The mechanism of urocanase action*

The enzymic pathway whereby histidine functions as a one-carbon donor involves its conversion to urocanic acid (UCA)** and then to formamidinoglutaric acid, which, in turn, reacts with tetrahydrofolic acid in a formimino transfer reaction to give ultimately, N^{10}-formyltetrahydrofolic acid[1,2]. The formation of formamidinoglutaric acid from UCA by a soluble enzyme system has been shown to occur in mammalian liver extracts[3] and also in extracts of histidine-adapted *Pseudomonas fluorescens*[4] and *Aerobacter aerogenes*[5].

We now wish to present some results which have revealed some of the properties of urocanase and the nature of the primary attack of this enzyme on UCA. Urocanase activity was measured by following the disappearance of the ultraviolet absorption of UCA[6]. An aqueous extract of cat liver acetone powder, purified about five-fold by repeated salt precipitation and removal of inactive proteins on $Ca_3(PO_4)_2$ gel, was used as a source of enzyme. When $3.3 \cdot 10^{-5} M$ UCA was incubated with such a preparation at pH 7.1, a linear decrease in absorption at 277 mμ was observed until about 80% of the substrate had disappeared.

The purified preparation has not been found to require any additions for full activity. No loss in activity was noted after treatment of the enzyme with Norit A at pH 6.3, with *iso*octane, or with Dowex-2-Cl or Dowex-2-formate, or after dialysis against $0.02 M$ ethylenediaminetetraacetate for 32 h. Preincubation of the enzyme with $10^{-2} M$ azide, fluoride, or pyrophosphate or with $10^{-4} M$ quinacrine led to no diminution in activity. However, preincubation with $10^{-5} M$ *p*-mercuribenzoate, $10^{-3} M$ cyanide, $10^{-5} M$ hydrosulfite, $10^{-5} M$ bisulfite or $10^{-4} M$ hydroxylamine caused a large decrease in activity. Except in the case of hydroxylamine, these inhibitors were far less effective when added after urocanic acid. All attempts to reverse these inhibitions have been unsuccessful.

In the presence of UCA, urocanase promoted the reduction of oxidized 2,6-dichlorophenol indophenol. Other oxidized indophenols and indamines were also reduced but the rate of reduction seemed to depend on the ionization state of the indophenol at pH 7.2 as well as the oxidation potential. In addition, the coupled reduction of ferricyanide was observed but no reduction was found with thionine, gallocyanine, methylene blue, diphosphopyridine nucleotide, triphosphopyridine nucleotide or ferricytochrome *c*.

The rate of reduction of oxidized 2,6-dichlorophenol indophenol was considerably lower than the rate of UCA disappearance when equimolar concentrations were used. With increasing concentrations of dye, the rate of dye reduction approached that of UCA disappearance (Table I). As the pH was increased above the optimum, the ratio of the rate of dye reduction to the rate of urocanate disappearance increased. At and about the pH optimum, addition of dye had no effect on the rate of UCA disappearance, but at higher pH values stimulation was noted.

TABLE I

UROCANATE DEGRADATION AND COUPLED DYE REDUCTION

Additions		*pH*	*Initial rate in mμmole/min*	
UCA (μmole)	*Dye (μmole)*		*UCA disappearance*	*Dye reduction*
0.1	—	7.12	2.61	
0.1	0.07	7.12	2.59	0.48
0.1	0.105	7.12	—	0.74
0.1	0.14	7.12	—	1.01
0.1	—	9.23	0.55	
0.1	0.094	9.23	0.73	0.58
0.1	—	9.83	0.33	
0.1	0.094	9.83	0.46	0.39

Experiments performed in 3 ml total volume in Beckman cuvettes at 37°. Rates calculated using molar extinction coefficients of 18,500 and 19,100 for UCA at 277 mμ[6] and oxidized 2,6-dichlorophenol indophenol at 600 mμ[9], respectively.

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** *trans*-Imidazole-4-acrylic acid.

