} + H_2O \xrightarrow[HCO_3^-,\ K^+]{ATP,\ Mg^{2+}} \text{glutamate} + NH_2OH$$

The effect of ATP concentration on the rate of hydrolysis of γ-glutamyl hydroxamate is similar to the sigmoidal curve describing the rate of the over-all reaction as a function of ATP concentration (P. M. Anderson and A. Meister, 1966, unpublished data). The data given in Figure 4 show that the rate of this reaction is also affected by IMP and UMP and that the effects of these nucleotides are similar to those on the over-all reaction.

$$\text{ATP} + H_2O \xrightarrow[K^+]{Mg^{2+},\ HCO_3^-} \text{ADP} + P_i$$

As reported previously, the rate of this reaction as a function of ATP concentration is described by a hyperbolic curve; half-maximal velocity is obtained with an ATP concentration of about 7×10^{-4} M (Anderson and Meister, 1966). IMP has relatively little effect on the rate of this reaction, particularly at low concentrations of ATP (Figure 5). The small inhibitory effect of UMP diminishes at low concentrations of ATP.

Discussion

Piérard *et al.* (1965) have reported genetic and other evidence indicating that there is a single enzyme in *E. coli* for the synthesis of carbamyl phosphate used for both the arginine and pyrimidine pathways, and that this enzyme is subject to a double end-product control involving cumulative repression of its synthesis by arginine and uracil as well as partial feedback inhibition of its activity by UMP. The effects of pyrimidine and purine nucleotides on carbamyl phosphate synthetase described in this paper confirm the feedback inhibition by UMP. The data show also that the enzyme is subject to both positive and negative feedback control which might function in maintaining a balance between the rates of purine and pyrimidine biosynthesis.

The interaction between the two pathways is illustrated schematically in Figure 6. An increase in the concentration of purine nucleotides would be expected to result in an increased rate of formation of carbamyl phosphate (assuming that the ATP concentration is not saturating). The studies of Gerhart and Pardee (1962)

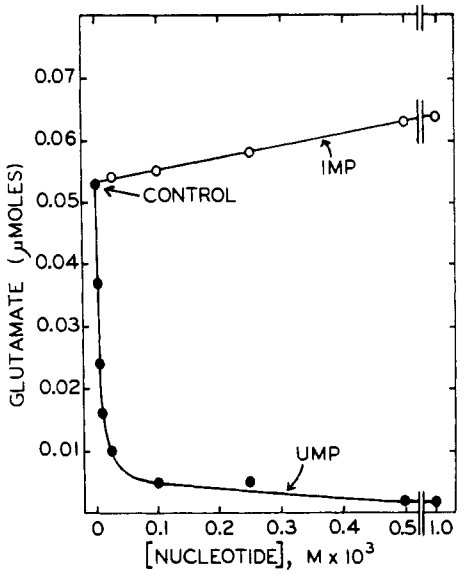

FIGURE 4: Effect of various concentrations of IMP and UMP on the rate of hydrolysis of γ-glutamyl hydroxamate. The reaction mixtures contained L-γ-glutamyl hydroxamate (1.3 μmoles), ATP (0.9 μmole), $MgCl_2$ (0.9 μmole), $NaHCO_3$ (3 μmoles), potassium phosphate buffer (20 μmoles, pH 7.8), enzyme (0.03 mg), and IMP and UMP as indicated in a final volume of 0.3 ml. The amount of γ-glutamyl hydroxamate hydrolyzed after incubation for 10 min at 37° was determined.

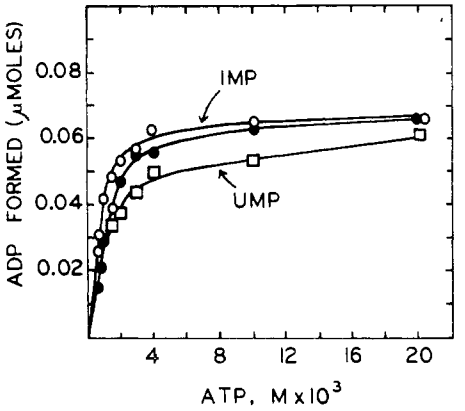

FIGURE 5: Effect of IMP and UMP on bicarbonate-dependent ATPase activity at various concentrations of ATP. The reaction mixtures contained $MgCl_2$ concentrations that were equimolar with ATP, $NaHCO_3$ (6 μmoles), Tris-HCl (30 μmoles), KCl (30 μmoles), enzyme (0.014 mg), and IMP and UMP as indicated (0.3 μmole) in a final volume of 0.3 ml. The ADP formed after incubation for 10 min at 37° was determined.

