Wheat-Germ Aspartate Transcarbamoylase

THE EFFECTS OF LIGANDS ON THE INACTIVATION OF THE ENZYME BY TRYPSIN AND DENATURING AGENTS

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In the absence of added ligands aspartate transcarbamoylase (EC 2.1.3.2) from wheat germ is inactivated fairly rapidly by trypsin, by heat (60°C), by highly alkaline conditions (pH 11.3) and by sodium dodecyl sulphate. Addition of UMP alone, at low concentrations, decreases the rate of inactivation by each of these agents significantly. Carbamoyl phosphate alone does not alter the rate of inactivation by trypsin and by the detergent, but it antagonizes the effect of UMP in protecting the enzyme against these agents. These results have been interpreted to mean that two conformational states are reversibly accessible to the enzyme, namely an easily inactivated state favoured in the presence of carbamoyl phosphate and a more resistant state favoured in the presence of UMP. In the absence of ligands the enzyme is in the easily inactivated conformation. At very high concentrations L-aspartate also protects the enzyme but to a smaller extent than UMP. Some implications of these results are discussed.

Aspartate transcarbamoylase (EC 2.1.3.2) catalyses the transfer of the carbamoyl group from carbamoyl phosphate to L-aspartate to form *N*-carbamoyl-aspartate, an early step in the biosynthetic pathway by which pyrimidine nucleotides are formed. The enzyme in higher plants may be involved in the control of the pathway by feedback inhibition, since it is inhibited *in vitro* by low concentrations of a pyrimidine nucleotide, UMP, in extracts of lettuce seedlings (Neumann & Jones, 1962), mung-bean (*Phaseolus aureus*) radicles (Ong & Jackson, 1970) and wheat germ (Yon, 1971). In the wheat-germ enzyme catalytic activity and inhibition by UMP both showed an unusual pH-dependence. Maximum catalytic activity and maximum sensitivity to UMP both occurred at pH 10.5, whereas at near-neutral pH values only low catalytic activity and no effect of UMP (at 0.2 mM) were observed (Yon, 1972*b*). The physiological significance of this pH-dependence is not understood. Alkaline conditions are unlikely to be of general occurrence in the developing or dormant wheat embryo or in the developing seedling. Possibly activating conditions, at present unknown, exist in the cell which are able to reverse the effects of neutral pH observed *in vitro*. Alternatively the enzyme may be spatially confined to an alkaline micro-environment. It has recently been reported (Ong & Jackson, 1972) that another higher-plant aspartate transcarbamoyl-

ase, that of *P. aureus*, also exhibits a pH maximum for enzyme activity at pH 10.5. The authors showed that a change from pH 10.5 to 8.5 affected $V_{\max}$, but not the K_m values. Unlike the wheat-germ enzyme, the *P. aureus* enzyme is equally sensitive to UMP at pH 8.5 and at 10.5 (Ong & Jackson, 1972). The two plant enzymes are very similar in other kinetic properties and in molecular size. In both enzymes UMP interacts kinetically with one of the substrates, carbamoyl phosphate, in a typically allosteric manner, each ligand showing a sigmoid saturation curve in the presence of a constant concentration of the other (Ong & Jackson, 1972; Yon, 1972*b*). In the wheat-germ enzyme the kinetic interactions at pH 10 were shown to be compatible, to a large extent, with the two-conformation allosteric model of Monod *et al.* (1965).

During purification trials with the wheat-germ enzyme it was observed that the enzyme had increased stability under alkaline conditions if UMP was present. In view of a possible connexion between this protective effect and the presumed conformational changes in the enzyme, it was decided to investigate the effects of ligands on enzyme stability in some detail. These investigations are reported in the present paper.

Experimental

Materials

Aspartate transcarbamoylase was partially purified from commercial wheat germ as described previously

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(Yon, 1972b). Carbamoyl phosphate (dilithium salt), UMP (disodium salt), carbamoylaspartic acid, L-malate (sodium salt), sodium dodecyl sulphate, bovine pancreatic trypsin (twice recrystallized), soyabean trypsin inhibitor (recrystallized) and ox liver catalase were from the Sigma (London) Chemical Co., London S.W.6, U.K. L-Aspartic acid (chromatographically homogeneous) and succinic acid were from British Drug Houses, Poole, Dorset, U.K. D-Aspartic acid was from Calbiochem Ltd., London W1, U.K. Sephadex G-200 and Blue Dextran 2000 were from Pharmacia (G.B.) Ltd., London W.13, U.K. All other chemicals used as reagents or buffers were of analytical-reagent grade.

