Meloche, H. P. (1967) *Biochemistry 6*, 2273–2280.
Michaels, G., Milner, Y., & Reed, H. G. (1975) *Biochemistry 14*, 3213–3219.
Milner, Y., & Wood, H. G. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 2463–2468.
Milner, Y., & Wood, H. G. (1976) *J. Biol. Chem. 251*, 7920–7928.
Milner, Y., Michaels, G., & Wood, H. G. (1975) *Methods Enzymol. 42*, 199–212.
Milner, Y., Michaels, G., & Wood, H. G. (1978) *J. Biol. Chem. 253*, 878–883.
Mukrasch, L. L., & McGilvery, R. W. (1956) *J. Biol. Chem. 221*, 909–917.
Powell, J. T., & Brew, K. (1976) *Biochemistry 15*, 3499–3505.
Spronk, A. M., Yoshida, H., & Wood, H. G. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 4415–4419.
Yoshida, H., & Wood, H. G. (1978) *J. Biol. Chem. 253*, 7650–7655.

Substrate-Mediated Inactivation of Urocanase from *Pseudomonas putida*. Evidence for an Essential Sulfhydryl Group†

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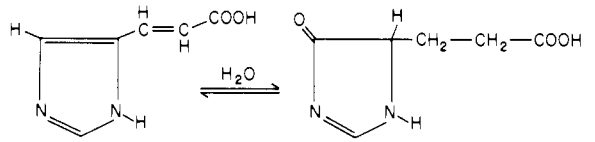
ABSTRACT: Incubation of urocanase from *Pseudomonas putida* with either its substrate, urocanic acid, or product, 4′(5′)-imidazolone-5′(4′)-propionic acid, resulted in an oxygen-dependent inhibition of enzyme activity. Coincident with the inactivation was the stoichiometric incorporation of radioactivity from [^{14}C]urocanate into the protein. NAD^+ which is required for activity of urocanase was not directly involved in the inactivation process. The inactivation of urocanase was irreversible, could be partially blocked by the competitive inhibitor imidazolepropionate, and involved the modification of a single active-site thiol. The inhibition resulted from oxidative decomposition of 4′(5′)-imidazolone-5′(4′)-propionate but was not due to the formation of the major degradative product, 4-ketoglutaramate, since this compound was not an irreversible inactivator of urocanase although it did produce some inhibition at high concentrations. A mechanism is presented in which a reactive imine intermediate in the decomposition scheme is subject to nucleophilic attack by an active-site thiol, thereby generating a covalent enzyme–thioaminal adduct. These results emphasize the importance of a catalytic center sulfhydryl group for urocanase activity.

Urocanase (EC 4.2.1.49) catalyzes the second step in the histidine catabolic pathway, namely, the conversion of urocanic acid to 4′(5′)-imidazolone-5′(4′)-propionic acid:



UROCANIC ACID — IMIDAZOLONE PROPIONIC ACID

The urocanase from *Pseudomonas putida* (Egan & Phillips, 1977; Keul et al., 1979) as well as the enzyme from beef liver (Keul et al., 1979) contains a tightly bound NAD^+ that is essential for catalysis. Other studies have suggested the importance of sulfhydryl residues in catalysis for urocanases from *P. putida* (Hug & Roth, 1973), *Pseudomonas testosteroni* (Hacking et al., 1978), and beef liver (Feinberg & Greenberg, 1959).

In the course of an investigation into the participation of NAD^+ in the mechanism of the urocanase reaction, we observed that the enzyme from *P. putida* became completely inactivated under conditions where large amounts of substrate were being converted to product. Kaminskas et al. (1970) had reported a similar inactivation of the enzyme from *Bacillus subtilis*; however, no mechanism was offered to account for this observation. Our interest in the chemistry of the active site led us to examine this substrate-derived inactivation in greater detail so as to understand its molecular basis and relationship to catalysis. In this report, we present evidence for the role of substrate in the inactivation of urocanase and additionally substantiate the importance of an active-site thiol for catalysis.

