EFFECT OF POTASSIUM CYANATE ON THE CATALYTIC ACTIVITIES OF CARBAMYL PHOSPHATE SYNTHETASE[1]

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SUMMARY. Potassium cyanate has the following effects on the catalytic activities exhibited by carbamyl phosphate synthetase (from E. coli): Glutamine-dependent carbamyl phosphate synthesis and ATP- and bicarbonate-dependent L-γ-glutamyl hydroxamate hydrolysis activities are inhibited, bicarbonate-dependent ATPase activity is stimulated by nearly 3-fold, synthesis of ATP from carbamyl phosphate and ADP is not affected, and the apparent K_m value for ammonia in the ammonia-dependent carbamyl phosphate synthesis reaction is decreased. Saturation kinetics are obtained when the effect of cyanate concentration is measured, and half maximal effect of cyanate is observed with a concentration of about 2 mM. These effects of potassium cyanate thus closely resemble those observed after treatment of this enzyme with L-2-amino-4-oxo-5-chloropentanoic acid. The present studies strongly suggest that cyanate, like the chloroketone, reacts at the glutamine binding site on the light subunit of the enzyme.

INTRODUCTION. In addition to the synthesis of carbamyl phosphate in which the source of nitrogen can be either glutamine or ammonia, carbamyl phosphate synthetase catalyzes the following "partial" reactions: 1) bicarbonate-dependent hydrolysis of ATP (ATPase), 2) stoichiometric synthesis of ATP from ADP and carbamyl phosphate, and 3) ATP- and bicarbonate-dependent hydrolysis of L-γ-glutamyl hydroxamate (1,2). The glutamine-dependent synthetase and

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the L-γ-glutamyl hydroxamate hydrolysis activities can be selectively inhibited by reaction of the enzyme with 2-amino-4-oxo-5-chloropentanoic acid (chloroketone), an active-site-directed irreversible inhibitor which has been shown to react with an SH group in the glutamine binding site of the enzyme; inhibition of these two reactions is accompanied by retention of full activity of both the ammonia-dependent synthetase and the ATP synthesis activities, and 3-fold activation of the ATPase activity (3,4).

In this communication we report that potassium cyanate at relatively low concentrations has effects on the catalytic activities of carbamyl phosphate synthetase which are similar to those of the chloroketone.

METHODS AND MATERIALS. Carbamyl phosphate synthetase was isolated from Escherichia coli B by the procedure described by Anderson et al. (5) as modified by Matthews and Anderson (6). Escherichia coli B cell paste (washed, 3/4 log phase, grown on minimal media) was obtained from Grain Processing Corp.

The ammonia- and glutamine-dependent synthetase activities were determined by measuring the rate of [^{14}C]carbamyl phosphate formation from [^{14}C]$NaHCO_3$ as previously described (2). ATPase, ATP synthesis and L-γ-glutamyl hydroxamate hydrolysis activities were determined by measuring the rate of formation of ADP, the rate of formation of ATP, and the rate of decrease in L-γ-glutamyl hydroxamate concentration, respectively, as previously described (2).

RESULTS. The effect of cyanate concentration on the different catalytic activities of carbamyl phosphate synthetase is shown in Figure 1. Similar results were obtained when the enzyme was preincubated with KOCN for 30 min at 22° before the assay reagents were added, indicating that the reaction with KOCN is rapid compared to the assay time (except as noted in the legend to Figure 1). The reaction with

