THE NATURE OF LIPOTEICHOIC ACID CARRIER (LTC) IN *STAPHYLOCOCCUS AUREUS* H

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1. Introduction

The biosynthesis of wall teichoic acids in several bacteria involves both prenol phosphate derivatives [1–3] and a non-prenol lipid called lipoteichoic acid carrier (LTC) [4]. In *Staphylococcus aureus* H the main chain, comprising poly(*N*-acetylglucosaminyl-ribitol phosphate), is assembled by transfer of residues from nucleotide precursors to LTC, and the linkage unit containing a chain of three glycerol phosphate residues [5] attached to the 4-position of an *N*-acetylglucosamine phosphate (J. Coley, unpublished observations) is assembled on a prenol phosphate [6,7]. It is presumed that the main chain is then transferred to the terminal glycerol of the linkage unit–prenol intermediate, and the completed assembly is subsequently attached to peptidoglycan. The present communication is concerned with the identity of LTC.

A soluble form of poly(ribitol phosphate) polymerase can be extracted [4,8] from membranes of *S. aureus* H with Triton X-100. Synthesis of poly-(ribitol phosphate) from CDP-ribitol in the presence of enzyme is dependent upon the addition of LTC which acts as an acceptor. LTC has been stated [9] to be identical to the membrane teichoic acid of the organism, and has been reported to consist of a chain of 12–14 glycerol phosphate residues attached to a monoglucosylglyceride [9]. It was first isolated from membrane preparations by using buffers containing Triton X-100 [4,8] but it can also be extracted with aqueous phenol. This latter procedure has in the past been used to isolate the membrane teichoic acids from many Gram-positive bacteria [10]. The membrane teichoic acid from *S. aureus* H, isolated by this method, has been fully characterized [11], and in contrast to the previously proposed structure for LTC it contains a chain of 28–30 glycerol phosphate residues attached to a gentiobiosyldiglyceride. There have been conflicting reports [4,8,9,12] on the ability of the material isolated using the phenol method to act as acceptor in the synthesis of poly-(ribitol phosphate).

2. Methods

Preparation of membranes from *S. aureus* H and isolation and purification of both LTC and poly-(ribitol phosphate) polymerase with Triton X-100 was carried out exactly as described by Fiedler and Glaser [4,8]. Membrane teichoic acid was isolated from membranes and also from the cell contents of disrupted organisms, after removal of the walls, by the phenol procedure [10]. Purified LTC and membrane teichoic acid were analysed by methods described previously [13].

3. Results and discussion

The material extracted from membranes with buffers containing 0.1% of Triton X-100 was purified by ion-exchange chromatography on DEAE-cellulose (fig.1a). Two major phosphate-containing fractions (peaks I and IV) were eluted together with two smaller fractions. LTC was obtained from the fraction corresponding to peak I after repeated ultrafiltration (Amicon XM-50 membrane) to remove salts and Triton; this fraction alone showed acceptor activity in the procedure described using soluble polymerase [4].

The products of acid hydrolysis (2 M HCl, 100°C, 3 h) of material corresponding to peak I included glycerol monophosphate, glycerol diphosphate, glycerol and glucose. Methanolysis of LTC (0.5 M HCl–MeOH, 65°C, 2 h), followed by gas–liquid chromatography (g.l.c) analysis of the ether extract showed the presence of fatty acids in the following proportions: C_{14}, 6.8%; *anteiso* C_{15}, 13.6%; C_{16}, 32.8%; *anteiso* C_{17}, 14%; C_{18}, 32.8%. The same fatty acids were found in similar proportions in the membrane glycolipid. The total amount of fatty acid was determined by g.l.c. with elaidic acid as an internal standard. After enzymic dephosphorylation of the acid hydrolysate with alkaline phosphatase