Materials and Methods

Chemicals. [^{14}C]Urocanic acid was prepared from L-[U-^{14}C]histidine (270 mCi/mmol) according to the procedure of Mehler et al. (1955). Imidazolonepropionic acid, both unlabeled and ^{14}C labeled, was prepared and purified as described by Brown & Kies (1959). 4-Ketoglutaramic acid was prepared by the method of Hassall & Greenberg (1963). *N*-Formylisoglutamine was synthesized as described by Borek & Waelsch (1953). All other chemicals were obtained from commercial sources.

Enzyme Preparation and Assay. Urocanase was isolated from *Pseudomonas putida*, ATCC 12633, essentially as described by George & Phillips (1970). The purified enzyme had a specific activity of 1.8–2.2 μmol min^{-1} mg^{-1} and was of 95% purity or greater as determined by polyacrylamide gel electrophoresis at pH 8.3 (Davis, 1965) and sodium dodecyl sulfate gel electrophoresis (Weber & Osborn, 1969).

The urocanase assay was the spectrophotometric measurement of urocanate disappearance at 277 nm, as described by George & Phillips (1970). Protein determinations were performed according to the method of Groves et al. (1968), with bovine serum albumin as the standard.

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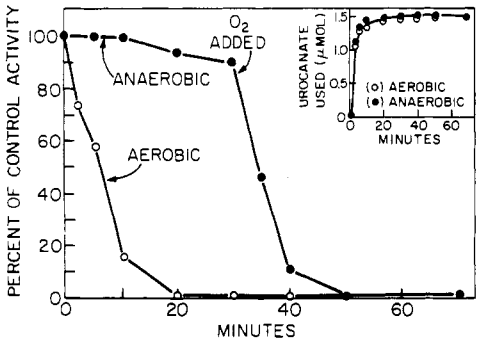

FIGURE 1: Effect of oxygen on the inactivation of urocanase by urocanic acid. Urocanase (0.1 mg) was incubated at 21 °C with 1.5 μmol of urocanic acid in 0.05 M potassium phosphate, pH 7.5, and 0.2 mM EDTA (total volume, 0.5 mL). For both incubations, 20 μL was removed at the indicated times and diluted to 0.4 mL in 0.05 M potassium phosphate, pH 7.5. From this dilution, 20 μL was used in the standard urocanase assay and the remainder examined for absorbance at 277 nm to determine the concentration of urocanate remaining (inset, ϵ = 18 800 M^{-1} cm^{-1}; Tabor & Mehleer, 1955). As indicated, the anaerobic reaction was opened to air after 30 min. Activity is expressed relative to a control sample incubated aerobically but without urocanate.

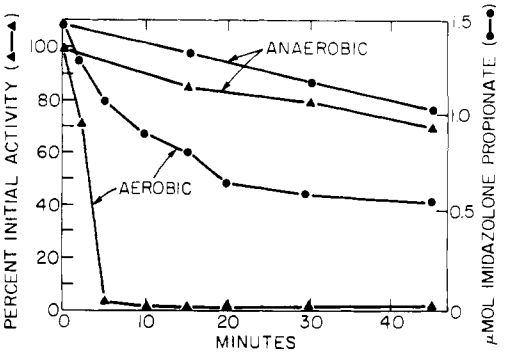

FIGURE 2: Incubation of urocanase with imidazolonepropionate under anaerobic and aerobic conditions. Urocanase (0.1 mg) was incubated at 21 °C with 1.5 μmol of imidazolonepropionic acid in 0.05 M potassium phosphate, pH 7.5, plus 0.2 mM EDTA (total volume, 0.5 mL). At the indicated times 20 μL was removed from the reaction and diluted to 0.2 mL in 0.1 M HCl. The absorbance at 234 nm of this solution was measured to determine the concentration of imidazolonepropionate (ϵ = 2860 M^{-1} cm^{-1}; Cohn et al., 1975). For activity assays, 10 μL was removed from the reaction solution and diluted to 0.10 mL in 0.05 M potassium phosphate, pH 7.5, and 20 μL of this dilution was used in the standard activity assay.