With the aid of ^{14}C-UCA, the products of the reaction in the presence and absence of dye have been investigated. In the absence of dye, the product of the reaction appeared to be L-formamidinoglutaric acid as judged by its behavior upon ionophoresis on paper and its conversion to L-glutamic acid. In the presence of dye, a new substance was found which behaved upon ionophoresis as a neutral molecule at pH 2 and as a divalent anion at pH 10. The oxidized nature (relative to urocanic acid) of this compound was demonstrated by its conversion to α-ketoglutaric acid upon treatment with acid. These properties suggest that the compound formed in the presence of dye is 5-imidazolone-4-acrylic acid. We have previously suggested[3,7] that in the absence of dye the product of urocanase action is 5-imidazolone-4-propionic acid.

Although our results indicate that an oxidation and subsequent reduction are involved in UCA degradation, the absence of an easily dissociable cofactor (which could act as an electron carrier) makes it likely that a single protein catalyzes both reactions. It appears that urocanase oxidizes UCA and that oxidized UCA is then either reduced without detachment from the enzyme or, in the presence of dye, liberated as imidazolone acrylic acid. In summary, we suggest the following mechanism for urocanase action:

$$\text{urocanate} + \text{enzyme} \longrightarrow [\text{oxidized urocanate} \cdot \text{reduced enzyme}] \qquad (1)$$

$$[\text{oxidized urocanate} \cdot \text{reduced enzyme}] \longrightarrow \text{imidazolone propionic acid} + \text{enzyme} \qquad (2a)$$

or in the presence of dye:

$$[\text{oxidized urocanate} \cdot \text{reduced enzyme}] + \text{dye} \longrightarrow \text{imidazolone acrylic acid} + \text{reduced dye} + \text{enzyme} \qquad (2b)$$

We have also observed that urocanase prepared from histidine-adapted *Pseudomonas fluorescens*[8] required no easily dissociable cofactor and catalyzed dye reduction in the presence of UCA, results which suggest that the mechanism of action of the bacterial enzyme is similar to that of mammalian urocanase.

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1 A. MILLER AND H. WAELSCH, *Arch. Biochem. Biophys.*, 63 (1956) 263.
2 H. TABOR AND J. C. RABINOWITZ, *J. Am. Chem. Soc.*, 78 (1956) 5705.
3 B. A. BOREK AND H. WAELSCH, *J. Biol. Chem.*, 205 (1953) 459.
4 H. TABOR AND A. H. MEHLER, *J. Biol. Chem.*, 210 (1954) 559.
5 B. MAGASANIK AND H. R. BOWSER, *J. Biol. Chem.*, 213 (1955) 571.
6 A. H. MEHLER AND H. TABOR, *J. Biol. Chem.*, 201 (1953) 775.
7 H. WAELSCH AND A. MILLER, in W. D. MCELROY AND B. GLASS, *Amino Acid Metabolism*, Johns Hopkins Press, Baltimore, 1955, p. 407.
8 H. TABOR AND O. HAYAISHI, *J. Biol. Chem.*, 194 (1952) 171.
9 R. E. BASFORD AND F. M. HUENNEKENS, *J. Am. Chem. Soc.*, 77 (1955) 3873.

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Chromatographic purification of T2r bacteriophage

The recent development of cellulose ion-exchange resins[1] which permit the chromatography of nucleic acids from various sources[2,3,4] suggested that such resins would be of use in the purification of nucleoproteins, such as bacteriophage, and bacteriophage nucleic acids. Successful fractionation of Tobacco Mosaic Virus has been reported[5].

Preliminary experiments showed that it is possible to chromatograph the components of lysed cultures of *Escherichia coli* and, therefore, the following method was devised for the preparation of T2r from such lysates. *E. coli* B/1,5 was grown in 1 l of nutrient broth (Difco) containing 0.4% glucose and 0.5% sodium chloride to a concentration of $5 \cdot 10^8$ cells/ml. The culture was then infected with T2r bacteriophage in a multiplicity of one. After lysis had occurred the culture was dialysed against 20 l of water overnight. There was no decrease in the virus titre after dialysis. To the dialysed suspension was added 10 g of ECTEOLA-SF cellulose ion-exchange resin,