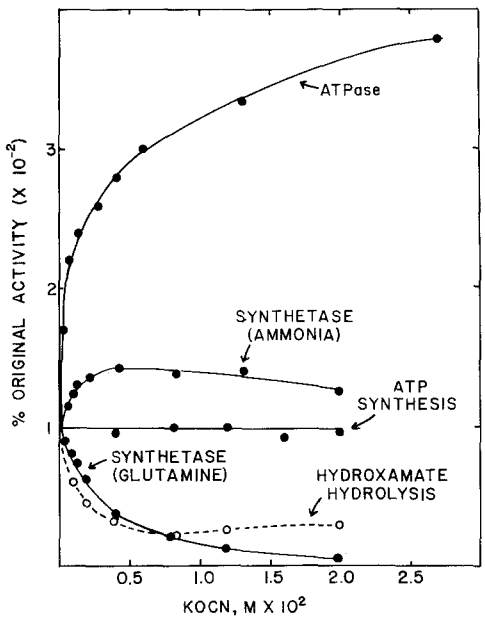

Figure 1. Effect of potassium cyanate concentration on different catalytic activities of carbamyl phosphate synthetase. The reactions were initiated by the addition of enzyme, and the μmoles of product formed after incubation for 10 min at 37° were determined for each activity as described in the text. The composition of the reaction mixtures and the original activities (μmoles product/10 min in the absence of KOCN) for the different activities were as follows. ATPase: ATP (10 mM), $MgCl_2$ (10 mM), $NaHCO_3$ (10 mM), potassium phosphate buffer (0.16 M, pH 7.8), and enzyme (20 μg) in a final volume of 0.3 ml; 0.08 μmoles ADP/10 min. ATP synthesis: ADP (20 mM), $MgCl_2$ (20 mM), dilithium carbamyl phosphate (12 mM), potassium phosphate buffer (0.12 M, pH 7.8), and enzyme (40 μg) in a final volume of 0.2 ml; 0.11 μmoles ATP/10 min. L-γ-glutamyl hydroxamate hydrolysis: L-γ-glutamyl hydroxamate (4 mM), ATP (10 mM), $MgCl_2$ (10 mM), $NaHCO_3$ (10 mM), potassium phosphate buffer (0.09 M, pH 7.8), and enzyme (15 μg) in a final volume of 0.3 ml; 0.5 μmoles L-γ-glutamyl hydroxamate hydrolyzed/10 min. Ammonia-dependent synthetase: ATP (20 mM), $MgCl_2$ (20 mM), [^{14}C]$NaHCO_3$ (20 mM, 4.6 x 10^5 cpm), NH_4Cl (0.1 M), potassium phosphate buffer (0.09 M, pH 7.8), and enzyme (16 μg) in a final volume of 1.0 ml; 0.34 μmoles [^{14}C]carbamyl phosphate/10 min. Glutamine-dependent synthetase: ATP (20 mM), $MgCl_2$ (20 mM), [^{14}C]$NaHCO_3$ (10 mM, 19,600 cpm), glutamine (5 mM), potassium phosphate buffer (0.09 M, pH 7.8), and enzyme (16 μg) in a final volume of 1.0 ml; 0.8 μmoles [^{14}C]carbamyl phosphate/10 min [The rate of product formation in this reaction in the presence of KOCN decreases with time after adding enzyme, reaching a constant value within 4 min which is dependent on the KOCN concentration (7). Consequently, the reaction was initiated with only unlabelled $NaHCO_3$ present. [^{14}C]-$NaHCO_3$ was added after 5 min and the reaction was allowed to proceed for 15 min rather than the usual 10 min; thus, the activity represents the linear rate observed during the latter 10 min of the 15 min assay]. The reaction mixtures also contained KOCN as indicated in the graph.

KOCN results in a decrease in the apparent K_m for ammonia from 115 mM to 20 mM, which is analogous to that observed when the enzyme is treated with chloroketone (4); the increase in synthetase activity with ammonia

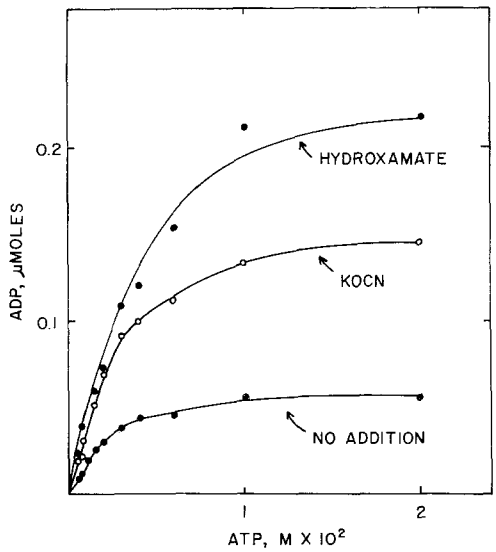

Figure 2. Effect of ATP concentration on the rate of ATPase activity in the presence and absence of potassium cyanate. The reaction mixtures contained $MgCl_2$ (equimolar with ATP), $NaHCO_3$ (10 mM), potassium phosphate buffer (0.07 M, pH 7.8), enzyme (15 μg), L-γ-glutamyl hydroxomate where indicated (0.02 M), KOCN where indicated (0.02 M), and ATP as indicated in a final volume of 0.3 ml. The ADP formed after incubation for 10 min at 37° was determined as described in the text.

as nitrogen donor as shown in Figure 1 is therefore expected, since a saturating concentration of ammonia is not used in the assay.

