Selective Inactivation of the Glutamine Binding Site of *Escherichia coli* Carbamyl Phosphate Synthetase by 2-Amino-4-oxo-5-chloropentanoic Acid*

Ezra Khedouri,† Paul M. Anderson,‡ and Alton Meister

ABSTRACT: L-2-Amino-4-oxo-5-chloropentanoic acid, a chloroketone analog of L-glutamine, was synthesized and found to inhibit the synthesis of carbamyl phosphate by *Escherichia coli* carbamyl phosphate synthetase when glutamine is the nitrogen donor. Inhibition, which is virtually complete in 10 min with a chloroketone concentration of 4×10^{-4} M, is not reversible by dialysis. The ability of the enzyme to catalyze carbamyl phosphate synthesis from ammonia is not impaired by treatment with the chloroketone, nor is the activity responsible for synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and carbamyl phosphate affected. The treated enzyme exhibits about three times as much carbon dioxide dependent ATPase as the untreated enzyme, but the treated enzyme does not catalyze hydrolysis of γ-glutamyl hydroxamate. Inhibition by the chloroketone is prevented by L-glutamine, L-γ-glutamyl hydroxamate, and to a much smaller extent by ATP, Mg^{2+}, and bicarbonate. The findings indicate that the chloroketone selectively reacts with the enzyme site that normally accepts glutamine and that it probably alkylates a specific nucleophilic group of the enzyme. DL-3-Amino-5-oxo-6-chlorohexanoic acid was also synthesized; this chloroketone is about 25% as active in inhibiting the enzyme as L-2-amino-4-oxo-5-chloropentanoic acid.

Carbamyl phosphate synthetase of *Escherichia coli* catalyzes the following over-all synthesis reactions (Anderson and Meister, 1965a,b, 1966a):

$$\text{L-glutamine} + CO_2 + 2ATP^1 \longrightarrow \text{L-glutamate} + 2ADP + \text{carbamyl phosphate} + P_i \quad (1)$$

$$NH_3 + CO_2 + 2ATP \longrightarrow 2ADP + \text{carbamyl phosphate} + P_i \quad (2)$$

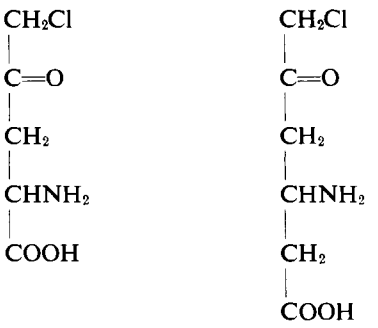
Although reaction 2 can proceed at a rate close to the maximal velocity of reaction 1, much higher concentrations of NH_4Cl are needed as compared to glutamine. The enzyme is thus similar to several others that catalyze reactions involving transfer of the amide nitrogen atom of glutamine but which can utilize ammonia in place of glutamine (*e.g.*, synthesis of DPN from deamido-DPN, synthesis of guanylate from xanthylate; for a review, see Meister, 1962). Previous studies in this laboratory (Anderson and Meister, 1966a) have shown that carbamyl phosphate synthetase can catalyze several additional reactions each of which seems to reflect an individual step in the over-all reaction, *i.e.* (1) bicarbonate-dependent cleavage of ATP, (2) ATP- and bicarbonate-dependent hydrolysis of γ-glutamyl hydroxamate, and (3) synthesis of ATP from ADP and carbamyl phosphate. Other studies have indicated that the enzyme can combine with L-glutamine (in the absence of ATP) to yield a glutamyl–enzyme–ammonia complex (P. M. Anderson and A. Meister, unpublished). In the synthesis reaction with ammonia (reaction 2) it is probable that an enzyme–ammonia complex is formed directly and that under these conditions synthesis does not require utilization of the glutamine (or glutamyl) binding site. It would therefore seem possible to design a reagent which would react with the glutamine binding site of the enzyme without interfering with its other catalytic functions; such treatment might then convert the enzyme from one which can utilize both glutamine and ammonia to one which can use only ammonia. This goal has been attained through synthesis of a chloroketone analog of glutamine, L-2-amino-4-oxo-5-chloropentanoic acid (referred to in this paper as chloroketone I). This compound was prepared by converting *N*-carbobenzoxy-L-aspartyl-α-benzyl ester to the corresponding β-chloride, which was treated with diazomethane to give the β-diazoketone. The latter compound was treated with hydrogen chloride to obtain the desired compound (chloroketone I). DL-3-Amino-5-oxo-6-chlorohexanoic acid (chloroketone II) was synthesized

* From the Department of Biochemistry, Tufts University School of Medicine, Boston, Massachusetts 02111. *Received July 22, 1966.* Supported in part by the National Science Foundation and the National Institutes of Health, U. S. Public Health Service.

