MsmE, a Lipoprotein Involved in Sugar Transport in *Streptococcus mutans*

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Metabolic labelling by [^{14}C]palmitic acid showed that growth of *Streptococcus mutans* LT11 in raffinose, an inducer of the *msm* operon, resulted in increased production of a 45-kDa lipoprotein corresponding to MsmE, which is believed to be a sugar-binding protein. MsmE was also labelled when an *msmE* clone was expressed in *Escherichia coli*. The presence of a lipid anchor on MsmE provides a likely explanation of how the sugar-binding protein component of the *msm* binding protein-dependent multiple sugar transport system is retained at the cell surface.

The oral bacterium *Streptococcus mutans* is extremely versatile in its mechanisms for metabolism of carbohydrates, the acid end products of which can contribute to tooth demineralization and the disease of dental caries (9). The major means of uptake for a number of sugars appears to be the phosphoenol pyruvate transport system, although additional pathways for uptake of glucose and sucrose are known to exist (13). We have recently described a novel operon, named the *msm* (multiple sugar metabolism) operon, which encodes a transport system for melibiose, raffinose, and isomaltosaccharides (16, 17); the components of the transport system show strong homology to components of binding protein-dependent periplasmic transport systems of gram-negative bacteria, the so-called traffic ATPases (2, 11). In gram-negative bacteria, a binding protein with high affinity for the substrate is located between the inner and outer membranes and serves to trap the substrate before presenting it to the membrane-bound components. In gram-positive bacteria, such as *S. mutans*, it is not immediately apparent how the analogous binding protein could remain in close association with the cell in the absence of a restricting outer membrane. However, on the basis of sequence data, Gilson et al. (8) suggested that the binding proteins could be retained at the cytoplasmic membrane by means of an NH$_2$-terminal lipo-amino acid anchor. Known prokaryotic lipoproteins (from both gram-positive and gram-negative organisms) have a glyceride linked to a cysteine residue and the following consensus sequence around the cysteine in the prolipoprotein: Leu-Ala-Ala/Gly-Cys (4, 10, 18). The *msmE* gene product has an NH$_2$-terminal 22-residue peptide containing the sequence Leu-Ala-Ala-Cys, and there is no other cysteine in the sequence. This information suggested that MsmE is a lipoprotein, and this report describes the experimental demonstration that this is indeed so.

The total lipoproteins of *S. mutans* LT11 were labelled by inoculating 5 ml of brain heart infusion (Oxoid) from an overnight culture and incubating it for 1 h at 37°C. [^{14}C]palmitic acid (Amersham) was added to 1 μCi/ml, and growth continued for 3 h. Cells were harvested, washed with phosphate-buffered saline, and solubilized in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

sample buffer, and the insoluble material was cleared by centrifugation. Supernatant fractions were dialyzed, and proteins were precipitated with 66% (vol/vol) ethanol; precipitated material was redissolved in SDS-PAGE sample buffer. Cell extracts and supernatants were analyzed by SDS-PAGE and prepared for fluorography by soaking in Amplify (Amersham). Dried gels were exposed to Amersham Hyperfilm MP for up to 6 weeks at −80°C. Autoradiography of [^{14}C]palmitic acid-labelled *S. mutans* LT11 proteins identified at least 10 lipoproteins (Fig. 1a). Two major and one minor lipoprotein bands were observable at approximately 45 kDa, the predicted size of mature MsmE (16).

Globomycin inhibits cleavage of the prelipoprotein signal peptide by a signal peptidase II enzyme in other bacterial species (10, 12) and also appears to modify processing of lipoproteins in *S. mutans*, as inclusion of 150 μg of globomycin per ml resulted in the migration of each lipoprotein at a slightly increased molecular weight (Fig. 1b). Supernatants showed a lipoprotein profile closely matching that of cell extracts (Fig. 1c).

The *msm* operon is induced by a number of sugars, of which raffinose is the most efficient (16). For induction of MsmE, labelling was therefore performed as described above, except that cells were grown to the mid-exponential phase on semidefined medium containing raffinose as the sole carbon source. A major band at 45 kDa was clearly induced by growth in raffinose, showing a marked increase in the intensity of labelling compared with other bands (Fig. 2).

The *msmE* gene was cloned as a 1.9-kb fragment behind the *lac* promoter in pUC18 to form recombinant plasmid pSF112. Labelling of lipoproteins in recombinant *Escherichia coli* expressing *msmE* from pSF112 was performed essentially as described above, except that cells were grown in 500 μl of Luria-Bertani broth and [^{14}C]palmitic acid was added at 2 μCi/ml. The recombinant showed strong labelling of a band at 45 kDa, markedly increased over the labelling of a coincident band in the parental *E. coli*, which was also labelled with [^{14}C]palmitate (Fig. 3). The recombinant MsmE protein is thus recognized by the *E. coli* enzymes which perform the posttranslational lipid modification.