Methods

General experimental conditions and enzyme assay.

Since the protective effects of UMP were most pronounced under alkaline conditions (see Table 1) as far as possible all experiments were done at pH 10. Two exceptions to this were the inactivation of the enzyme at pH 11.3 and the inactivation at 60°C. The latter experiment was performed in glycine buffer which had been adjusted to pH 10 at 22°C; it was calculated that this buffer would have a pH of 9.01 at 60°C. In the remainder of this paper 'standard glycine buffer' means 0.1M-glycine adjusted to pH 10.0 at room temperature (22°C) with 5M-NaOH.

All of the following inactivation experiments were designed to yield 2.6ml of diluted, possibly partly inactivated enzyme at pH 10.0 after the inactivation treatment. The residual enzyme activity was determined by adding 0.2ml of 15mM-L-aspartate, previously adjusted to pH 10.0 and 0.2ml of 15mM-carbamoyl phosphate in water, the addition of carbamoyl phosphate being used to start the reaction. When one of the substrates was used in the inactivation experiment, its concentration was proportionately decreased in the assay. After a reaction time of 10min at 25°C the enzyme was completely inactivated by the addition of 3.0ml of the colorimetric reagent of Prescott & Jones (1969) and the determination of carbamoylaspartate was carried out according to method 1 of these authors.

To establish the stability of enzyme activity at pH 10.0 and 25°C in the absence of any of the inactivating agents used below, enzyme activity under these conditions, and at a dilution of 50-fold relative to the partially purified preparation, was determined at intervals over a period of 48h. The activity was found to decrease slowly, with a half-life of about 24h. No correction was applied for this slow inactivation in the inactivation experiments below, none of which lasted for more than 30min.

Whenever carbamoyl phosphate was included in an inactivation experiment it was made up from a frozen 15mM stock solution in water and added to

the experimental system just before timing was started, to decrease as far as possible the loss of this ligand by decomposition.

Inactivation by heat. Standard glycine buffer (49.5ml), with or without the added ligand, was equilibrated at 60°C in a water bath. At zero time 0.5ml of partially purified aspartate transcarbamoylase was added with efficient mixing. At timed intervals 2.6ml portions were transferred to precooled test tubes on ice. To establish the true zero-time activity 4.95ml of buffer or buffered ligand and 0.05ml of partially purified enzyme were mixed without heating and a 2.6ml portion taken. All test tubes and contents were equilibrated at 25°C for 10min and then assayed for residual enzyme activity.

Inactivation by alkaline pH. Glycine (0.2M), with or without the added ligand, was adjusted to pH 11.3 with 10M-NaOH. A portion (2.7ml) of this mixture was equilibrated at 25°C and at zero time 0.3ml of partially purified enzyme was added. No change of pH was recorded, by using a glass electrode and pH-meter assembly, when the enzyme was added showing that the buffering capacity of glycine at pH 11.3, though weak, was adequate for the present purpose. At timed intervals 0.2ml portions were transferred into tubes containing 2.4ml of standard glycine buffer at 25°C, with efficient mixing. The true zero-time activity was found by using enzyme at the same final concentration in standard glycine buffer, which had not been taken to pH 11.3. The contents of all tubes were assayed for residual enzyme activity.

Inactivation by sodium dodecyl sulphate. Sodium dodecyl sulphate was dissolved in standard glycine buffer to a concentration of 2mM. A portion (2.7ml) of this mixture, with or without the added ligand, was equilibrated at 25°C. At zero time 0.3ml of partially purified enzyme was added with efficient mixing. At timed intervals 0.3ml portions of the mixture were transferred into test tubes which contained 2.3ml of 1μM-UMP in standard glycine buffer. If sufficient UMP was transferred from the inactivation experiment, UMP was omitted from the assay test tubes. In the presence of 1μM-UMP the diluted detergent (now at approx. 0.2mM) produced negligible further inactivation provided the residual activity was determined within the next 30min. True zero-time activity was determined in tubes containing 2.3ml of 1μM-UMP and 0.2mM-sodium dodecyl sulphate in standard glycine buffer, to which 0.3ml of 10-fold-diluted enzyme (partially purified) was added just before assay. Residual activity was then determined in all test tubes.

