LIPOTEICHOIC ACID AND LIPOTEICHOIC ACID CARRIER IN *STAPHYLOCOCCUS AUREUS* H

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1. Introduction

Synthesis of poly(ribitol phosphate) by a purified polymerase from *Staphylococcus aureus* H has been shown to proceed by transfer of ribitol phosphate units from CDP-ribitol to a 'lipoteichoic acid carrier' onto which the ribitol phosphate chain is assembled [1–3]. Lipoteichoic acid carriers, active with the *S. aureus* polymerase, have been extracted from several Gram-positive bacteria and the material isolated from *S. aureus* also acts as carrier in the synthesis of poly(glycerol phosphate) by a polymerase from *Bacillus subtilis* [2]. These lipoteichoic acid carriers have usually been isolated by extraction from de-fatted membrane preparations into buffers containing Triton. On the other hand, the lipoteichoic acids examined in this laboratory have usually been extracted with aq. phenol from de-fatted membrane or 'cell contents' preparations [4–6]; this treatment appears to extract all of the membrane teichoic acid, essentially all of which is present as lipoteichoic [6,7]. In all lipoteichoic acids so far examined the lipid moiety is an acylated diglycosylglycerol [4,5,7,8]. In contrast the lipoteichoic acid carrier from *S. aureus* H, which is stated [3] to be identical to the lipoteichoic acid present in the membrane of this organism, has been reported to consist of chains of 12–14 glycerol residues, one molecule of glucose and one molecule of fatty acid being present in each chain [1,3]. We now report that the lipoteichoic acid extracted from this organism by treatment with phenol consists of chains of approximately 28 glycerol phosphate units attached to an acylated diglucosylglycerol. Possible explanations for these differences are discussed.

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2. Results

Lipoteichoic acid, isolated from *Staphylococcus aureus* H by extraction with 80% aq. phenol, was purified by chromatography on Sepharose 6B as described elsewhere [6]. The purified material, analysed by methods described previously [4], contained phosphate, glycerol, glucose and fatty acids (expressed as methyl palmitate) in the proportions 1.0 : 1.0: 0.066 : 0.076. Small amounts of *N*-acetylglucosamine were also present. Structural studies similar to those reported by Toon, Brown and Baddiley [4] and by RajBhandary and Baddiley [9] showed that the teichoic acid was a 1,3-poly(glycerol phosphate) which was attached through a phosphodiester linkage to a diacylgentiobiosylglycerol. After degradation of the teichoic acid with HF [4] all of the glucose was present as material which was soluble in chloroform. Examination of this material by thin-layer chromatography [10] showed that the major component co-chromatographed with 1-*O*-β-D-gentiobiosyl-2,3-di-*O*-acylglycerol, which is the major free glycolipid of the bacterium [11]. Minor amounts of free glycosides and free fatty acids were also present. Deacylation of the glycolipid derived from the teichoic acid gave 1-*O*-β-D-gentiobiosylglycerol which was identified by chromatographic comparison with an

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authentic sample. These results indicate that the teichoic acid chain is covalently attached through a phosphodiester group to a glycolipid residue. This phosphodiester group is attached to the hydroxyl group at position 6 of the terminal glucose residue of the glycolipid since [^{3}H]glycerol phosphate was produced on acid hydrolysis of a sample of teichoic acid which had been oxidized with periodate and then reduced with [^{3}H]KBH_4 as described elsewhere [12]. The average chain-length of the teichoic acid, determined by measurement of the formaldehyde formed from the glycerol-terminal end of the chain by oxidation with sodium periodate [9], was 28 units.