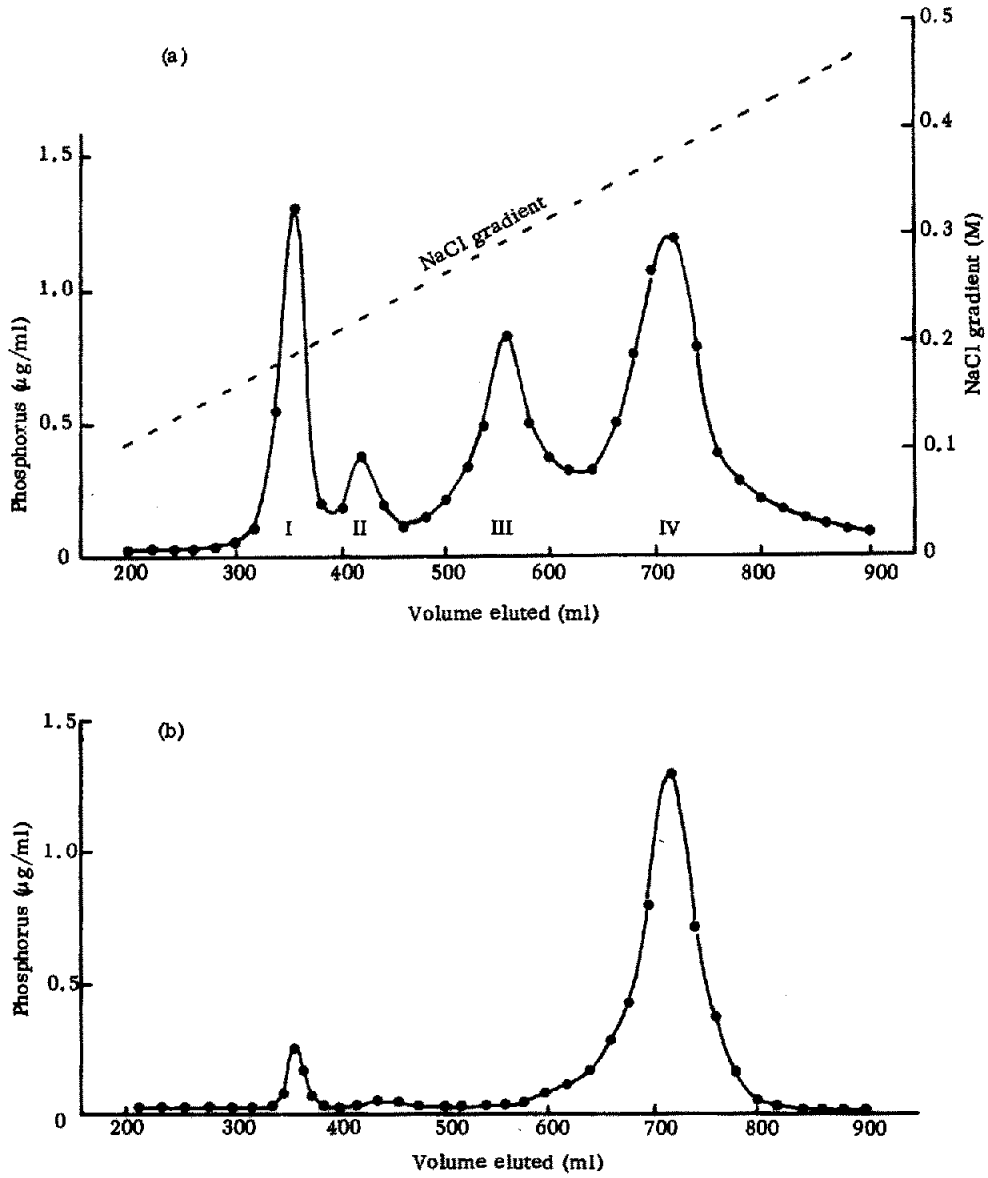

Fig.1. Triton extract from defatted membranes of *S. aureus* H eluted from DEAE-cellulose with a 0–0.5 M NaCl gradient in 10 mM Tris–HCl, pH 8.0, containing 0.1% (w/v) Triton X-100. Fig.1b. Membrane teichoic acid extracted from *S. aureus* H by the phenol procedure and eluted from DEAE-cellulose under the same conditions as described for fig.1a.

(Boehringer Corporation, London), the molecular proportions of phosphorus, glycerol, glucose and fatty acid were 1.0 : 1.04 : 0.096 : 0.143. These values are closely similar to those previously reported [4], (1.1 : 1.02 : 0.07 : 0.10, respectively).