Inactivation by proteolysis. Partially purified enzyme was diluted 50-fold in standard glycine buffer and ligands at appropriate concentrations were dissolved in this mixture (with the exception of carbamoyl phosphate; see under 'General experimental conditions and enzyme assay' above). Portions (2.2ml)

of this mixture were equilibrated at 25°C for 5 min in a series of test tubes. At zero time and 5 s intervals thereafter proteolysis was started in these tubes by adding 0.2 ml of 0.05% (w/v) trypsin in water, with efficient mixing. After suitable timed intervals proteolysis was stopped by adding 0.2 ml of 0.2% (w/v) soya-bean trypsin inhibitor in water. Zero-time activity was determined in tubes to which trypsin inhibitor was added before trypsin. Residual activity was determined in all tubes.

To exclude possible effects of the added ligands on trypsin, the tryptic digestion of casein (Laskowski, 1955) was carried out in the presence of 30 μ M-UMP, 19 mM-L-aspartate or 1 mM-carbamoyl phosphate. Tested separately, none of these compounds affected the rate of tryptic digestion of casein in 0.1 M-glycine buffer, pH 10, at 25°C.

Gel-filtration chromatography. Gel filtration was carried out on a column (1 cm $\times$ 95 cm) of Sephadex G-200 equilibrated and developed with standard glycine buffer at room temperature (22°C), with or without 60 μ M-UMP. The same gel was used to repack the column between repetitions of the experiment. In each repetition the sample consisted of 1.0 ml of partially purified aspartate transcarbamoylase containing 10 mg of catalase, 0.1 ml of 1% (w/v) Blue Dextran and 0.1 ml of 1 M-NaCl. The column was developed at 6 ml/h and 3 ml fractions were collected. Development of the column up to the emergence of the Cl^- peak took approx. 24 h. Effluent fractions were assayed for aspartate transcarbamoylase activity, catalase activity, Blue Dextran and Cl^- ions. Aspartate transcarbamoylase activity was assayed as for the inactivation experiments. Catalase activity was assayed by the method of Beers & Sizer (1952). Blue Dextran was determined by its extinction at 625 nm. Cl^- was determined by mixing 0.5 ml of the effluent fraction, 2 ml of water and 0.5 ml of 4% (w/v) AgNO_3 in 95% (v/v) ethanol and determining the extinction due to turbidity at 625 nm within 5 min of the precipitate forming.

The Blue Dextran and Cl^- peaks were used to determine the void volume and internal gel volume respectively. Assuming a Stokes radius of 5.2 nm for catalase (Siegel & Monty, 1966) the tables of Ackers (1964) were used to determine the effective gel-pore radius and the Stokes radius of aspartate transcarbamoylase.

Results

Changes produced by tryptic digestion in the sensitivity of the enzyme to inhibition by UMP

An experiment was performed to find out whether tryptic digestion affected the inhibition of enzyme activity by UMP. At different times during the course of proteolysis the residual activity was determined

Fig. 1. *Effect of partial proteolysis by trypsin on the inhibitory effectiveness of UMP*

The percentage inhibition by a constant concentration of UMP (60 μ M) of the enzyme activity remaining after various short periods of proteolysis by trypsin is shown. Activity was determined in the presence of 1.0 mM each of L-aspartate and carbamoyl phosphate, in 0.05 M-glycine buffer, pH 10.0 at 25°C. Proteolysis was carried out in the absence of UMP (●) and in the presence of 10 μ M-UMP (○).

both in the presence and in the absence of UMP and the percentage inhibition calculated. The results (●, Fig. 1) showed that as proteolysis proceeded the residual activity became progressively less sensitive to inhibition by UMP. Before proteolysis 60 μ M-UMP inhibited the activity by 40% at the substrate concentrations used in the assay. After 12 min of proteolysis the same concentration of UMP inhibited the residual activity by only 12% under the same conditions (the corresponding residual activity after 12 min was about 40%; see Fig. 2d). When the tryptic digestion was performed in the presence of 10 μ M-UMP, the decrease in sensitivity towards UMP (determined by the additional inhibition produced by a further 60 μ M-UMP in the assay system) was slowed down, but not abolished (○, Fig. 1).

These results suggest that UMP binds at a site (or sites) distinct from the active site. Making this assumption, the effects of trypsin may be accounted for by postulating a more rapid proteolytic attack on the UMP-binding site, or on the mechanism of interaction between the UMP-binding site and the active site, than on the active site itself. The result would be a time-dependent alteration of the percentage inhibition of enzyme activity by a constant concentration of UMP.