† Present address, Department of Chemistry, Inter American University, San Juan, Puerto Rico.

‡ Present address, Department of Chemistry, Southern Illinois University, Carbondale, Ill.

[1] Abbreviations: ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; DPN, diphosphopyridine nucleotide.



chloroketone I (2-amino-4-oxo-5-chloropentanoic acid)

chloroketone II (3-amino-5-oxo-6-chlorohexanoic acid)

in analogous fashion. Chloroketone I is a potent inhibitor of carbamyl phosphate synthetase when glutamine is the nitrogen donor (reaction 1) but does not inhibit the ammonia-utilizing reaction 2.

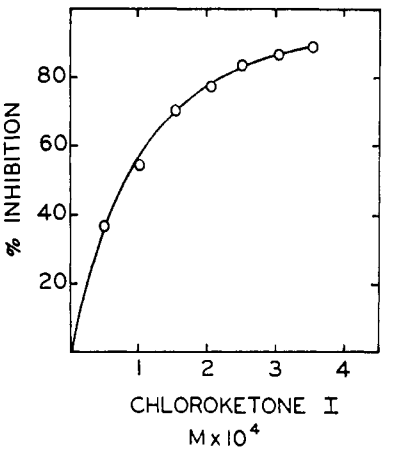


FIGURE 1: Effect of chloroketone I concentration on glutamine-dependent carbamyl phosphate synthetase. The reaction mixtures contained enzyme (0.016 mg), potassium phosphate buffer (pH 7.8, 10 μmoles), and chloroketone I in a final volume of 0.1 ml; after incubation at 37° for 10 min, 0.9 ml of a solution containing L-glutamine (20 μmoles), ATP (20 μmoles), $MgCl_2$ (20 μmoles), [^{14}C]$NaHCO_3$ (20 μmoles, 700,000 cpm), Tris–HCl (pH 8.2, 40 μmoles), and KCl (40 μmoles) was added. After incubation for 10 min at 37°, the [^{14}C]carbamyl phosphate formed was determined.

Experimental Section

Materials. Carbamyl phosphate synthetase was isolated from *E. coli* B as previously described (Anderson and Meister, 1965b, 1966a). The sources and methods of preparation of the reagents used for the determination of enzymatic activity were given previously (Anderson and Meister, 1966a). L-Azaserine and 6-diazo-5-oxo-L-norleucine were generously provided by Dr. John M. Buchanan.

Methods. Carbamyl phosphate synthetase activity was determined in the presence of L-glutamine and NH_4Cl as previously described (Anderson and Meister, 1966a); in this procedure the [^{14}C]carbamyl phosphate is converted to [^{14}C]urea which is determined after separation from $^{14}CO_2$. The other enzymatic activities were also determined as described (Anderson and Meister, 1966a).

Synthesis of Chloroketones. BENZYL-*N*-CARBOBENZOXY-L-2-AMINO-4-OXO-5-AZAPENTANOATE (III). *N*-Carbobenzoxy-L-aspartyl-α-benzyl ester (Cyclo Chemical Co.), 3 g, was dissolved in 10 ml of thionyl chloride in a round-bottom flask fitted with a $CaCl_2$ drying tube. The flask contents were warmed to 40° on a water bath for 30 min and the excess thionyl chloride was removed in a flash evaporator under high vacuum. *N*-Carbobenzoxy-L-aspartyl-β-chloro α-benzyl ester remained as a colorless oil. Diazomethane (2.1 molar equiv) in ether solution was then added; after shaking the mixture for 5 min, the mixture was allowed to stand in the hood overnight. The ether was removed on a flash evaporator and the oily residue was crystallized from ether–petroleum ether (bp 30–60°) to give 2.3 g (75% yield) of colorless crystals, mp 71–72°. The infrared spectrum in chloroform showed the characteristic diazoketone absorption at 4.78 μ. *Anal.* Calcd for $C_{20}H_{19}N_3O_5$: C, 63.0; H, 5.1. Found: C, 63.0; H, 5.0.

