Isolation of a Particulate (Membrane) Fraction from *Mycobacterium smegmatis* Showing Phosphorylation Coupled to Malate Oxidation

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Washed cells of *Mycobacterium smegmatis* grown for 18 h in the medium described by Brodie & Gray (1956) were incubated for 1 h in 0.5M-sucrose–20mM-tris–HCl buffer, pH 7.4, containing lysozyme and EDTA. They were then suspended in hypo-osmotic sucrose (50mM-sucrose 20mM-tris–HCl buffer, pH 7.4, containing Mg^{2+} and sonicated for 2 min. The particulate fraction obtained between 20000 and 144000g oxidized malate with FAD or NAD^+ or $NADP^+$ as a cofactor without the addition of supernatant fraction. There was phosphorylation coupled to malate oxidation. The P/O ratios were about 0.7. The system was sensitive to *p*-chloro-mercuribenzoate and uncoupling agents. Thus these preliminary results seem to indicate that the particulate fraction from *M. smegmatis* obtained by lysozyme treatment possesses factors responsible for both oxidation and phosphorylation with malate as the substrate, unlike the system in *Mycobacterium phlei* in which both particulate and supernatant fractions are necessary for malate oxidation by the NAD^+ pathway. Further, in *M. smegmatis* oxidative phosphorylation with malate occurs with $NADP^+$ also as a cofactor.

Brodie, A. F. & Gray, C. T. (1956) *J. Biol. Chem.* **219**, 853

Enzymic Interaction between Isoniazid and Nicotinamide–Adenine Dinucleotide

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The most recent proposal for the mechanism of the antitubercular action of the potent drug isoniazid (isonicotinic acid hydrazide) is based on an enzymic reaction between isoniazid and NAD leading to formation of a pigment precursor, observed in *Mycobacterium* BCG (Youatt, 1969). A similar reaction has been observed in whole cells and cell-free extracts of *Mycobacterium tuberculosis* H₃₇R_v, and the biochemical characterization of this reaction was therefore undertaken. The enzyme can utilize as substrate NADP or nicotinamide–hypoxanthine dinucleotide with equal efficiency in place of NAD, and NMN with 50% efficiency, whereas any other substitution or alteration of NAD (particularly of the

nicotinamide moiety) results in loss of activity. Modification of isoniazid also results in loss of enzyme activity. The pigment produced on acidification after the enzyme reaction can be extracted with ethanol or acetone, but is not soluble in most of the other organic solvents tested. The pigment is relatively stable in acid, but is labile in alkali or at high temperature.

Although the enzyme activity is present in isoniazid-sensitive cells of *M. tuberculosis*, two different types of isoniazid-resistant mutants (Sriprakash & Ramakrishnan, 1970) failed to show this activity. The significance of this enzyme reaction in explaining the antitubercular action of isoniazid will be discussed.

Sriprakash, K. S. & Ramakrishnan, T. (1970) *J. Gen. Microbiol.* **60**, 125

Youatt, J. (1969) *Amer. Rev. Resp. Dis.* **99**, 729

Enzymic Conversion of *p*-Coumarate into Caffeate by *Streptomyces nigrifaciens*

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An enzyme responsible for the conversion of *p*-coumarate into caffeate was isolated and purified 96-fold from *Streptomyces nigrifaciens* and was found to be homogeneous on polyacrylamide-gel electrophoresis; its molecular weight was 18000 as determined by Sephadex G-100 gel filtration. It exhibited *p*-coumarate hydroxylase activity as well as caffeate oxidase activity. The caffeate oxidase activity was competitively inhibited by *p*-coumarate, thus facilitating selective study of the hydroxylase reaction in the presence of excess of substrate.

The stoichiometry of the reaction indicated a typical mono-oxygenase-mediated catalysis consuming 1 mol of O₂/mol of substrate hydroxylated. The enzyme showed a requirement for an electron donor like NADH, NADPH, tetrahydrofolate or ascorbate, though at higher concentrations ascorbate was inhibitory. The *p*-coumarate hydroxylase was optimally active at about pH 7.7 and 40°C, with an activation energy of 39 kJ/mol (9.5 kcal/mol). In addition to *p*-coumarate, other monophenols such as *p*-hydroxyphenylpropionate, *p*-hydroxyphenylacetate, L-tyrosine and DL-*p*-hydroxyphenyl-lactate were also substrates for the enzyme. The enzyme was found to contain 0.38% copper in a bound form. Thiol-group inhibitors did not have any effect on the reaction.