3. Discussion

An early study that *Staphylococcus aureus* H contains an 'intracellular' or 'membrane' teichoic acid which was isolated by extraction of a 'cell contents' fraction of disrupted cells with cold trichloroacetic acid [9]. Material thus obtained consisted of chains of 1→3 poly(glycerol phosphate) with D-alanyl ester substituents attached to a proportion of the glycerol phosphate units. Small amounts of gentiobiosylglycerol and *N*-acetylglucosaminylglycerol residues were also present. Since it is now known that membrane teichoic acids are covalently attached to lipid, much of which would be removed during extraction into acid, the structure of the teichoic acid has been re-examined using material extracted with aqueous phenol under conditions which have been shown to give good yields of lipoteichoic acid [4–6]. Material obtained in this way differed from that obtained earlier in that it contained fatty acids, although the very labile D-alanyl ester residues [9] were lost during purification. However both preparations contained identical proportions of glucose (P: glucose ratio, 1 : 0.066). All of the glucose in the lipoteichoic acid was present as a diacylated 1-*O*-β-D-gentiobiosyl-glycerol which was isolated and identified after degradation of the teichoic acid with HF, a reagent which selectively cleaves phosphate ester linkages [13]. The teichoic acid chain was shown to be attached to the diacylgentiobiosylglycerol through a phospho-diester linkage between the chain and the 6-position of the non-reducing terminal glucose residue of the glycolipid. It follows from its glucose content that the lipoteichoic acid contains one molecule of gentio-biosylglycerol for every 30 glycerol phosphate units. Determination of the formaldehyde formed from the glycerol-terminal end of the chain on oxidation of the lipoteichoic acid with periodate showed that the average chain-length was 28 units. We conclude that the lipoteichoic acid of *S. aureus* consists of a chain of 28–30 units of glycerol phosphate linked at its phosphate-terminal end to the hydroxyl group at position 6 of the terminal glucose moiety of diacyl-gentiobiosylglycerol as shown in fig.1. This material is therefore similar in general structure to the lipo-teichoic acid of *Streptococcus faecalis* [4]. The glycolipid moiety of this latter teichoic acid is thought to possess a phosphatidyl substituent on one of its sugar residues; the presence of such a substituent in the lipoteichoic acid of *S. aureus* is not precluded by the present results.

Essentially all of the membrane teichoic acid is extracted by phenol and yet the purified material appears to differ structurally from the lipoteichoic acid carrier isolated [1,3] from the same organism; both preparations are similar however in overall composition. Thus the proportions of glycerol, phos-phorus, glucose and fatty acids [1] in lipoteichoic acid carrier (1.1 : 1.02 : 0.07 : 0.10 respectively) are almost identical to those present in the material studied by us. However the lipoteichoic acid carrier is considered [1,3] to have a chain-length of 12–14 units and to contain one glucose molecule per chain.

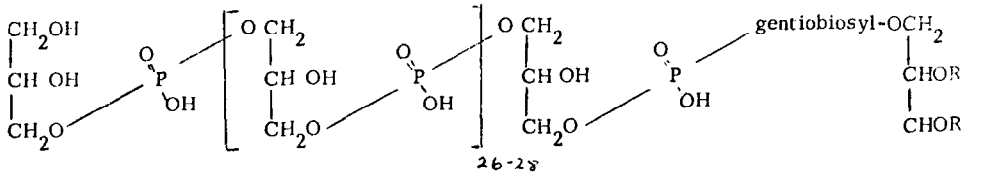

Fig.1.

After cleavage with HF this glucose moiety is soluble in butanol and the butanol extract contains glucose and glycerol in equal proportions, though the nature of the glucose-containing material has not been reported. Oxidation of the lipoteichoic acid carrier with periodate, followed by reduction with borohydride and acid hydrolysis gave ethylene glycol phosphate, derived from the glycerol-terminal end unit of the chain, and glycerol monophosphates, derived from the other glycerol units in the chain, in the ratio 1 : 23. This was considered to be consistent with the chain length of 12–14 units earlier deduced on the basis of the glucose content. It may be therefore, that the lipoteichoic acid which acts as carrier is chemically distinct from the bulk of the material which we have examined. However, Fiedler and Glaser have reported [1] that lipoteichoic acid carrier can be extracted from membranes by the phenol procedure and these authors consider that the carrier is identical to the membrane teichoic acid [3].