Fig. 2. Effects of UMP on the rates of inactivation of the enzyme by four destructive agents

Experimental conditions are described in the text. Inactivating agents are: (a) pH 11.3; (b) sodium dodecyl sulphate; (c) heat (60°C); (d), trypsin. Concentrations of UMP are: none (●); 1 μM (Δ); 3 μM (▲); 10 μM (□); 20 μM (■); 30 μM (○). No other ligands were included in these experiments.

Protective effects of UMP against various inactivating agents

UMP was found to abolish or retard the inactivation of the enzyme by each of the four different agents used in these experiments (Fig. 2). In the absence of UMP, enzyme activity was destroyed by a pH of 11.3 with a half-life of 20 min. Addition of 30 μM-UMP completely abolished this inactivation over the 30 min period of the experiment (Fig. 2a). Similarly the detergent inactivation (half-life of 15 min) was abolished by 10 μM-UMP (Fig. 2b). Addition of UMP retarded, but did not abolish, the inactivation by heat and by proteolysis. UMP (30 μM) increased the half-life of inactivation by heat from

3 min to 30 min (Fig. 2c) and the half-life of inactivation by proteolysis from 10 min to 25 min (Fig. 2d).

Inactivation of the enzyme by heat, by pH 11.3 and by detergent were apparently first-order reactions as indicated by linear semi-logarithmic time-plots. In contrast, inactivation by proteolysis was kinetically more complex, as indicated by semi-logarithmic plots which were concave downwards. Inspection of Figs. 2(c) and 2(d) shows another difference between inactivation by heat and by proteolysis. A concentration of UMP as low as 1 μM produced a marked change in the rate of inactivation by proteolysis but had no effect on the rate of inactivation by heat, which required higher concentrations of UMP (10 or 20 μM)

to produce significant changes in the inactivation rate. This difference in sensitivity towards UMP probably reflects a difference in binding affinity for the ligand under the different conditions of pH and temperature employed in the proteolytic and heat experiments.

Interaction between the effects of UMP and carbamoyl phosphate

Since the conditions of the heat and alkaline-pH experiments would rapidly decompose carbamoyl phosphate, only the detergent and proteolytic methods were used in studying the effects of carbamoyl phosphate. The results of these experiments are shown in Fig. 3.

Compared with the control inactivation in the absence of ligands, carbamoyl phosphate alone produced no significant change in the rate of inactivation by proteolysis at concentrations up to 1.2 mM. In the presence of 1.2 mM-carbamoyl phosphate and 30 μ M-UMP, trypsin inactivated the enzyme at the same rate as it did in the absence of all ligands or in the presence of carbamoyl phosphate alone. However, in the presence of a lower concentration of carbamoyl phosphate (0.1 mM) and the same concentration of UMP (30 μ M) inactivation by trypsin was slowed to almost the same rate as in the presence of UMP alone at 30 μ M (Fig. 3a). Thus the effects of increasing concentrations of UMP and of carbamoyl phosphate were antagonistic to each other.

Similar results were obtained for inactivation by the detergent, sodium dodecyl sulphate. Carbamoyl phosphate alone, at concentrations up to 0.5 mM, did not protect the enzyme against detergent inactivation when compared with the control inactivation in the absence of ligands. The effects of increasing concentrations of UMP and carbamoyl phosphate were, again, antagonistic to each other (Fig. 3b). However, the concentrations of ligands at which similar protective effects were achieved were much lower in the detergent experiments than in the proteolytic experiments, suggesting that the presence of the detergent had affected the binding affinities of, or the strength of interaction between, UMP and carbamoyl phosphate. Moreover, in a kinetic experiment it was found that the concentration of UMP required to produce 50% inhibition in the presence of 1 mM of each substrate was considerably decreased by sodium dodecyl sulphate, when the detergent was at a concentration low enough to preclude inactivation during the time of the experiment. Although these effects have yet to be explored in detail, it appears that this detergent may influence kinetic and other characteristics by an enzyme-detergent interaction which is rapid compared with the inactivating effect of the detergent. It is possible that the detergent

Fig. 3. Interaction between the effects of UMP and carbamoyl phosphate on the rates of inactivation of the enzyme by trypsin and by sodium dodecyl sulphate

Experimental conditions are described in the text. Ligand concentrations are as follows. For tryptic inactivation (a): none ($\bullet$); 1.2 mM-carbamoyl phosphate (Δ); 30 μ M-UMP ($\circ$); 30 μ M-UMP and 0.1 mM-carbamoyl phosphate ($\blacksquare$); 30 μ M-UMP and 0.4 mM-carbamoyl phosphate ($\square$); 30 μ M-UMP and 1.2 mM-carbamoyl phosphate ($\blacktriangle$). For sodium dodecyl sulphate inactivation (b): none ($\bullet$); 0.5 mM-carbamoyl phosphate (Δ); 10 μ M-UMP ($\circ$); 10 μ M-UMP and 0.05 mM-carbamoyl phosphate ($\square$); 10 μ M-UMP and 0.5 mM-carbamoyl phosphate ($\blacktriangle$).

binds at a hydrophobic site or sites suggested by experiments in affinity chromatography (Yon, 1972a).