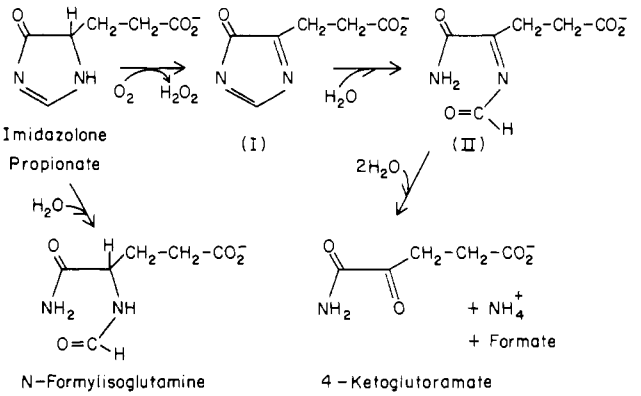

FIGURE 3: Scheme for the decomposition of imidazolonepropionate to 4-ketoglutaramic acid.

Anaerobic Conditions. For all anaerobic incubations, the reaction mixtures were thoroughly evacuated and flushed with nitrogen 3 times prior to enzyme addition. Enzyme solutions as well as transfer syringes were purged with nitrogen.

Determination of Sulfhydryl Groups. Sulfhydryl groups were assayed spectrophotometrically by using DTNB[1] as described by Habeeb (1972). To purified urocanase (0.2–0.3 mg, 0.18 mL) in 0.1 M sodium phosphate, pH 8.0, and 2% sodium dodecyl sulfate was added 20 μL of DTNB solution (40 mg of DTNB in 10 mL of 0.1 M sodium phosphate, pH 8.0). Absorbance was monitored at 412 nm on a recording spectrophotometer (ϵ = 13 600 M^{-1} cm^{-1}). The total cysteine content of the enzyme was assayed similarly after reduction with sodium borohydride according to the method of Cavallini et al. (1966).

Radioactivity Measurements. Radioactivity measurements were conducted in a Beckman LS 255B liquid scintillation counter with a scintillation fluid consisting of 5 g of 2,5-diphenyloxazole/L of toluene–Triton X-100 (2:1 v/v). Corrections for efficiency were made by internal standardization with [^{14}C]toluene.

Results

Requirement of Oxygen for the Inactivation of Urocanase. It has been demonstrated that the urocanase from *B. subtilis* is inactivated when incubated with urocanic acid (Kaminskas et al., 1970). We have observed a similar effect for the enzyme from *P. putida* and furthermore have established that the inactivation was markedly dependent on the availability of oxygen (Figure 1). In this experiment, after 20 min of reaction either with or without oxygen present, most of the substrate had been converted to product as estimated from urocanate disappearance. Enzyme activity remaining at this time, however, for the aerobic mixture was less than 1% of the control, and the anaerobic incubation retained greater than 95% of its original activity. When the anaerobic solution was opened to air (after 30 min), a complete abolition of enzyme activity was observed within 20 min of additional incubation. These results indicated that urocanate itself was not the inhibitory species, since inhibition could be observed even after most of the urocanate had been converted to imidazolonepropionate, and suggested that some combination of imidazolonepropionate and oxygen was responsible for the loss of activity.

Imidazolonepropionate was found to inactivate urocanase with a similar oxygen dependence (Figure 2). At an initial concentration of 3 mM imidazolonepropionate, activity was completely lost after 10 min in the presence of oxygen. Under anaerobiosis, however, the enzyme still retained 70% of its activity after 45 min. For both incubations, the loss of enzyme activity was accompanied by the disappearance of imidazolonepropionate, with a more rapid decomposition occurring in the aerobic incubation.

