by way of formaldehyde and formate to carbon dioxide. It has been shown (C. Anthony, unpublished work) that the purified methanol dehydrogenase and its prosthetic group are apparently identical with those first described in *Pseudomonas* sp. M27 by Anthony & Zatman (1964, 1965, 1967a,b). The dehydrogenases for the oxidation of methylamine (Eady & Large, 1968), formaldehyde and formate (Johnson & Quayle, 1964) in *Pseudomonas* AM1 have already been described. The formate dehydrogenase is the only enzyme involved in the oxidation of C₁ compounds that requires NAD as coenzyme. The prosthetic group of methanol dehydrogenase is probably a pteridine, but the prosthetic groups of methylamine dehydrogenase and formaldehyde dehydrogenase are unknown. This communication reports further work on the system for electron transport from C₁ compounds in this organism.

Spectroscopic studies of whole cells and of sonic extracts demonstrated the presence of typical cytochromes. Cytochrome *a* and cytochrome *b* were insoluble. The soluble cytochrome *c*, which has been purified, has α , β and γ absorption bands of a typical cytochrome *c* with an acid isoelectric point and a mol. wt. of 20000.

The extent of reduction of cytochrome *c* by a given substrate in whole cells was measured from difference spectra obtained with aerobic cells in the reference cuvette and with either substrate or dithionite as reductant. Methanol, methylamine, formaldehyde, formate, succinate and malate all reduced cytochrome *c*. Cytochrome *c* was not reduced by methanol, methylamine or malate when cells were grown in conditions that precluded the formation of the specific dehydrogenases for these substrates. Likewise methanol did not reduce cytochrome *c* in mutants lacking the methanol dehydrogenase.

Potassium cyanide (1 mM) inhibited the oxidation of all the substrates mentioned and in the presence of cyanide all substrates completely reduced the cytochrome *c*.

Sodium phosphate (100 mM, pH 7.0) inhibited the oxidation of methanol and formaldehyde but stimulated the oxidation of methylamine, and in parallel experiments phosphate prevented reduction of cytochrome *c* by methanol or formaldehyde but stimulated reduction by methylamine.

It is concluded that cytochrome *c* is an essential intermediate in the oxidation of methanol, methylamine, formaldehyde, formate, succinate and malate in *Pseudomonas* AM1.

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An Enzymically Active P-420-Type Cytochrome Involved in the Mixed-Function Dimethylamine Oxidase System of *Pseudomonas aminovorans*

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Pseudomonas aminovorans grown on a variety of amines, including dimethylamine or trimethylamine *N*-oxide, as sole carbon source contains an enzyme system catalysing the oxygen- and NAD(P)H-dependent conversion of dimethylamine into methylamine and formaldehyde (Eady & Large, 1969). The rather unstable system has been partially purified about 15-fold. It can be stabilized by the presence of 5% (v/v) ethanol. The K_m values for NADH and NADPH were respectively 6.5 and 13.2 μ M, with identical V_{max} values. Diethylamine, *N*-methyl-*n*-propylamine, *N*-ethyl-*n*-propylamine, and *N*-methyl-*n*-butylamine were also substrates. The enzyme was inhibited by carbon monoxide, cyanide, *p*-chloromercuribenzoate and mersalyl, and also by heavy metals. It was not inhibited by proadifen hydrochloride (SKF 525-A) or chelating agents.

The spectral maxima varied slightly (2–5 nm) from one preparation to another, but the following results are typical. The partially purified system had an absorption maximum at 412 nm, which shifted on reduction with dithionite to 422 nm, and with NADPH to 415 nm. Difference spectra of reduced minus oxidized enzyme had a peak at 430 nm and troughs at 400–410 and 470 nm, although the changes in extinction were less with NADPH as reducing agent. Treatment of the test sample with carbon monoxide before dithionite reduction gave an enhancement in the absorption maximum, with a shift to 420 nm. The difference spectrum (dithionite-reduced enzyme as blank) had a peak at 420 nm and a trough at 440 nm.