have shown that the enzyme catalyzing the next reaction in the pyrimidine pathway, aspartate transcarbamylase, is also subject to inhibition by certain end products of the pathway, particularly CTP, and that ATP stimulates activity. These observations are analogous to those presented here, *i.e.*, end-product inhibition by pyrimidine nucleotides and stimulation by an end product of purine biosynthesis. However, there are several important qualitative differences between the regulatory control of these enzymes. As illustrated in Figure 6, control of carbamyl phosphate synthetase is exerted maximally by the first nucleotides synthesized in each pathway, the effect decreasing as the number of steps required to synthesize each nucleotide from IMP or UMP, respectively, increases, whereas the activity of aspartate transcarbamylase is apparently affected maximally by the nucleotides synthesized in the terminal steps of each pathway.

All of the purine nucleotides tested stimulated activity and all of the pyrimidine nucleotides tested were either inhibitory or had no effect on the activity of carbamyl phosphate synthetase. The situation is somewhat more complicated in the case of aspartate transcarbamylase, since GTP is an inhibitor rather than an activator of this enzyme (Gerhart and Pardee, 1962). Therefore, the over-all effect of an increase in purine nucleotide biosynthesis would be expected to lead to an initial increase in the rate of formation of carbamyl phosphate; this could lead to an increase in the rate of synthesis of both arginine and pyrimidines. An increase in the concentration of GTP, however (as well as other purine nucleotides), would have the effect of increasing the rate of carbamyl phosphate formation but of decreasing the rate of its utilization for pyrimidine biosynthesis, thereby increasing the quantity of carbamyl phosphate available for arginine biosynthesis. It is interesting to speculate that the control of carbamyl phosphate synthetase as described here might serve as a partial regulatory link between the biosynthesis of proteins and nucleic acids.

The sigmoidal nature of the curve describing the effect of ATP concentration on the rate of carbamyl phosphate formation and the modification of the shape of this curve by end-product metabolites, thereby altering the activity of the enzyme, resemble the behavior of many regulatory enzymes which are subject to feedback controls (Atkinson, 1965; Monod *et al.*, 1965). Several models have been proposed to explain the kinetic properties of these enzymes. For example, it has been suggested that the regulatory metabolite binds at a site other than the catalytic site and influences the binding of substrates at the active site by a mechanism involving subunit–subunit interaction or a conformational change of the enzyme molecule (Atkinson *et al.*, 1965; Monod *et al.*, 1965; Koshland *et al.*, 1966). The initial rates of reaction of many of the enzymes exhibiting "substrate–substrate" interactions is described by the following equation (Atkinson *et al.*, 1965; Changeux, 1963), referred to as the Hill equation