Imidazolonepropionate is highly unstable in the presence of oxygen at neutral pH (Brown & Kies, 1959), decomposing with a half-life of 20 min at pH 7.2, 30 °C (Rao & Greenberg, 1961). Two products arise from this decomposition (Figure 3): *N*-formylisoglutamine, derived from a simple oxygen-independent hydrolysis of the imidazolone, and 4-ketoglutaramic acid, the formation of which has an obligatory requirement for oxygen (Hassall & Greenberg, 1963). The latter process involves both an oxidation and a hydrolytic ring opening. The hydrolysis of the imine intermediate (II) yields 4-ketoglutaramic acid as the final product.

Effect of 4-Ketoglutaramate on Urocanase. The oxygen dependence of the inactivation of urocanase by urocanate and imidazolonepropionate supports a relationship between inac-

[1] Abbreviation used: DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

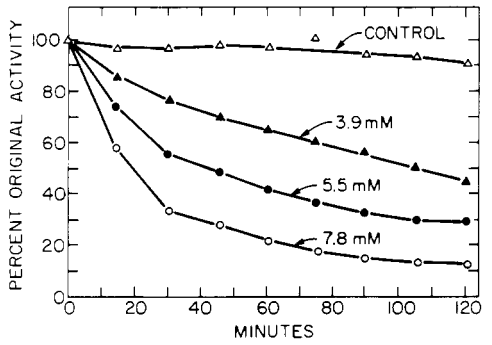

FIGURE 4: Inactivation of urocanase by 4-ketoglutaramic acid. Urocanase (2.6 μg) was incubated at 21 °C with 4-ketoglutaramic acid at the indicated concentrations in 0.05 M potassium phosphate, pH 7.5 (total volume, 0.15 mL). At the indicated times, 15 μL was removed and used directly in the standard activity assay. A control was prepared without 4-ketoglutaramic acid.

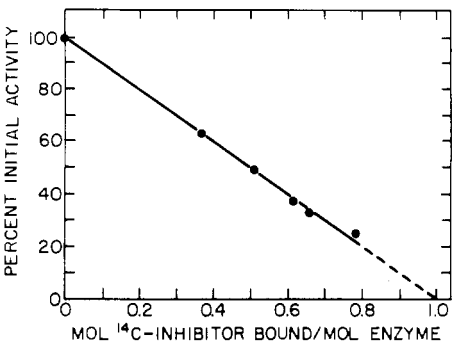

FIGURE 5: Stoichiometry of inhibitor binding to urocanase. Five milligrams of urocanase (45 nmol) was incubated at 21 °C with [^{14}C]urocanic acid (2.5 μmol; 0.45 μCi/μmol) in 0.5 mL of 0.05 M potassium phosphate, pH 7.5. At various times, 90 μL was removed from the reaction solution for activity assay and determination of bound radioactivity. For the latter determination, the protein was purified by gel filtration, and the appropriate fractions were pooled and counted.

tivation and the oxygen-induced decomposition of the latter. When 4-ketoglutaramate was tested for its inhibitory ability, a concentration-dependent inactivation was observed (Figure 4), but the extent of incubation after 120 min with 7.8 mM 4-ketoglutaramate was less than 100%, a marked reduction from that observed with either urocanate or imidazolonepropionate. Furthermore, extended incubation (10 h) did not result in complete inhibition of the enzyme (data not shown). It should be noted that the molar ratio of inhibitor to enzyme in this experiment (50 000:1 at 7.8 mM) far exceeded that in the experiments described in Figures 1 and 2 (1600:1). The failure to inactivate urocanase completely in the above experiment strongly suggested that 4-ketoglutaramate was not the major inhibitory species formed from urocanate and imidazolonepropionate.