The ATPase and ammonia-dependent synthetase activities of chloroketone-treated enzyme were found to be the same in the presence and absence of 10 mM KOCN, indicating that the effect of KOCN is eliminated by reaction with chloroketone. In this experiment, chloroketone-treated enzyme was prepared essentially as previously described (3,4). The reaction mixture contained chloroketone (1 mM), potassium phosphate buffer (0.15 M, pH 7.8), EDTA (0.5 mM), and enzyme (0.22 mg) in a volume of 1.0 ml. After incubation for 50 min, the mixture was dialyzed for 48 hr at 4° against the same buffer.

The concentration of ATP required to give a half-maximal velocity of ATPase activity is not changed by the presence of KOCN as shown in Figure 2. The similarity of the effects of ATP concentration on the

rate of ADP formation during the hydrolysis of L-γ-glutamyl hydroxamate and on the ATPase activity in the presence or absence of KOCN is also demonstrated in Figure 2.

DISCUSSION. Previous studies have shown that carbamyl phosphate synthetase is composed of two subunits of different molecular weight (6,8). Evidence has been published which indicates that the function of the light subunit is to bind glutamine and in effect act as a glutaminase, releasing ammonia from the amide group which subsequently reacts with intermediates and other substrates on the heavy subunit (4,8). Thus, the SH group which reacts specifically with the chloroketone is located in the light subunit and all of the reactions catalyzed by the enzyme referred to in this paper (including ammonia-dependent synthetase activity) which do not involve glutamine or a glutamine analog directly can be catalyzed by the heavy subunit alone; the light subunit alone has been shown to have glutaminase activity (4,8). Since the effects of KOCN on the activities catalyzed by carbamyl phosphate synthetase and on the apparent K_m for ammonia as described in this paper are virtually identical to those caused by the chloroketone, it is probable that the specific site of interaction with KOCN is also at the glutamine binding site on the light subunit.

The ability of cyanate to react with various nucleophilic groups in proteins is well established (9,10) and its ability to inactivate several different enzymes by carbamylation of a specific amino acid residue(s) has been described (11,12). In the cited studies, relatively high concentrations of cyanate and long reaction times were necessary to effect complete inactivation and the reactions were shown to be first order with respect to cyanate. In contrast, the results reported in this paper show that the effects of cyanate on the catalytic activities of carbamyl phosphate synthetase are observed at relatively low cyanate concentrations, are not dependent on long reaction times, and saturation

kinetics are obtained when the effect of cyanate concentration on activity is measured. These observations together with the observed similarity of the effects of cyanate and chloroketone indicate that the inhibitory action of cyanate probably involves an initial binding of cyanate at the glutamine binding site.

The hydrolysis of L-γ-glutamyl hydroxamate proceeds at a rate which is close to that of glutamine hydrolysis in the glutamine-dependent synthetase reaction. However, although hydrolysis of this glutamine analog is dependent on ATP and bicarbonate, the reaction is not accompanied by stoichiometric formation of ATP (presumably the hydroxylamine moiety which is released from L-γ-glutamyl hydroxamate fails to react with the normal intermediate) (2). The results reported in this paper suggest that the small amount of ADP formation previously observed to accompany this reaction is the result of binding of L-γ-glutamyl hydroxamate at the glutamine binding site on the light subunit with stimulation of the ATPase activity analogous to that caused by cyanate or chloroketone.

These studies with cyanate support previous observations that there is a functional interaction between the heavy and light subunits of this enzyme (4,13). Thus, the reaction of chloroketone, L-glutamine, L-γ-glutamyl hydroxamate, and presumably, KOCN, at the glutamine-binding site on the light subunit of carbamyl phosphate synthetase has a substantial effect on the ATPase activity and the affinity for ammonia (with no effect on the ATP synthesis reaction), all of which are functions of the heavy subunit. Additional evidence for such an interaction is the observation that binding at the heavy subunit can apparently affect the properties of the light subunit, since the presence of ATP plus bicarbonate, which presumably bind to site(s) on the heavy subunit, results in the availability of a specific SH group in the light subunit for reaction with N-ethylmaleimide (6,14).

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