L-2-AMINO-4-OXO-5-CHLOROPENTANOIC ACID HYDROCHLORIDE (I). Compound III (2.3 g) was dissolved in 15 ml of a chloroform–ether mixture (1:1) and dry hydrogen chloride was bubbled in slowly for 15 min. The solvents were removed on a flash evaporator and the remaining oil (whose infrared spectrum in chloroform showed complete loss of the diazoketone absorption at 4.78 μ) was suspended in 75 ml of 6 N HCl and heated on a steam bath for 24 hr. The solution was reduced in volume to 10 ml on a flash evaporator and then extracted with ether (two 20-ml portions) and chloroform (two 20-ml portions). The extracts were discarded and the aqueous layer was evaporated to dryness. The residue was dissolved in 20 ml of water, decolorized with the minimum amount of Norit, and filtered. The filtrate was evaporated to dryness; on addition of dry acetone, the residue quickly crystallized. The product was recrystallized from water–acetone to yield colorless crystals, mp 151–152° dec, yield 0.6 g (60%). The infrared spectrum (KBr) showed the expected (CCl) absorption at 12.8 μ. *Anal.* Calcd for $C_5H_9Cl_2NO_3$: C, 29.7; H, 4.5; Cl, 35.1; N, 6.9. Found: C, 29.9; H, 4.7; Cl, 35.1; N, 7.2.

BENZYL-*N*-CARBOBENZOXY-DL-3-AMINO-5-OXO-6-AZAHEXANOATE (IV). This compound was prepared from *N*-carbobenzoxy-DL-3-aminoglutaryl monobenzyl ester (Khedouri *et al.*, 1964) in 75% yield in the same manner as described for III; mp 93°. *Anal.* Calcd for $C_{21}H_{21}N_3O_5$: C, 63.8; H, 5.3; N, 10.6. Found: C, 63.7; H, 5.6; N, 10.6.

DL-3-AMINO-5-OXO-6-CHLOROHEXANOIC ACID HYDROCHLORIDE (II). This compound was prepared from the corresponding diazoketone IV in 70% yield in the same manner as described for I; mp 165–167° dec. *Anal.*

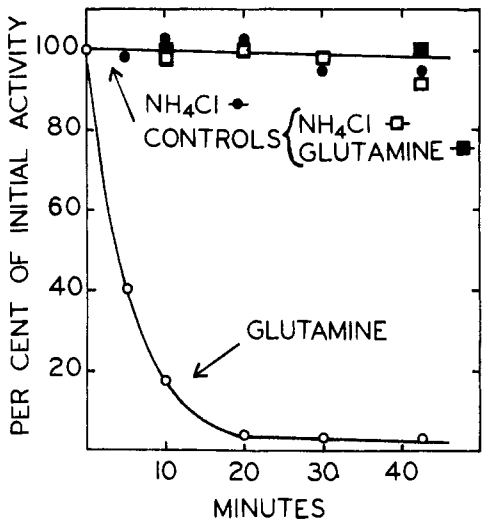


FIGURE 2: Effect of chloroketone I as a function of time on the activity of carbamyl phosphate synthetase with NH_4Cl or glutamine as amino donor. A solution (0.5 ml) containing enzyme (0.3 mg), potassium phosphate buffer (100 μmoles, pH 6.9), potassium chloride (100 μmoles), and EDTA (0.25 μmole) was added to 0.5 ml of a solution containing chloroketone I (0.25 μmole) at 0°. The mixture was then warmed rapidly to 37° and maintained at this temperature. At the indicated intervals 0.05-ml aliquots were added to separate test tubes and frozen in a Dry Ice–acetone bath. The activity of the enzyme samples thus removed was then determined with NH_4Cl or glutamine by adding 0.95 ml of a mixture containing ATP (20 μmoles), $MgCl_2$ (20 μmoles), [^{14}C]$NaHCO_3$ (20 μmoles, 800,000 cpm), potassium phosphate buffer (80 μmoles, pH 7.8), and L-glutamine (10 μmoles) or potassium phosphate buffer (40 μmoles, pH 7.8) and NH_4Cl (100 μmoles). The [^{14}C]carbamyl phosphate formed after incubation for 10 min at 37° was determined. The control experiments were carried out in the same way, except that chloroketone I was omitted. The initial activities were 0.28 and 0.49 μmole of [^{14}C]carbamyl phosphate formed, respectively, with NH_4Cl and glutamine.