Other properties of the protective effect of UMP against trypsin

The effect of UMP on the proteolytic inactivation rate was studied at pH 7.5, 8.5 and 10 (Table 1). Only

Table 1. *Effect of UMP on the tryptic digestion rate at various pH values*

Tryptic digestion was carried out in the following buffers: at pH 7.5 and 8.5, 0.05M-Tris-HCl; and at pH 10, 0.05M-glycine-NaOH. Residual activity was determined at the same pH as the tryptic digestion, in each case. Other conditions are described in the text.

pH	% of activity remaining after 10 min digestion	
	No UMP	10 μ M-UMP
7.5	57	57
8.5	39	42
10.0	42	70

at pH 10 was there a pronounced protection by UMP. At pH 8.5 there was a slight protection, and at pH 7.5 there was no effect. The protective effect therefore occurs under quite strongly alkaline conditions, and in this respect resembles the inhibitory effect of UMP.

The protective effect was also highly specific for UMP. At 50 μ M none of the following structural analogues of UMP gave any protective effect at pH 10: uridine, UDP, UTP, TMP and ribose 5'-phosphate. CMP gave a slight protective effect.

Effects of L-aspartate and other dicarboxylic acid anions

At high concentrations L-aspartate also provided some protection against inactivation by heat and by trypsin (Fig. 4). At the two highest concentrations of L-aspartate used (19 mM and 28.4 mM) the inactivation rates were identical or very similar, indicating a saturation effect by the ligand. However, the inactivation under these conditions was still faster than in a concurrent experiment in the presence of 30 μ M-UMP. Thus the protective effect of L-aspartate, compared with that of UMP, was less complete and only achieved at very much higher ligand concentrations.

There was also a high degree of specificity in the protective effect of L-aspartate. Of three other dicarboxylic acids which were tried (succinate, D-aspartate and L-malate) none gave the degree of protection which L-aspartate did when all these compounds were used at the same concentration (19 mM) (Fig. 5). L-Malate was marginally more protective than succinate or D-aspartate, both of which had only a small effect compared with the control experiment in the absence of ligands.

Molecular-size characteristics

Gel filtration on Sephadex G-200 was used to study possible size variations in the enzyme at pH 10. The

Fig. 4. *Effects of the concentration of L-aspartate on the rates of inactivation of the enzyme by trypsin (a) and by heat (60°C) (b)*

The effects of various concentrations of L-aspartate are compared with the effect of 30 μ M-UMP. Experimental conditions are described in the text. Ligand concentrations are: none (●); 30 μ M-UMP (○); 4.75 mM-L-aspartate (Δ); 19.0 mM-L-aspartate (▲); 28.4 mM-L-aspartate (□).

elution profiles (Fig. 6) showed that enzyme activity was associated mainly with a single peak suggesting that most of the enzyme consisted of a uniform-size species. However the shoulder on one side of the main peak showed that a small proportion of the activity was associated with one or more enzyme species of larger size. Estimates of the area under the main peak (assumed to be symmetrical) showed that 80% of the activity recovered was associated with the predominant-size species in the absence of UMP, and 82% in the presence of UMP. Similar behaviour was observed at pH 7.5 (Yon, 1971).

Fig. 5. Effects of various dicarboxylic acids on the rates of inactivation of the enzyme by trypsin (a) and by heat (60°C) (b)

Experimental conditions are described in the text. Dicarboxylic acid concentrations are: none (●); 19 mM-L-aspartate (□); 19 mM-L-malate (■); 19 mM-succinate (▲); 19 mM-D-aspartate (△).

The inclusion of 60 μ M-UMP in the eluting buffer increased the overall yield of activity but did not significantly change the shape of the elution profile. The increase in yield was most likely due to protection by UMP against slow inactivation at pH 10. Since the profile shape was unchanged, UMP appeared not to affect the state of aggregation of the enzyme. The possibility that UMP might stabilize an inactive aggregate or subunit (which would not be evident in an activity profile) was rendered unlikely by the observation that UMP protected the main peak against inactivation, suggesting that the binding of UMP was predominantly to this enzyme species.