Two hydrolytic degradation products of imidazolonepropionate, namely, *N*-formylisoglutamine and formate (see Figure 3), were also examined for their possible effects on urocanase. In data not presented, *N*-formyl-L-isoglutamine failed to inhibit urocanase when incubated with enzyme under the same conditions described in Figure 4, except the concentration of *N*-formylisoglutamine was 15 mM (10^5 molar excess over enzyme). Formate, on the other hand, was a competitive inhibitor of urocanase, having a K_i of 0.54 mM. Because the formate inhibition was competitive and reversible, it could not contribute to the permanent loss of activity observed upon incubation of urocanate or imidazolonepropionate with enzyme.

Inactivation Does Not Involve NAD$^+$. In view of the possibility that inactivation by imidazolonepropionate might affect NAD$^+$, either by covalently attacking the nicotinamide ring or by conversion of bound NAD$^+$ to NADH in a dead-end form, it was necessary to establish whether the NAD$^+$ on inactivated urocanase had been altered in some way. Enzyme which had been completely inactivated by incubation with urocanate was treated with perchloric acid to extract NAD$^+$, and then portions were neutralized for testing of the NAD$^+$ content with yeast alcohol dehydrogenase (Egan & Phillips, 1977). The results were consistently between 0.8 and 1.0 mol of NAD$^+$/mol of enzyme, in agreement with the expected NAD$^+$ content. Since perchloric acid degradation of NADH does not produce NAD$^+$ (Kim & Chaykin, 1968; Burton & Kaplan, 1963), no appreciable amount of NADH was present on the inactivated enzyme.

Urocanase containing NADH can be produced by $NaBH_4$ reduction (Egan & Phillips, 1977). When fully reduced, and therefore inactive, enzyme was incubated with [^{14}C]-imidazolonepropionate essentially as described in the legend to Figure 5, radioactivity was incorporated into protein. Although activity loss could not be monitored and [^{14}C]-imidazolonepropionate degraded rapidly during the reaction, incorporation of 0.5 mol of ^{14}C-labeled inhibitor/mol of enzyme was achieved. This finding argues against inhibitor binding to the oxidized nicotinamide ring of NAD$^+$.

Table I: Effect of Dithiothreitol and Imidazolepropionate on the Inactivation of Urocanase by Imidazolonepropionate[a]

additions[b]	act. (nmol/min)	% inhibition
control (no additions)	0.521	
IOPA	0.085	84
IOPA + 0.2 mM IPA	0.213	59
IOPA + 0.8 mM IPA	0.245	53
IOPA + 2.0 mM IPA	0.298	43
IOPA + 1 mM DTT	0.468	10
KGA	0.106	80
KGA + 1 mM DTT	0.457	12

[a] Incubations were performed as described in the legend for Figure 4 with activity determinations after 60 min. The concentration of imidazolonepropionate was 0.24 mM and 4-ketoglutaramate was 7.8 mM. [b] The abbreviations used are as follows: DTT, dithiothreitol; IPA, imidazolepropionate; IOPA, 4'-imidazolone-5'-propionate; KGA, 4-ketoglutaramic acid.

Stoichiometry and Irreversibility of the Inactivation by Urocanic Acid. When urocanase was incubated with [^{14}C]-urocanate in the presence of oxygen, the loss of catalytic activity proceeded with a proportional incorporation of radioactivity into the protein. Extrapolation of the data shown in Figure 5 revealed that complete inhibition would correspond to the binding of 1 mol of inhibitor/mol of enzyme. The binding of inhibitor was apparently irreversible, since radioactivity remained bound throughout exhaustive dialysis and gel filtration. Under no circumstances was catalytic activity regained from inactive enzyme, in contrast to what was seen upon dialysis of enzyme inhibited by 4-ketoglutaramate where much activity was regained.

Effect of Imidazolepropionate and Dithiothreitol on the Inactivation of Urocanase by Imidazolonepropionate. To examine the question of whether the inhibitor reacted at the catalytic center of the protein, we included the substrate analogue imidazolepropionate (K_i = 0.9 mM at pH 7.5; Phillips et al., 1977) in an incubation of imidazolonepropionate and urocanase (Table I). The data are consistent with a protective effect against inactivation, with increasing imidazolepropionate concentrations providing greater protection.

