relationships and various matters of detail are similarly difficult to account for, and are the subject of further studies.

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Studies on the Reaction of Glutamate Dehydrogenase with Ethoxyformic Anhydride

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The use of ethoxyformic anhydride for studying the acylation of histidine residues has considerable advantages over other similar reagents. The extent of the reaction can be followed spectrophotometrically by measuring the increase in absorption at 242 nm due to the formation of ethoxyformylhistidine residues (Ovadi *et al.*, 1967). The reagent is, however, not specific for histidine residues, but is susceptible to nucleophilic attack from tyrosine, cysteine, lysine and tryptophan residues (Rosen & Fedorcsak, 1966; Múhlrad *et al.*, 1967), although at pH 6.1 it reacts preferentially with histidine residues. The reagent has proved particularly useful in the present studies on glutamate dehydrogenase.

Reaction of the reagent at pH 6.1 in 0.1 M-sodium acetate-acetic acid buffer containing 0.1 M-NaCl produced a rapid loss of about 40% of the initial activity as a result of the acylation of a single histidine residue. Over a longer period of time two further residues could be modified without any further loss of activity. When the enzyme was treated with the reagent at pH 7.5 in 0.1 M-tris-HCl buffer containing 0.1 M-NaCl complete loss of enzymic activity occurred after modification of only two histidine residues. When the enzyme was first allowed to react at pH 6.1 and the pH then adjusted to 7.5 only a single residue reacted at the latter pH, although this resulted in the complete loss of the enzymic activity. If the excess of ethoxyformic anhydride was removed after the pH 6.1 treatment by gel filtration loss of activity was not observed on adjusting the pH to 7.5 unless further anhydride was added.

The reagent appeared to react only with histidine residues at both pH 6.1 and 7.5. Analysis of the difference spectrum produced on modification of the enzyme showed none of the characteristics associated with ethoxyformylation of the aromatic

residues. In addition, no decrease in the number of cysteine residues present was observed. It was not possible to exclude reaction with all the lysine residues present in the molecule, although lysine-97, which reacts specifically with pyridoxal phosphate (Piszkiwicz *et al.*, 1970) and *N*-(*N*'-acetyl-4-sulphamoylphenyl)maleimide (Holbrook & Jeckel, 1969), was not acylated.

Both the loss of activity at pH 7.5 and the extent of ethoxyformylation were decreased by the presence of glutamate. This protection was not observed at pH 6.1, suggesting that the residue protected was important for activity but was not available for acylation at pH 6.1. At pH 7.5 NAD⁺ did not affect the loss of activity but it decreased the extent of modification of the enzyme.

The implications of these results will be discussed.

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- Holbrook, J. J. & Jeckel, R. (1969) *Biochem. J.* **111**, 689
Múhlrad, A., Hegyi, G. & Tóth, G. (1967) *Acta Biochim. Biophys. Acad. Sci. Hung.* **2**, 19
Ovadi, J., Libor, S. & Elodi, P. (1967) *Acta Biochim. Biophys. Acad. Sci. Hung.* **2**, 455
Piszkiwicz, D., Landon, M. & Smith, E. L. (1970) *J. Biol. Chem.* **245**, 2622
Rosen, C. G. & Fedorcsak, I. (1966) *Biochim. Biophys. Acta* **130**, 401

The Reduced Nicotinamide-Adenine Dinucleotide Phosphate- and Oxygen-Dependent *N*-Oxygenation of Trimethylamine by *Pseudomonas aminovorans*

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Extracts of *Pseudomonas aminovorans* grown on trimethylamine contain an enzyme catalysing the NADPH- and oxygen-dependent conversion of trimethylamine into trimethylamine *N*-oxide. The specific activity of the enzyme in cells grown on other methylamines or succinate was only about 2-3% of the activity in trimethylamine-grown cells.

The enzyme was assayed spectrophotometrically by measuring the rate of trimethylamine-dependent oxidation of NADPH spectrophotometrically at 340 nm. A number of other tertiary amines were active substrates, including ethyldimethylamine, diethylmethylamine, *NN*-dimethylethylenediamine, *NN*-dimethylethanolamine, triethylamine, *NN*-dimethylpropylenediamine, 3-dimethylaminopropan-1-ol, 3-dimethylaminopropan-2-ol and *NN*-dimethyl-2-chloroethylamine. All substrates showed inhibition at concentrations above about 1 mM. NADPH was