Determinations of the Stokes radius of the hydrated

Fig. 6. Gel-filtration chromatography of the enzyme in the absence and in the presence of UMP

Experimental conditions are described in the text. Chromatography was carried out in the absence of UMP (●, ↓) and in the presence of 60 μ M-UMP (○, ↓). The arrows show the peak positions of internal markers. The activity is given relative to the most active fraction.

enzyme at pH 10 were made by the gel-filtration technique of Ackers (1964) by using catalase as the reference protein. In three determinations in the absence of UMP values of 4.22, 4.16 and 4.16 nm were obtained, and in two determinations in the presence of 60 μ M-UMP values of 4.19 and 4.20 nm were obtained. These values refer to the enzyme in the main peak. Thus the addition of UMP was not accompanied by any change in hydrodynamic behaviour large enough to detect by gel filtration.

Discussion

The most likely explanation for the effects produced by ligands in these experiments is that conformational changes are induced in the enzyme as a result of ligand binding. Such effects are well known in proteins. If this explanation is assumed then the following conformational properties are indicated by these experiments. In the absence of ligands the enzyme exists in a conformation that is inactivated fairly rapidly by trypsin and a variety of denaturing agents. The addition of carbamoyl phosphate to the free enzyme either does not change its conformational state (in which case the free enzyme is in a conformation with high affinity for this ligand) or changes it to another conformation which is inactivated at the same rate. The first effect is considered to be more likely. In the presence of UMP the enzyme changes to a more resistant conformation. When the two

ligands are present together the enzyme may be distributed dynamically between the two states. Alternatively, the binding of both ligands may force the enzyme into one or more additional conformations of intermediate stability. Because of the high concentrations of L-aspartate required to produce noticeable protective effects, it is probable that under most conditions any conformational effects caused by this ligand are small in comparison with those caused by UMP and carbamoyl phosphate.

Assuming the enzyme is a typical regulatory enzyme with subunits, it is important to differentiate between ligand-induced conformational changes which occur independently in each subunit and those that are transmitted between subunits and may result in the co-operative or sigmoidal ligand-binding curves characteristic of allosteric proteins. In the experiments described in this paper there is no evidence that the presumed conformational changes are of the latter type, and it is therefore open to question whether these results are relevant to an understanding of the regulatory mechanism of this enzyme. Nevertheless, there are similarities between the co-operative conformational model suggested in a previous paper to account for the kinetic interactions of the enzyme (Yon, 1972*b*) and the conformational properties suggested by the inactivation behaviour of the enzyme. Thus both studies suggest that UMP and carbamoyl phosphate bind to different conformational states which are reversibly accessible to the enzyme, and that the free enzyme is in the conformation which binds carbamoyl phosphate. The low concentrations of UMP at which its protective

effects become noticeable are of the same order of magnitude (about 10^{-6} M) as the K_t value of the enzyme-UMP complex determined from kinetic studies. Further, the protective effects of UMP and its kinetic inhibitory action are both highly specific for UMP and both are enhanced by alkaline conditions. All these similarities suggest that the protective and kinetic interactions between UMP and carbamoyl phosphate derive from the same underlying causes.

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References

- Ackers, G. K. (1964) *Biochemistry* **3**, 723-730
- Beers, R. F. & Sizer, I. W. (1952) *J. Biol. Chem.* **195**, 133-140
- Laskowski, M. (1955) *Methods Enzymol.* **2**, 26-36
- Monod, J., Wyman, J. & Changeux, J.-P. (1965) *J. Mol. Biol.* **12**, 88-118
- Neumann, J. & Jones, M. E. (1962) *Nature (London)* **195**, 709-710
- Ong, B. L. & Jackson, J. F. (1970) *Proc. Aust. Biochem. Soc.* **3**, 17
- Ong, B. L. & Jackson, J. F. (1972) *Biochem. J.* **129**, 571-581
- Prescott, L. M. & Jones, M. E. (1969) *Anal. Biochem.* **32**, 408-419
- Siegel, L. M. & Monty, K. J. (1966) *Biochim. Biophys. Acta* **112**, 346-362
- Yon, R. J. (1971) *Biochem. J.* **121**, 18P-19P
- Yon, R. J. (1972*a*) *Biochem. J.* **126**, 765-767
- Yon, R. J. (1972*b*) *Biochem. J.* **128**, 311-320