Calcd for $C_6H_{11}Cl_2NO_3$: C, 33.3; H, 5.1; Cl, 32.8; N, 6.5. Found: C, 33.6; H, 5.2, Cl, 32.1; N, 6.6.

Paper Electrophoresis. The chloroketones moved as homogeneous compounds when subjected to paper electrophoresis for 90 min on Whatman 3MM paper in 6.7% formic acid under the conditions previously described (Khedouri *et al.*, 1964); II and I moved 97 and 65 cm, respectively, in the direction of the negative electrode.

Enzymatic Studies. EFFECT OF CHLOROKETONE I ON CARBAMYL PHOSPHATE SYNTHETASE ACTIVITY. Preincubation of the enzyme with chloroketone I (0.5–4 × 10^{-4} M) for 10 min at 37° led to a marked decrease in glutamine-dependent carbamyl phosphate synthetase activity. Under these conditions about 50% inhibition was observed with 10^{-4} M chloroketone I (Figure 1). Dialysis of the inhibited enzyme did not restore activity. The inhibitory effect of chloroketone I was substantially the same at values of pH from 6.5 to 9.0. Chloroketone I produced considerably more inhibition than did chloroketone II, L-azaserine, or 6-diazo-5-oxo-L-norleucine (Table I).

TABLE 1: Inhibition of Glutamine-Dependent Carbamyl Phosphate Synthetase by Chloroketone I and Other Compounds.[a]

Compound	% Inhibn
Chloroketone I	55[b]
Chloroketone II	15[b]
6-Diazo-5-oxo-L-norleucine	15
L-Azaserine	4

[a] The enzyme (0.4 mg) was incubated in 0.5 ml of a solution containing potassium phosphate buffer (pH 7.7, 38 μmoles), KCl (25 μmoles), and inhibitor (L 0.125 μmole, DL 0.25 μmole) at 37°. After 5 min a sample (0.05 ml) of the incubation mixture was removed and added to 0.5 ml of a solution at 0° containing L-glutamine (10 μmoles) and potassium phosphate buffer (pH 7.8, 200 μmoles). The activity of the enzyme was determined by adding 0.5 ml of a solution containing [^{14}C]-$NaHCO_3$ (20 μmoles), ATP (20 μmoles), $MgCl_2$ (20 μmoles), Tris–HCl (pH 8.2, 40 μmoles), and KCl (40 μmoles); after incubation for 10 min at 37° the reaction was stopped and the [^{14}C]carbamyl phosphate formed was determined. [b] No inhibition was observed when the initial incubation was carried out in the presence of 0.03 M L-glutamine.

In contrast to the marked inhibition of glutamine-dependent synthesis (reaction 1), incubation of the enzyme with chloroketone I had no effect on the synthesis of carbamyl phosphate from CO_2 and ammonia (reaction 2). Thus, as described in Figure 2, virtually all glutamine-dependent activity was abolished by incubation of the enzyme with 5 × 10^{-4} M chloroketone I for 20 min; on the other hand, no decrease in activity toward ammonia was observed after 40 min of incubation.

EFFECT OF CHLOROKETONE I ON OTHER REACTIONS CATALYZED BY THE ENZYME. Incubation of the enzyme with 2.5 × 10^{-4} M chloroketone I for 30 min followed by extensive dialysis gave a preparation that showed virtually no glutamine-dependent activity but exhibited unchanged activity toward ammonia. This preparation exhibited more than three times the CO_2-dependent ATPase activity of the control, but it did not catalyze detectable hydrolysis of γ-glutamyl hydroxamate. The treated preparation and the control catalyzed ATP synthesis from ADP and carbamyl phosphate at the same rate (Table II).