The neutral products of alkaline hydrolysis (1 M NaOH, 100°C, 1 h) of LTC were shown by paper chromatography (butan-1-ol/pyridine/H_2O, 6 : 4 : 3, v/v/v) to contain a glycerol 1-glucoside with the same R_F as an authentic sample of 1-*O*-β-D-gentiobiosylglycerol obtained from the membrane glycolipid of *S. aureus* H [14]. Confirmation of this result was obtained by g.l.c. analysis of the trimethylsilyl derivative of the 1-glucoside. No monoglucosylglycerol was detected. These results show that all of the glucose in LTC is present as gentiobiosylglycerol. Consequently, the molecule must possess a chain of 22–24 glycerol phosphate units and three fatty acid residues.

When LTC was treated with HF, which selectively hydrolyses phosphate mono- and di-esters, it had been shown [9] that the glucose was soluble in the butanol phase, and that the butanol contained glucose and glycerol in equimolar amounts. It was concluded that LTC contained 1 glucose, 1 fatty acid and 12–14 glycerol phosphate residues, although the molar ratio of fatty acid to glucose in the material studied was 1.5 : 1.0. Since we have now shown that LTC contains a disaccharide residue, the material studied previously must therefore have contained 3 fatty acid residues; this agrees exactly with our present results. It is thus possible that a phosphatidyl or lysophosphatidyl residue occurs in LTC, and this might explain the glucose : glycerol ratio of 1 : 1 in the butanol-soluble fraction obtained after HF treatment.

For comparison purposes membrane teichoic acid was isolated from *S. aureus* H by the usual method of extraction with 80% aqueous phenol, and purified by chromatography on Sepharose 6B. The purified material, which showed variable acceptor activity, contained phosphorus, glycerol, glucose and fatty acid (expressed as methyl palmitate) in the proportions 1.0 : 1.0 : 0.66 : 0.076 [11]. Structural studies were in agreement with the previously established structure in which a 1,3-poly(glycerol phosphate) chain of 28 units is attached through a phosphodiester linkage to the 6-hydroxyl of the terminal glucose of a 1-*O*-β-D-gentiobiosyl-2,3-di-*O*-acylglycerol [11].

The chromatographic properties of LTC and the membrane teichoic acid were compared on DEAE-cellulose. Thus a Triton extract of membranes, which possessed acceptor activity, gave four peaks (fig.1a). Peak I represented LTC with acceptor activity in enzymic assays. The material corresponding to peak IV showed no acceptor activity. Chemical analysis of IV gave identical molar proportions of phosphorus, glycerol, glucose and fatty acids to those obtained from analysis of the phenol-extracted membrane teichoic acid. The membrane teichoic acid which had been extracted using the phenol procedure, was chromatographed on a column of DEAE-cellulose under identical conditions; two phosphate peaks were observed (fig.1b). The major peak occurred in the same region as peak IV, which is therefore presumably membrane teichoic acid, and the minor component co-chromatographed with LTC (peak I).

The material corresponding to peak IV represents essentially all of the cellular membrane teichoic acid. This was confirmed by phenol extraction of the remaining cell contents and washings from the membrane preparation, which yielded no teichoic acid. Similarly, none remained in the membrane residue after extraction with Triton X-100. The membrane teichoic acid content of the defatted membrane before Triton extraction was 0.6% (w/w). The yield of LTC obtained after repeated ultrafiltration of the material corresponding to peak I of the Triton extract was 0.038% (w/w of defatted membranes). Thus there is a 16-fold excess of membrane teichoic acid over LTC in the membrane.

In an earlier communication [11] we had questioned either the structure claimed [9] for LTC or its identity with the membrane teichoic acid. We have now shown that the two membrane components are indeed structurally different and separable from each other on DEAE-cellulose. Moreover, LTC has a higher mobility than does membrane teichoic acid on polyacrylamide gel electrophoresis under conditions identical to those previously described [4]. These differences in properties are consistent with the observed chemical structural differences, i.e., LTC has a poly(glycerol phosphate) chain of 22–24 units and three long-chain fatty ester residues, whereas the membrane teichoic acid has a slightly longer chain (28 units) and only two fatty ester residues. Evidence

for the possible presence of a phosphatidyl or lysophosphatidyl residue in LTC, or for the presence of unidentified components must await further fractionation and purification studies.

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