When 1 mM dithiothreitol was included in an incubation of imidazolonepropionate (or 4-ketoglutaramate) and uroca-

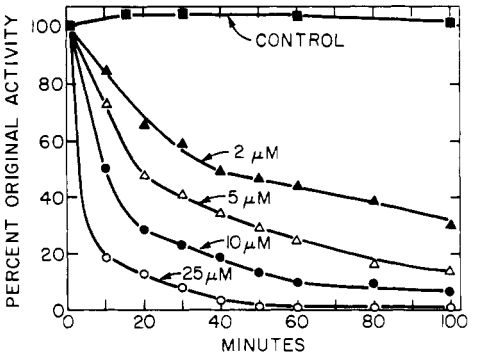

FIGURE 6: Effect of *p*-(chloromercuri)benzoate on urocanase. Urocanase (4.8 μg/mL) was incubated at 21 °C with the indicated concentrations of *p*-(chloromercuri)benzoate in 0.05 M potassium phosphate, pH 7.5. Activity was determined by removing 30 μL from the incubation and adding it directly to a 0.2-mL cuvette containing all the components for the activity assay.

Table II: Determination of Urocanase Sulfhydryl Content[a]

enzyme treatment	nmol of urocanase/mL[b]	nmol of thiol/mL	mol of thiol/mol urocanase
untreated[c]	10.0	80.5 ± 1.2	8.1
urocanate inactivated[c]	10.0	71.9 ± 1.9	7.2
borohydride reduced	1.11	12.8 ± 0.2	11.5

[a] Urocanase, 4.1 mg, was incubated in 0.05 M potassium phosphate, pH 7.5, with or without 5 μmol of potassium urocanate for 1 h at 21 °C (total volume, 0.62 mL). After dialysis for 12 h against 0.1 M sodium phosphate, pH 8.0, plus 0.2 mM EDTA (2 × 2 L), activity assays and sulfhydryl determinations were performed as described under Materials and Methods. For the determination of the total sulfhydryl content, 0.73 mg of urocanase was reduced with sodium borohydride and titrated with DTNB as described under Materials and Methods. [b] Based on a molecular weight of 110 000 (George & Phillips, 1970). [c] Specific activities for the preparations were 1.97 units/mg for the untreated enzyme and 0.05 units/mg after treatment with potassium urocanate.

nase, practically no loss of catalytic activity was evident after 60 min (Table I). While this is indicative of a cysteine residue as the locus for inhibitor binding, it is noteworthy that dithiothreitol failed to effect any reversal of the inactivation by imidazolonepropionate (data not shown).

Effect of p-(Chloromercuri)benzoate on Urocanase. Hug & Roth (1973) have reported that the urocanase from *P. putida* is inhibited by cupric and mercuric ions, suggesting the importance of sulfhydryls to catalysis even though George & Phillips (1970) found that *N*-ethylmaleimide was not inhibitory. We examined this question further by incubating the enzyme with *p*-(chloromercuri)benzoate and looking for an effect on enzyme activity. As depicted in Figure 6, this reagent irreversibly inactivated urocanase in a concentration-dependent and time-dependent fashion.

Determination of Sulfhydryl Groups in Native and Inhibited Urocanase. Native urocanase and enzyme which had been completely inactivated by incubation with urocanate were each titrated with DTNB in the presence of detergent (Table II). Of the twelve half-cystines in urocanase, as determined by amino acid analysis (George & Phillips, 1970) and by DTNB titration following reduction of disulfide bonds with sodium borohydride (Table II), eight SH groups in unmodified urocanase were reactive toward DTNB under these conditions. The remaining four sulfhydryls were apparently involved in disulfide linkages and thus were unavailable for reaction. For the enzyme inactivated by urocanate, the number of reactive