TABLE II: Effect of Chloroketone I on the Reactions Catalyzed by Carbamyl Phosphate Synthetase.[a]

Expt	Reaction	Specific Activity: Control Enzyme	Specific Activity: Treated Enzyme	% Change
1a	Over-all reaction (glutamine)	220	10	−95
1b	Over-all reaction (NH_4Cl)	125	125	0
2	CO_2-dependent ATPase	13	43	+230
3	ATP synthesis from ADP and carbamyl phosphate	20	20	0
4	Hydrolysis of γ-glutamyl hydroxamate	290	<15	>−95

[a] The enzyme (0.9 mg) was incubated at 37° for 30 min in 1 ml of a solution containing chloroketone I (0.25 μmole) (omitted in the control), potassium phosphate buffer (100 μmoles, pH 6.9), KCl (100 μmoles), and EDTA (0.5 μmole); the solution was then dialyzed at 5° against two changes of 1 l. each of 0.2 M potassium phosphate buffer (pH 7.8) containing 5×10^{-4} M EDTA for 18 hr. The reaction mixtures used for assay of the several activities were as follows. *expt 1:* ATP (20 μmoles), $MgCl_2$ (20 μmoles), [^{14}C]$NaHCO_3$ (20 μmoles, 800,000 cpm), L-glutamine (20 μmoles) or NH_4Cl (100 μmoles), potassium phosphate buffer (100 μmoles, pH 7.8), potassium chloride (10 μmoles), EDTA (0.05 μmole), and enzyme (0.018 mg) in a final volume of 1 ml. *Expt* 2: ATP (3 μmoles), $MgCl_2$ (3 μmoles), $NaHCO_3$ (3 μmoles), potassium phosphate buffer (30 μmoles, pH 7.8), and enzyme (0.034 mg) in a final volume of 0.3 ml. *Expt* 3: ADP (3 μmoles), lithium carbamyl phosphate (3 μmoles), potassium phosphate buffer (30 μmoles, pH 7.8), and enzyme (0.034 mg) in a final volume of 0.3 ml. *Expt 4*: ATP (3 μmoles), $MgCl_2$ (3 μmoles), $NaHCO_3$ (3 μmoles), L-γ-glutamyl hydroxamate (0.9 μmole), potassium phosphate buffer (30 μmoles, pH 7.8), and enzyme (0.0045 mg) in a final volume of 0.3 ml. The products formed after incubation for 10 min at 37° were determined.

PROTECTION OF THE ENZYME AGAINST INACTIVATION BY CHLOROKETONE I. As indicated by the findings given in Table III, L-glutamine and L-γ-glutamyl hydroxamate effectively protected the enzyme from inhibition by chloroketone I, while D-glutamine, NH_4Cl, ATP, ADP, $NaHCO_3$, and $MgCl_2$ (in various combinations) offered much less or no protection.

Discussion

The data indicate that chloroketone I is a potent and selective inhibitor of the enzyme and that such inhibition is apparently irreversible. Chloroketone I is substantially more active than azaserine and 6-diazo-5-oxonorleucine, which are known as inhibitors of a variety of reactions involving transfer of the amide nitrogen atom of glutamine (Meister, 1962). Glutamine-dependent carbamyl phosphate synthesis and hydrolysis of γ-glutamyl hydroxamate are the only reactions that are inhibited by chloroketone I, and both L-glutamine and L-γ-glutamyl hydroxamate protect against inhibition. These observations lead to the conclusion that chloroketone I binds to the enzyme site that normally accepts glutamine (and can also combine with γ-glutamyl hydroxamate). That this site is different from that which binds ammonia seems evident from the unimpaired ability of the chloroketone-treated enzyme to utilize ammonia. These considerations together with the finding that incubation of the enzyme with L-[^{14}C]glutamine (in the absence of other substrates) yields a glutamyl–enzyme complex that can be separated from free [^{14}C]glutamine by gel filtration on Sephadex columns (P. M. Anderson and A. Meister, unpublished) suggest that the reaction between enzyme and glutamine is accompanied by transfer of the glutamine amide N atom to a site on the enzyme. It seems probable that this site can also accept free ammonia (or ammonium ion). This hypothesis is illustrated in Figure 3, in which it is postulated that a nucleophilic group (X) of the enzyme attacks the electrophilic center on C_5 of glutamine (Figure 3A and B) or of chloroketone I (Figure 3C and D). Reaction with the latter reagent results in irreversible alkylation of the enzyme. Schoellmann and Shaw (1963) have reported the synthesis of a specific chloroketone which combines with chymotrypsin in a similar manner.

