The degradation of cholesterol by *Pseudomonas* sp NCIB 10590 under anaerobic conditions

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The aerobic metabolism of sterols by bacteria has been comprehensively documented by Sih *et al.* (1967a, b; 1968, b). Fujimoto *et al.* (1982a, b) and by Owen *et al.* (1978, 1983, 1985). The metabolism of sterols by bacteria under anaerobic conditions however is less well documented. Although transformation of cholesterol to co-products is a common reaction mediated by the human intestinal flora, especially strains of the genus *Eubacterium* (Owen & Hill, 1982), there is very little evidence (Goddard & Hill, 1974) of cholesterol side-chain cleavage mediated by bacteria under anaerobic conditions. Because *Ps* sp 10590 has proved to be a potent sterol-degrader under aerobic conditions, a study has been conducted to establish whether or not the organism can degrade cholesterol under anaerobic conditions. The anaerobic biotransformation of cholesterol by *Ps* sp 10590 was conducted in a buffered mineral salts medium comprising (g/l): cholesterol, 0.5; K₂HPO₄, 7.0; KH₂PO₄, 3.0; NaCl, 2.0; (NH₄)₂SO₄, 1.0; KNO₃, 0.5; niacin, 0.01; biotin, 0.125; MgSO₄.7H₂O, 0.01; CaCl₂ (dried), 0.05 and FeSO₄.7H₂O, 0.005; final pH 7.0.

Cells obtained from a 500 ml aerobic culture of *Ps* sp 10590 by centrifugation were used to inoculate 500 ml of the culture medium. The culture was incubated anaerobically for 3 weeks at 28°C under 90% H₂/10% CO₂ (with palladium catalyst to remove residual oxygen). At the end of the fermentation the culture was extracted anaerobically for 3 weeks at 28°C under 90% H₂/10% CO₂ (with palladium catalyst to remove residual oxygen). The products detected were the neutral metabolites cholesterol, cholest-4-en-3-one, cholesta-1,4-diene-3-one (10 mg) and androsta-1,4-diene-3,17-dione (10 mg).

The products were identified by reference to t.l.c., g.l.c. and n.m.r. and mass spectra of authentic standards as described previously (Owen & Bilton, 1983).

The results show (Fig. 1) that *Ps* sp 10590 is capable of extensive metabolism of cholesterol under anaerobic conditions. The products detected were the neutral metabolites cholesterol, cholest-4-en-3-one (2 mg), cholesta-1,4-diene-3-one (3 mg) and androsta-1,4-diene-3,17-dione (4 mg). Acidic metabolites were not detected, which is similar to the aerobic metabolism of cholesterol by the same organism in the absence of specific enzyme inhibitors (Owen *et al.*, 1983). The added metabolites produced anaerobically probably reflecting slower degradation of the steroid nucleus.

The mechanism of cholesterol side-chain cleavage under aerobic conditions has been fully elucidated (Owen *et al.*, 1983). The present study, whilst showing that cholesterol side-chain cleavage can occur under anaerobic conditions, however does not indicate the exact mechanism. If indeed side-chain cleavage of cholesterol by *Ps* sp 10590 is identical under both aerobic and anaerobic conditions then it would suggest that the organism is capable of hydroxylating the terminal methyl group at C₅ in the absence of molecular oxygen. Such a phenomenon is not without exception. It has been shown that during the dehalogenation of 4-chlorobenzoate to 4-hydroxy-benzoate by an *Arthrobacter* sp, the hydroxyl group which is substituted onto the aromatic nucleus is derived from water and not molecular oxygen (Marks *et al.*, 1984).

Alternatively side-chain cleavage may be occurring at C₁₇/C₂₇ yielding androsta-1,4-diene-3,17-dione directly from cholesta-1,4-diene-3-one (Horvath & Kramli, 1947).

Studies are in progress to elucidate the exact mechanism of cholesterol side-chain cleavage by *Ps* sp 10590 under anaerobic conditions, involving selected enzyme inhibitors and O-labelled water and nitrate.

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Abbreviation used: *Ps* sp 10590, *Pseudomonas* sp NCIB 10590.

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Fig. 1. Products of cholesterol metabolism by *Pseudomonas* sp NCIB 10590 under anaerobic conditions

(1) Cholesterol, (2) cholest-4-en-3-one, (3) cholesta-1,4-diene-3-one, (4) androsta-1,4-diene-3,17-dione.
Nucleotide binding to type-M pyruvate kinase monitored by protection against trinitrobenzene sulphonate inhibition

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Pyruvate kinase (EC 2.7.1.40) catalyses the essentially irreversible transfer of a phosphoryl group from phosphoenolpyruvate to ADP to yield the products pyruvate and ATP. The reaction requires the presence of both a univalent and divalent metal ion, usually K⁺ and Mg²⁺.

The binding of the nucleotide ADP to the enzyme has been measured using a variety of procedures in different experimental conditions. From the results obtained dissociation constants have been calculated for the nucleotide.

The values reported vary over two orders of magnitude. Part of the difficulty in analysing data in the literature is that some of the techniques employed require the dissociation constant for nucleotide may depend, not only on the presence and absence of metal ions, but appears to strengthen that for MgADP.

In the absence of K⁺ and Mg²⁺, ADP protected against loss of activity, indicating that ADPₚ can bind to the protein. Between pH 6.2 and 8.1 the dissociation constant of the enzyme–ADP binary complex was independent of pH, the constant being approx. 2.1 mM. At higher pH values, however, the affinity of the enzyme for ADP decreased such that a plot of pKₐ against pH had a slope of -1. This observation is consistent with a model where the binding of ADP is dependent upon the state of protonation of a group on the enzyme–nucleotide complex is responsible for the pH profile, such that Kₚ decreases as pH increases.

Similar results were obtained when ATP was used in the presence of and absence of magnesium. Magnesium alone was found to bind to the system, and afford protection, with a Kₚ of 22 mM at pH 7.5, an observation consistent with the findings of Hollenberg et al. (1971).

In conclusion our results show that ADPₚ does bind to the enzyme, although the binding is not as strong as that reported for MgADP. It is interesting to note that increasing pH weakens the binding of ADPₚ to the enzyme, but appears to strengthen that for MgADP.

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Abbreviation used: TNBS, trinitrobenzene sulphonate.

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