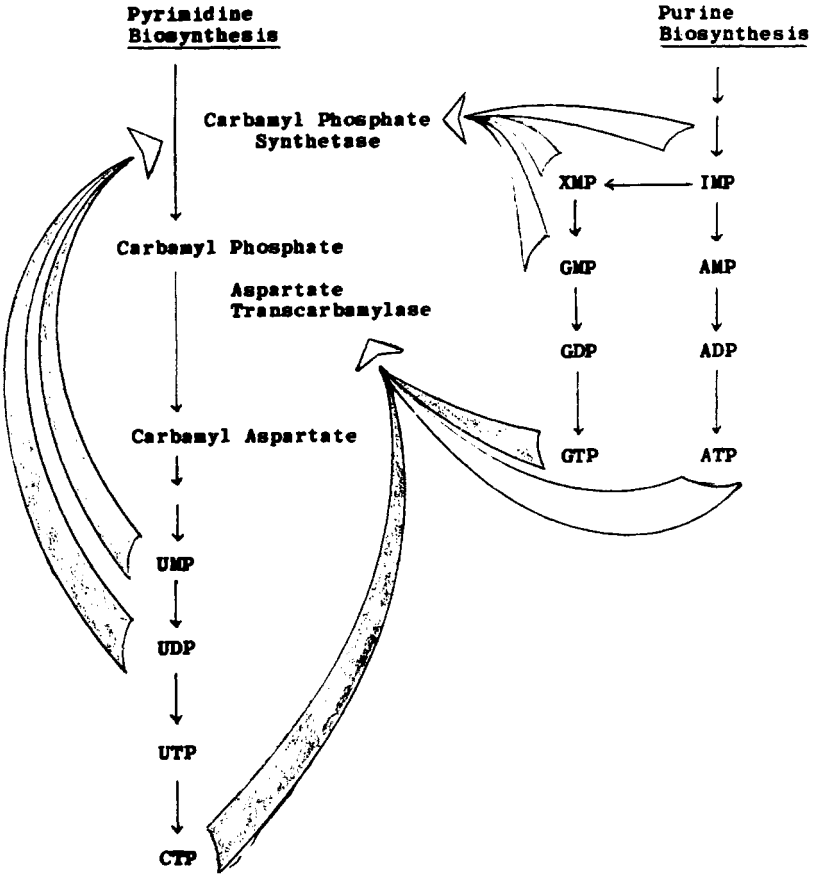

FIGURE 6: Diagram of the feedback inhibition of carbamyl phosphate and carbamyl aspartate synthesis by pyrimidine and purine nucleotides. Shaded arrows, inhibition; open arrows; activation.

$$\log \frac{v}{V - v} = n \log S - \log k$$

where v is the initial velocity, V is the maximum velocity, S is the substrate concentration, and k is a constant. A plot of $\log (v/(V - v))$ *vs.* log of substrate concentration yields a straight line with the slope equal to n. The value of the slope is thought to be a function of the number of interacting substrate-binding sites per molecule of enzyme and of the strength of such interactions. As shown in Figure 7, when the data of Figure 1 are plotted in this manner, a straight line is obtained at intermediate values of S with a slope equal to 1.7. Sanwal and Cook (1966) have pointed out, however, that sigmoidal curves can result from a variety of causes and have suggested that a straight line obtained when the data are plotted in this way cannot necessarily be regarded as proof of substrate binding at multiple sites and subsequent subunit interaction between the sites. It seems pertinent to note that *E. coli* carbamyl phosphate synthetase has a relatively high molecular weight,[2] a finding which is consistent with a subunit structure. Studies on this aspect of the enzyme will be reported at a later date.

Previous studies have provided evidence that the mechanism of action of carbamyl phosphate synthetase involves the following sequence of reactions (Anderson and Meister, 1965b, 1966)

$$\text{enzyme} + \text{ATP} + HCO_3^- \rightleftharpoons \text{enzyme–}[CO_3PO_3^{3-}] + \text{ADP}$$

$$\text{enzyme–}[CO_3PO_3^{3-}] + \text{glutamine} + H_2O \longrightarrow \text{enzyme–}[NH_2CO_2^-] + \text{glutamate} + P_i$$

$$\text{enzyme–}[NH_2CO_2^-] + \text{ATP} \rightleftharpoons \text{enzyme} + \text{carbamyl phosphate} + \text{ADP}$$

The bicarbonate-dependent ATPase activity exhibited by the enzyme (reaction 2) seems to be a reflection of the first step in this sequence (Anderson and Meister,

[2] The enzyme exhibits a sedimentation coefficient ($s_{20,w}$) of 13.6 (protein concentration, 1%; in 0.1 M KCl and 0.1 M potassium phosphate, pH 7.6). We are indebted to Dr. Rudy H. Haschemeyer and Mr. Jack Wilkinson for this determination.

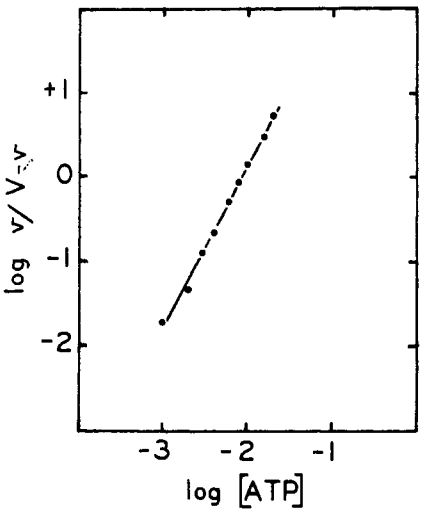

FIGURE 7: Hill equation plot of the data obtained for the control experiment in Figure 1.

1966). The effect of ATP concentration on this activity differs markedly from the effect of ATP (or ADP) concentration on the other reactions (reactions 3 and 4) catalyzed by carbamyl phosphate synthetase (Anderson and Meister, 1966). Similarly the purine and pyrimidine nucleotides tested here have substantial effects on the rates of reactions 1, 3, and 4, but exert little effect on the ATPase activity. Since reactions 3 and 4 seem to reflect the later steps in the over-all synthesis sequence, it would therefore appear that the major effect of the nucleotides observed on the over-all reaction rate is at an enzyme site which does not involve the first step of the reaction.

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