The observation that the synthesis of ATP from ADP and carbamyl phosphate is not affected by chloroketone I indicates that this reaction (and presumably the analogous postulated intermediate step in the over-all synthesis reaction) takes place on a separate site of the enzyme. A similar conclusion may be drawn concerning the CO_2-dependent ATPase reaction; however, the observed increase in this activity after reaction of the enzyme with chloroketone suggests that there may be interaction between the site that binds glutamine and that for activation of carbon dioxide. In this connection it seems pertinent to note that the hydrolysis of γ-glutamyl hydroxamate catalyzed by the enzyme requires both ATP and carbon dioxide and that hydrolysis of the hydroxamate is not accompanied by stoichiometric formation of ADP (An-

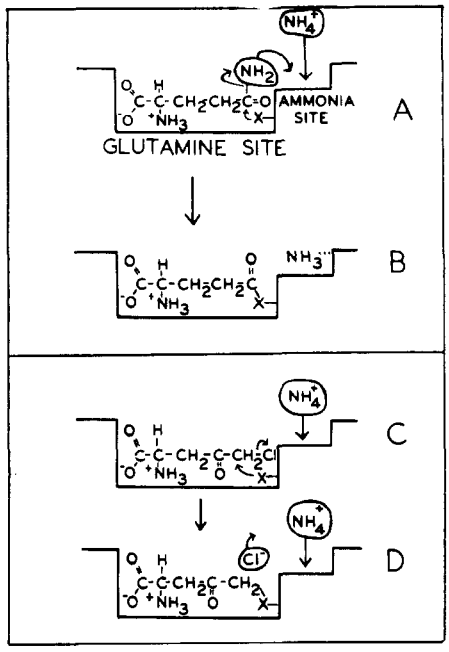


FIGURE 3: Schematic diagram of the reaction of the enzyme with glutamine and chloroketone I.

TABLE III: Protection of the Enzyme against Inactivation by Chloroketone I.[a]

Compd Added during Preincubation (M)	Carbamyl Phosphate Formed (μmoles): Control Expt	In Presence of Chloroketone I	% Inhibn
None	0.67	0.04	94
L-Glutamine (0.03)	0.65	0.65	0
D-Glutamine (0.03)	0.65	0.05	92
NH_4Cl (0.03)	0.69	0.06	91
NH_4Cl (0.10)	0.72	0.11	85
ATP (0.02), $MgCl_2$ (0.02), $NaHCO_3$ (0.02)	0.63	0.19	70
$NaHCO_3$ (0.02)	0.61	0.03	95
ATP (0.02), $MgCl_2$ (0.02)	0.64	0.06	91
ADP (0.02), $MgCl_2$ (0.02)	0.57	0.03	95
L-γ-Glutamyl hydroxamate (0.02)	0.60	0.59	1.7

[a] The enzyme (15 μg) was preincubated at 37° for 10 min in 0.1 ml of a solution containing potassium phosphate buffer (10 μmoles, pH 6.9), KCl (10 μmoles), EDTA (0.025 μmole), chloroketone I (0.06 μmole), and additional compounds as indicated in the table. Then 0.9 ml of a solution containing ATP (20 μmoles), $MgCl_2$ (20 μmoles), [^{14}C]$NaHCO_3$ (20 μmoles, 800,000 cpm), L-glutamine (20 μmoles), and potassium phosphate buffer (100 μmoles, pH 7.8) was added; after incubation at 37° for 10 min the amount of [^{14}C]carbamyl phosphate formed was determined.

derson and Meister, 1966a). It is also of note that ATP, Mg^{2+}, and $NaHCO_3$ afford some protection against chloroketone I; this effect is greater than that observed with either MgATP or $NaHCO_3$ alone (Table III). These considerations suggest that the first and second steps in the mechanism postulated previously (activation of CO_2 and reaction of the activated CO_2 with glutamine to yield enzyme-bound carbamate; Anderson and Meister, 1965b) are closely associated and relatively independent of the third step (phosphorylation of carbamate).