To provide further information about the site of lipid modification, pSF112 was digested with the restriction endonuclease *Sph*I and ligated with fusion vector pMTL22 (5).
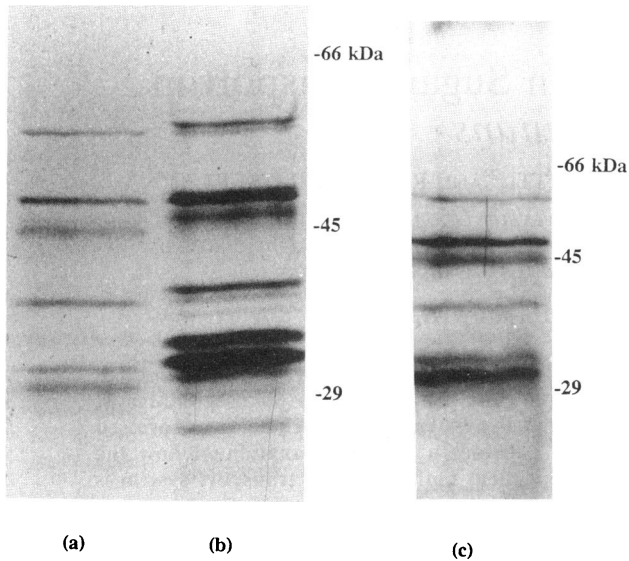
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FIG. 1. Lipoproteins of *S. mutans* metabolically labelled with [^{14}C]palmitic acid in the absence (a) or in the presence (b) of globomycin. Panel c shows lipoproteins from the culture supernatant.

The cleavage site for *Sph*I lies within the cysteine codon, so in the resultant plasmid the first 22 residues of MsmE were replaced by an in-frame 32-residue sequence from pMTL22 (which has none of the characteristics of a signal peptide), while the cysteine is retained. When this new construct was subjected to [^{14}C]palmitate labelling, the MsmE protein was not labelled (data not shown), indicating either that the protein must be secreted to be acylated or that it is the amino acid sequence surrounding the cysteine residue which determines its acylation.

The nature of the lipoproteins besides MsmE remains to be determined, but it seems probable that some are the
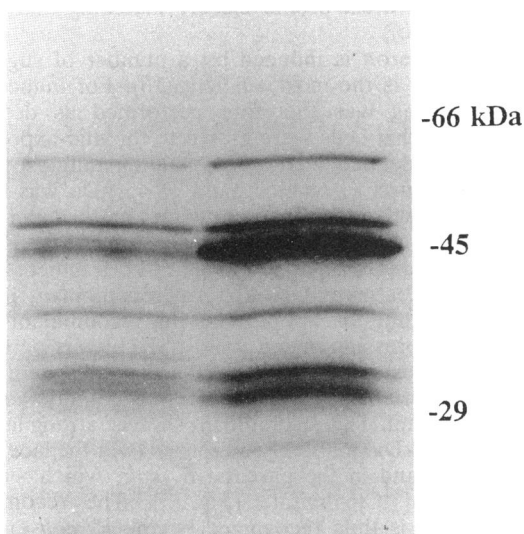

FIG. 2. Lipoproteins in *S. mutans* grown in glucose (left-hand lane) or raffinose (right-hand lane). Note that the total sample loading in the right-hand lane was approximately twice that in the left-hand lane.
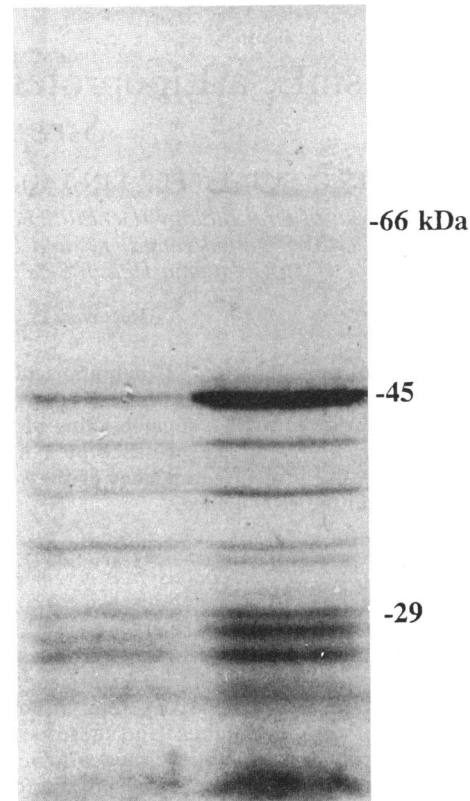

FIG. 3. Lipoproteins metabolically labelled with [^{14}C]palmitic acid in an *E. coli* control (left-hand lane) and in *E. coli* expressing *msmE* (right-hand lane). Slightly heavier loading was used in the right-hand lane.

binding components of other transport systems. In *Mycobacterium tuberculosis*, the 38-kDa antigen has been shown to be a lipoprotein concerned with phosphate uptake (3, 6, 19), and in both *Streptococcus pneumoniae* and *Bacillus subtilis*, lipoproteins corresponding to the *opp* peptide-binding protein of *Salmonella typhimurium* have been found (1, 11, 15). The *malX* gene of *S. pneumoniae* may similarly correspond to the maltose-binding protein of *E. coli* (8). It is interesting that some, but not all, of the lipoproteins which have been identified in gram-positive bacteria are found in both cell-bound and free forms (3, 15). In *S. mutans*, all of the lipoproteins found on intact cells (Fig. 1a and b) were also found in concentrated culture supernatant (Fig. 1c) but the significance of this for their function is unknown. The fact that the free proteins still retain lipid may indicate that characteristics of the sequence in addition to possession of a lipid substitute are involved in their localization, as has been shown for *E. coli* lipoproteins (7).

Jenkinson (14) has suggested that one or more lipoproteins may be significant contributors to streptococcal cell surface hydrophobicity. It remains to be determined whether this is due to properties of the peptide component or to exposure of the lipid moiety of free lipoproteins transiently associated with the cell surface prior to release into the supernatant. The extent to which lipoproteins, such as MsmE, are involved in determining overall surface properties remains to be investigated. However, it appears that lipoproteins are accessible to antibodies (the 38-kDa protein of *M. tubercu-*

losis is a major antigen [19]), and this suggests that they may be suitable as constituents of vaccines.

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