Since all of the glucose in the lipoteichoic acid isolated by us is present as gentiobiosylglycerol it is clear that our teichoic acid cannot contain only one glucose residue per molecule. The difference between the reported properties of the two preparations might, however, be resolved on the basis that the lipoteichoic acid carrier contains two molecules of glucose per chain. None of the published evidence would necessarily exclude this possibility. Thus the equimolar ratio of glucose: glycerol found in the butanol-soluble portion of the HF-degraded lipoteichoic acid carrier could be explained by the presence of acylated glycerol in addition to acylated diglucosylglycerol. Also the proportions of ethylene glycol and glycerol monophosphates produced on hydrolysis of the oxidized and reduced teichoic acid can be used to give a measure of the chain length only if it is known how much of the ethylene glycol present is released as its phosphate and how much of the glycerol present is released as its monophosphates.

Although it is possible that the lipoteichoic acid carrier is chemically distinct from our material it is also possible that it is in fact 24–28 units long and contains two glucose residues, thus being closely similar in structure to the material studied by us. This would have interesting implications on the nature of the group in the lipoteichoic acid carrier which accepts ribitol phosphate. Fiedler and Glaser [3] have shown that ribitol phosphate is transferred from [^{32}P]CDP-ribitol to a glycerol residue in the carrier and that, after removal of the poly(ribitol phosphate) chain by periodate oxidation, acid hydrolysis gives glycerol [^{32}P]monophosphates; this shows that the first ribitol phosphate to be added to the chain was attached to this glycerol through a phosphodiester bond, the phosphate in which was derived from the nucleotide. As mentioned above, the glycerol-terminal end of a lipoteichoic acid chain is susceptible to oxidation with periodate and, after reduction of the resulting glycolaldehyde moiety with borohydride, acid hydrolysis gives ethylene glycol and its phosphate derived from the terminal unit. Using [^{32}P]lipoteichoic acid, Fiedler and Glaser found that the yield of ethylene glycol [^{32}P]phosphate resulting from this sequence of operations was unaffected by prior assembly of poly(ribitol phosphate) onto the carrier. This shows that the glycerol moiety at the glycerol-terminal end of the chain is not the glycerol to which ribitol phosphate becomes attached, since such attachment would render that glycerol residue resistant to periodate oxidation so preventing its conversion to ethylene glycol phosphate. It was therefore tentatively concluded that the ribitol phosphate must be transferred to a glycerol residue at the other end of the chain. It was also concluded that this glycerol residue must be resistant to periodate oxidation even when not substituted by ribitol phosphate, since otherwise such substitution would again alter the amount of ethylene glycol phosphate formed during the sequence of operations described above. This latter argument presupposes that the glycerol residue to which ribitol phosphate can be attached is itself attached to the rest of the lipoteichoic acid carrier directly through a phosphodiester linkage; only in this case could it give ethylene glycol phosphate during the sequence of operations described.

However we do not consider that this has been established. Thus it is reported that no glycerol [^{32}P]diphosphate is produced on acid hydrolysis of lipoteichoic acid carrier which has first been charged with poly(ribitol [^{32}P]phosphate) and then oxidized with periodate. If the acceptor glycerol residue were held in the chain by direct attachment through phosphate, the above procedure would be expected to give glycerol [^{32}P]diphosphate, i.e. it is unlikely that hydrolysis of a phosphodiester linkage connecting the

acceptor glycerol residue to the rest of the lipoteichoic acid chain would proceed exclusively by cleavage of the linkage between the phosphate and this glycerol residue; cleavage between the phosphate and the rest of the chain would give glycerol [^{32}P]diphosphate. The presence of relatively stable substituents on the acceptor glycerol residue might block the formation of the cyclic intermediates through which acid hydrolysis of phosphodiester groups proceeds preferentially [14], but the formation of such intermediates is not a pre-requisite of hydrolysis in acid and so it might be expected that at least some glycerol diphosphate would be formed. Of course very small amounts might escape detection. However, in the absence of detectable glycerol [^{32}P] diphosphate in the products there is no evidence that the glycerol to which ribitol phosphate becomes attached is itself held in the carrier molecule directly through a phosphate linkage. If the lipoteichoic acid carrier is similar in structure to the lipoteichoic acid isolated by us one obvious possibility is that the 'acceptor' glycerol moiety is that which is substituted by gentiobiose, although, presumably, one of the fatty acid substituents would first have to be removed.

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