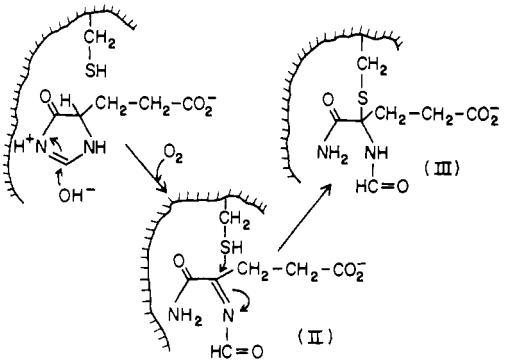

FIGURE 7: Proposed mechanism for the inactivation of urocanase by imidazolonepropionate.

sulfhydryls was reduced by one, to approximately seven sulfhydryl groups per mol of enzyme.

Discussion

The oxygen requirement for the inactivation of urocanase by either its substrate or its product, coupled with the demonstrated instability of the latter, leads us to conclude that some oxidative degradation product of imidazolonepropionate is responsible for the loss of catalytic activity. The failure of 4-ketoglutaramate to elicit an inhibition response comparable to that of either urocanate or imidazolonepropionate indicates that 4-ketoglutaramate itself is not the inhibiting species generated from imidazolonepropionate.

In Figure 3, a scheme for the decomposition of imidazolonepropionate is depicted. We propose that the actual inhibiting species is the reactive imine intermediate (II). Such a proposal is consistent with the aerobic requirement for the inactivation process. In the normal hydrolysis reaction, the reactive imine (II) would be subject to direct nucleophilic attack by water. This would facilitate hydrolysis, thereby generating 4-ketoglutaramate, formate, and ammonia. In the relatively apolar active site, however, the reaction of II with a nucleophile other than water is quite likely. Although intermediate I could also act as the inhibiting species, ring opening subsequent to reaction with an enzyme nucleophile would yield the same adduct as if II had actually reacted. Consequently, no distinction can be made between I and II with regard to the mechanism for inactivation.

An active-site thiol would be aptly suited for reaction with II. The existence of a cysteine sulfhydryl group essential for catalysis is indicated from the inhibition of urocanase by *p*-(chloromercuri)benzoate (Figure 6) and mercuric and cupric ions (Hug & Roth, 1973). The loss of a single titratable sulfhydryl upon incubation of urocanase with urocanate virtually confirms that a thiol is the locus for inhibitor binding.

A mechanism for the inactivation of urocanase upon incubation with imidazolonepropionate is depicted in Figure 7. Once the imidazolone is in the urocanase active site, oxidation and hydrolytic ring opening generate II. Thiol attack at the imino carbon yields a stable product, a glutaramate thioaminal (III). The proposed compound III has a structure somewhat like that of a linear amino sulfide, known examples of which are (diethylamino)methyl ethyl sulfide (Et_2NCH_2SEt; Renshaw & Searle, 1937) and *N*-(ethylmercaptomethyl)lauramide, ($C_{11}H_{23}CONHCH_2SEt$; Austin & Frank, 1943). Such thioethers are more stable than their oxy counterparts and show little tendency to dissociate to the mercaptan in aqueous media. Also related to compound III are the condensation products of carbonyls with the cysteine sulfhydryl group. With formaldehyde, cysteine combines to give thiazolidine-4-carboxylic

acid (Dann & Gates, 1957; Ratner & Clarke, 1937), while with pyruvic acid, 2-methylthiazolone-2,4-dicarboxylic acid results (Dann et al., 1957). Both thiazolidine derivatives show alkali and acid stability, leading us to expect compound III to be stable under our experimental conditions.

It seems probable that 4-ketoglutaramate inactivates urocanase by an analogous mechanism. The adduct generated, a glutaramate hemithioacetal, however, would be expected to dissociate reversibly (Jencks, 1969). Consistent with this is the failure of 4-ketoglutaramate to completely inactivate urocanase as well as a partial reactivation upon dialysis, indicative of the establishment of equilibrium binding.