The enzymic properties of the system resemble in many ways those of the *Pseudomonas putida* camphor methylene hydroxylase (Katagiri, Ganguli & Gunsalus, 1968) and the *n*-alkane hydroxylase systems (Peterson, Basu & Coon, 1966; Cardini & Jurtshuk, 1968). Although the instability of the system has so far precluded further purification or

resolution, preliminary evidence for the presence of both non-haem and haem iron and flavin has been obtained. The present system is unusual in that the spectral properties of the active preparation resemble microsomal cytochrome *P*-420 (Ohmura & Sato, 1964) rather than the *P*-450 type observed by Katagiri *et al.* (1968). Crude extracts that have not been exposed to ethanol show the dithionite-carbon monoxide-treated peak at 417–418nm. Although ethanol is known to convert microsomal cytochrome *P*-450 into *P*-420 (Ichikawa, Yamano & Fujishima, 1969), this in other systems is accompanied by a loss of enzyme activity not observed in the present system.

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Enzymic Reduction of the C-Terminal Dehydroalanyl-lysine Sequence in Nisin

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Inactivation of the polypeptide antibiotics nisin and subtilin by a non-proteolytic enzyme from several species of *Bacillus* has been reported, but no significant molecular changes were observed in the enzyme-inactivated nisin (Jarvis, 1967a,b). Gross, Morrell & Craig (1969) have shown that both nisin and subtilin contain the C-terminal sequence dehydroalanyl-lysine; loss of biological activity results from the hydrolytic cleavage of this terminal sequence in nisin (Gross & Morrell, 1967). It seemed possible, therefore, that the 'nisinase' might act as a dehydroalanine reductase or as a dehydropeptidase.

Purified nisin (25mg) was incubated for 72h at 30°C in phosphate buffer (0.1M, pH 6.8) containing 100 μ M-Co²⁺ and -Ca²⁺, with an enzyme preparation from *Bacillus cereus* (B. Jarvis, unpublished work). Controls containing heat-inactivated enzyme were incubated concurrently. After incubation the mixtures were acidified to pH 1 and heated for 5min at 100°C. Bioassay showed a net inactivation of 76% in the test

compared with the controls. After partial hydrolysis (110°C for 10min; M-HCl in acetic acid) (Gross & Morrell, 1967) the elution patterns of the nisin preparations from Sephadex G-25 (80cm $\times$ 2.5cm; 0.2M-acetic acid) showed no differences. However, t.l.c. of the pyruvyl-lysine peak (derived from dehydroalanyl-lysine) revealed the presence of alanyl-lysine in the eluate from the enzyme-inactivated nisin.

Semiquantitative high-voltage electrophoresis of acid hydrolysates of the nisin preparations treated for 10 days at room temperature with an excess of methylmercaptoacetate under N₂ (Gross & Morrell, 1968) showed a decrease in the carboxymethylcysteine content of the enzyme-inactivated nisin relative to the controls. Treatment of the nisin preparations (1.5nmol) with 2.5 μ Ci of L-[¹⁴C]cysteine (18.3 μ Ci/ μ mol) at pH 7.0 (0.1M-phosphate buffer) and 30°C for 24h showed a 23% decrease in the binding of cysteine to the inactivated nisin (13950 d.p.m.) compared with the controls (18040 d.p.m.). The theoretical decrease in binding is 26%, assuming that the 76% inactivation results from the reduction of only one of the three $\alpha\beta$ -unsaturated amino acid residues/mol of nisin. The [¹⁴C]cysteine-labelled nisins, mixed with 5mg of carrier nisin, were subjected to chromatography on Sephadex G-25 after partial hydrolysis (see above) and the eluate was monitored for extinction and radioactivity. Considerably less radioactivity was associated with the 'pyruvyl-lysine' peak in the inactive nisin than in the controls, but similar amounts of radioactivity were observed in the de-(dehydroalanyl-lysine)-nisin peaks. These results are consistent with the hypothesis of enzymic reduction of the C-terminal dehydroalanyl-lysine sequence to alanyl-lysine.

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