Incorporation of Radioactive Mevalonate into C₅₀ and C₅₅ Prenols by *Streptococcus mutans*

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Growing cells of *Streptococcus mutans* Ingbritt incorporate radioactive mevalonate into unsaponifiable lipid. Of the radioactive lipid 40% was shown by chromatography and mass spectrometry to be C₅₀ and C₅₅ prenol.

Bacterial prenols, lipid carriers in the biosynthesis of several cell-wall polymers (Lennarz & Scher, 1972), are somewhat inert substances, recognizable mainly by their characteristic nuclear-magnetic-resonance and mass spectra (Thorne & Kodicek, 1966; Stone *et al.*, 1967; Gough *et al.*, 1970). The ability of species of *Lactobacillus* to incorporate radioactive mevalonate from the growth medium and to use it predominantly for the synthesis of C₅₅ prenol (Thorne & Kodicek, 1966; Gough *et al.*, 1970) has provided a convenient label for investigations of prenol function in this micro-organism.

The formation of radioactive prenol from [¹⁴C]mevalonate has now been demonstrated in *Streptococcus mutans* Ingbritt (a gift from Dr. S. J. Challacombe, Guy's Hospital, London SE1 9RT, U.K.). *S. mutans* was grown from a small inoculum in 100 ml of a semi-synthetic medium (Thorne & Kodicek, 1962) containing 1 μ Ci (140 nmol) of DL-[2-¹⁴C]mevalonate for 24 h. The cells were collected by centrifuging for 10 min at 1500g, washed once with 0.9% (w/v) NaCl and saponified with 4 ml of saturated KOH solution and 16 ml of ethanol for 10 min at 100°C. After cooling 25 ml of water was added and the unsaponifiable lipid was extracted with 3 $\times$ 25 ml of diethyl ether, and the ether extracts were washed with 4 $\times$ 25 ml of water. It was found that 4.4% (97000 d.p.m.) of the mevalonate of the growth medium was incorporated by the cells, of which 63.4% (58400 d.p.m.) was in the unsaponifiable lipid. The lipid was fractionated by column chromatography on alumina (Thorne & Kodicek, 1966); 40% (23400 d.p.m.) of the radioactive lipid was recovered in the fraction eluted with 20% (v/v) benzene in *n*-hexane (fraction II) and could be prenol. The remainder was less polar, and was eluted in the first fraction (fraction I) with *n*-hexane.

The prenol nature of fraction II was shown by three methods: by t.l.c., by chromatography on paraffin-impregnated plates and by mass spectrometry. T.l.c. of fraction II from the alumina column on silica gel G with 10% (v/v) ethyl acetate in *n*-heptane showed a single radioactive substance, *R_F* 0.31, which co-chromatographed with authentic

C₅₅ prenol from *Lactobacillus plantarum* and which stained blue with *p*-anisaldehyde (McSweeney, 1963). Fraction I contained two substances with *R_F* 0.55 and 0.90. Chromatography with paraffin-saturated acetone containing 10% (v/v) water on paraffin-impregnated silica gel G, which separates prenols of different molecular weights (Dunphy *et al.*, 1967), demonstrated some similarity between fraction II from *S. mutans* and C₅₅ prenol from *L. plantarum*. *L. plantarum* C₅₅ prenol had *R_F* 0.87, whereas *S. mutans* contained two radioactive compounds with *R_F* 0.87 and 0.93. The latter could be C₅₀ prenol. This was confirmed by mass spectrometry.

A bulk quantity of prenol was prepared for mass spectrometry. *S. mutans* was grown in 15 litres of Todd-Hewitt medium (Oxoid Ltd., London SE1 9HF, U.K.) for 24 h and collected with a Sharples continuous centrifuge. The cells were saponified with 20 ml of saturated KOH solution and 80 ml of ethanol for 20 min at 100°C. The unsaponifiable lipid was extracted with diethyl ether and washed free of KOH. Radioactive lipid, from cells grown in 100 ml of semi-synthetic medium with 1 μ Ci of [2-¹⁴C]mevalonate, was added to label the bulk lipid during its purification. The method of purification was column chromatography on alumina, acetylation with acetic anhydride, and re-chromatography on alumina (Thorne & Kodicek, 1966).

Mass spectra were obtained with an MS9 (A.E.I., Manchester, U.K.) mass spectrometer. The assignment of peaks from prenols has been described by Wellburn *et al.* (1967). The molecular ion of the acetylated lipid was *m/e* 808, in agreement with a prenol containing 11 isoprene units. The next peak, at *m/e* 748, came from the deacetylated prenol. The presence of a peak at *m/e* 680 showed the occurrence of some C₅₀ prenol. The relative proportions were 62% C₅₅ prenol and 38% C₅₀ prenol. The successive loss of isoprene units, mass 68, produced two series, one derived from dehydrated [C₅H₈]₁₀-OH (*m/e* 679, 611, 543, 475, 407, 339, 271, 203, 135) and the other from CH₂-[C₅H₈]₉-OH (*m/e* 625, 557, 489, 421, 353, 285, 217, 149). Peaks at *m/e* 95, 83, 81, 69, 67, 55, 43 and 41 are

derived from the isopropyl, ω -end of the molecule. This spectrum is consistent with a lipid consisting of both C₅₅ prenol and C₅₀ prenol.

It is concluded that *S. mutans* incorporates radioactive mevalonate readily from the growth medium. Unlike *Lactobacillus* only one-quarter of the incorporated mevalonate is used for prenol synthesis. Nevertheless this provides a convenient tool for studying the possible involvement of prenols in the biosynthesis of the extracellular polysaccharides, which form a major component of dental plaque (Challacombe *et al.*, 1972).

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