The results presented here confirm the existence of an active-site thiol in the urocanase from *P. putida*, first indicated by the results of Hug & Roth (1973). The demonstration of a reactive sulfhydryl group in other urocanases suggests its conservation in the active sites of these enzymes and points to a function for this group in the catalytic process. Very recent work by O'Donnell et al. (1980) also reveals the existence of sulfhydryl groups at the active site of urocanase from *P. putida*. Their results indicate, moreover, that the enzyme contains an essential sulfhydryl group which is involved in NAD^+ binding, as shown by the ability of NAD^+ to reactivate enzyme which has been inhibited by *p*-(chloromercuri)-benzoate.

References

Austin, P. R., & Frank, C. E. (1943) U.S. Patent 2 323 111; (1944) *Chem. Abstr. 38*, 121.

Borek, B. A., & Waelsch, H. (1953) *J. Biol. Chem. 205*, 459.

Brown, D. D., & Kies, M. W. (1959) *J. Biol. Chem. 234*, 3188.

Burton, R. M., & Kaplan, N. O. (1963) *Arch. Biochem. Biophys. 101*, 150.

Cavallini, D., Grazioni, M. T., & Duprê, S. (1966) *Nature (London) 212*, 294.

Cohn, M. S., Lynch, M. C., & Phillips, A. T. (1975) *Biochim. Biophys. Acta 377*, 444.

Dann, J. R., & Gates, J. W. (1957) *J. Am. Chem. Soc. 79*, 1650.

Dann, J. R., Oliver, G. L., & Gates, J. W. (1957) *J. Am. Chem. Soc. 79*, 1644.

Davis, B. J. (1965) *Ann. N.Y. Acad. Sci. 121*, 404.

Egan, R. M., & Phillips, A. T. (1977) *J. Biol. Chem. 252*, 5701.

Feinberg, R. H., & Greenberg, D. M. (1959) *J. Biol. Chem. 234*, 2670.

George, D. J., & Phillips, A. T. (1970) *J. Biol. Chem. 245*, 528.

Groves, W. E., Davis, F. C., & Sells, B. H. (1968) *Anal. Biochem. 22*, 195.

Habeeb, A. F. S. A. (1972) *Methods Enzymol. 25*, 457.

Hacking, A. J., Bell, M. V., & Hassall, H. (1978) *Biochem. J. 171*, 41.

Hassall, H., & Greenberg, D. M. (1963) *J. Biol. Chem. 238*, 1423.

Hug, R. H., & Roth, D. (1973) *Biochim. Biophys. Acta 293*, 497.

Jencks, W. P. (1969) *Catalysis in Chemistry and Enzymology*, p 500, McGraw-Hill, New York.

Kaminskas, E., Kimhi, Y., & Magasanik, B, (1970) *J. Biol. Chem. 245*, 3536.

Keul, V., Kaeppeli, F., Ghosh, C., Krebs, T., Robinson, J. A., & Rêtey, J. (1979) *J. Biol. Chem. 254*, 843.

Kim, C. S. Y., & Chaykin, S. (1968) *Biochemistry 7*, 2339.

Mehler, A. H., Tabor, H., & Hayaishi, O. (1955) *Biochem. Prep. 4*, 50.

O'Donnell, P. S., Hug, D. H., & Hunter, J. K. (1980) *Arch. Biochem. Biophys. 202*, 242.

Phillips, A. T., LaJohn, L., & Lewis, B. (1977) *Arch. Biochem. Biophys. 184*, 215.

Rao, D. R., & Greenberg, D. M. (1961) *J. Biol. Chem. 236*, 1758.

Ratner, S., & Clarke, H. T. (1937) *J. Am. Chem. Soc. 59*, 200.

Renshaw, R. R., & Searle, D. E. (1937) *J. Am. Chem. Soc. 59*, 2056.

Tabor, H., & Mehler, A. H. (1955) *Methods Enzymol. 2*, 228.

Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406.