A number of enzymes are now known which catalyze the transfer of the amide nitrogen atom of glutamine, and most of these can utilize ammonia in place of glutamine. The possibility that the affinity of such an enzyme for glutamine might be altered during the procedure used for isolation has been considered (Meister, 1962). The present studies indicate that the affinity of carbamyl phosphate synthetase for glutamine can be selectively altered by treatment with chloroketone I, and it is conceivable that a similar result might be produced in other ways. Preiss and Handler (1958) reported that azaserine appeared to be a competitive inhibitor of both glutamine and ammonia in the enzymatic conversion of deamido-DPN to DPN. They also reported that preincubation of the enzyme with azaserine, deamido-DPN, ATP, Mg^{2+}, and K^+ led to increased inhibition when glutamine was the amide donor, while there was a smaller increase in inhibition when ammonia was used. It seems possible that azaserine inhibits this enzyme by more than one mechanism. It would be of interest to study the effect of the chloroketones described here on this system and on other enzymes that catalyze reactions involving transfer of the amide nitrogen atom of glutamine. Studies on other chloroketone analogs, *e.g.*, 2-amino-5-oxo-6-chlorohexanoic acid, would also be of interest. Attempts to synthesize the latter compound are now being made in this laboratory.

The present findings indicate that chloroketone I reacts with a specific enzyme site; studies with this reagent therefore seem to offer a promising experimental approach to an understanding of the amino acid structure of this portion of the enzyme. Studies along these lines are in progress.

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The Substitution of 1-Methylhydrazine for Ammonia in the Glutamine Synthetase System*

James E. Willis†

ABSTRACT: 1-Methylhydrazine was found to serve as a substrate for sheep brain glutamine synthetase. The Michaelis constant determined for 1-methylhydrazine is approximately 100 times larger than that for ammonia, although the maximum velocity is the same. Paper chromatography of deproteinized incubation mixtures revealed a major and a minor ninhydrin-positive, hydrazine-containing substance in the complete mixture, but absent from controls.

The enzymic products were separated from glutamic acid, glutamine, and 5-glutamylmethylamide in two solvent systems. A chromatographic reference material was prepared by condensation of pyrrolidonecarboxylic acid with 1-methylhydrazine. Two ninhydrin-positive, hydrazine-containing substances were obtained which were not separated from the corresponding major and minor components of the enzymic product. Experiments with [1-^{14}C]methylhydrazine showed that both the major and minor enzymic products contained radioactivity.

The metabolism of hydrazine and of alkylhydrazines is of current interest since these compounds are used as rocket fuels. Information concerning the biochemistry of hydrazines may be of value in the design of effective antidotes against the toxic effects of these substances (O'Brien *et al.*, 1964). At the time this study was begun little was known about the metabolism of 1-methylhydrazine. Recently, experiments with rats have shown that 45% of the administered radioactivity of an intraperitoneal dose of [1-^{14}C]methylhydrazine was expired as methane and another 40% of the radioactivity appeared as unidentified urinary products in a 24-hr period (Dost *et al.*, 1965). Since hydrazine is known to participate in the glutamine synthetase system (Speck, 1949), it was of interest to determine whether 1-methylhydrazine substitutes for ammonia to form an analog of glutamine.

Materials and Methods

The following reagents were obtained from commercial sources:[1] L-glutamine and ADP[2] (Sigma Chemical Co.), ATP (P-L Biochemicals, Inc.), L-glutamic acid (Calbiochem), 1-methylhydrazine (free base), methylamine and 1,1-dimethylhydrazine (99% pure) (Matheson Coleman and Bell), 1-methylhydrazine sulfate (Eastman Organic Chemicals), and [1-^{14}C]methylhydrazine (Nuclear Research Chemicals, Inc., Orlando, Fla.).

Recrystallization of 1-Methylhydrazine Sulfate. A yellow contamination develops on the surface of crystals of 1-methylhydrazine on standing (McKennis and Yard, 1954). The salt was recrystallized, when necessary, by the following procedure. 1-Methylhydrazine sulfate (10 g) was dissolved in approximately 20 ml of warm water and the solution was filtered rapidly by suction in the hood. Warm methanol (approximately 40 ml) was added to the cloud point and the preparation was stored under N_2 in the cold overnight. The crystals were collected by vacuum filtration, washed with cold methanol–water (2:1, v/v), and dried *in vacuo* over P_2O_5. The yield is 6–7 g.

Determination of 1-Methylhydrazine. 1-Methylhydrazine was determined colorimetrically by the method

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† Present Address: P-L Biochemicals, Inc., Milwaukee, Wis.

[1] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Air Force over other firms or similar products not mentioned.

[2] Abbreviations used: ADP, adenosine diphosphate; ATP, adenosine triphosphate.