THE ALANINE ESTER SUBSTITUTION OF LIPOTEICHOIC ACID (LTA) IN *STAPHYLOCOCCUS AUREUS*

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1. Introduction

Although LTA were discovered ten years ago [1] and a variety of biological functions has since been ascribed to them [2–4], native polymers that retain their alanine ester substitution had not been isolated until recently [5]. It because evident that alanine ester substitution strongly affects the biological properties, as it can block lipoteichoic acid carrier (LTC) activity [5] and reduce the inhibitory effect on autolytic enzymes (W. F. et al., unpublished). As a basis for further studies of these modifying effects we have investigated the variability of alanine ester content of LTA through growth conditions and elucidated the range of substitution of the polymers obtained thereby. *Staphylococcus aureus* was chosen because the structure of its LTA is well documented [5–9] and the aforementioned experiments on LTC-activity and autolysins were carried out with enzymes from this organism.

2. Materials and methods

S. aureus DSM 346 and 20233 (a derivative of *S. aureus* H) were grown in a 20 liter fermentor at 37°C to the mid-logarithmic phase (A_{578} 1.0–1.6). The basal medium contained per liter: 10 g casein peptone, 7.5 g yeast extract, 2 g meat extract, 3 g K_2HPO_4, 2 g NaCl, and 10 g glucose. Immediately after harvesting the cells were suspended in 0.1 M citrate buffer of pH 3 and disrupted with glass beads in a Braun desintegrator. Extraction and purification of LTA were performed as in [5]. From an aliquot of non-disrupted cells the lipids were extracted, the defatted material was hydrolyzed in HF and the hydrolysate analyzed for alanyl glycerol and total glycerol [5]. Quantitative determinations were the same as in [9]. Alanine ester was removed from LTA at pH 8 [5].

Chromatography of LTA was performed on columns of DEAE–Sephacel (2.5 × 45 cm) which were equilibrated in 0.05 M sodium acetate buffer of pH 4, containing 0.05% Triton X-100, and eluted with a linear NaCl gradient in the same buffer. Fractions (6 ml) were collected at 12 ml/h flow rate. Prior to analysis, fractions of low LTA content were concentrated by ultrafiltration [5].

3. Results

Extraction of disrupted *S. aureus* cells with hot aqueous phenol [5] led to a recovery of 80–90% of the total non-lipid glycerol in the form of LTA. Since the purified polymer retained, in addition, most of the original alanine ester (table 1) it is largely representative of LTA in whole cells.

The variability of alanine ester substitution was studied by growing *S. aureus* in media of low and high [NaCl] which were known to cause extremely high and low alanine ester substitution of the wall teichoic acid in *S. aureus* [10]. As shown in table 1, the alanine ester content of LTA was influenced in a similar way, dropping from 0.7–0.3 when NaCl was increased from 2–75 or 100 g/l.

The range of substitution within these polymers was elucidated by column chromatography on DEAE–Sephacel. Fig.1A shows that native LTA separates clearly from its artificial alanine-free derivative. Chromatographed alone, the native polymer emerged as a single peak, and virtually no phosphorus was observed at the location of the alanine-free derivative (fig.1B). When this peak was fractionated it became apparent

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Table 1
Alanine ester substitution of LTA in defatted cells and purified samples

S. aureus strain	[NaCl] (g/l)	alaGro/Gro	
		Defatted cells	LTA
DSM 346	2	0.59	0.62 ± 0.02
DSM 20233	2	0.78 ± 0.02	0.73 ± 0.03
	75	0.36 ± 0.03	0.32 ± 0.02
	100	0.34 ± 0.02	0.30 ± 0.02

S. aureus strains were grown in the presence of the [NaCl] indicated. Molar ratios of alanylglycerol to total glycerol were determined in HF hydrolysates as in [5]

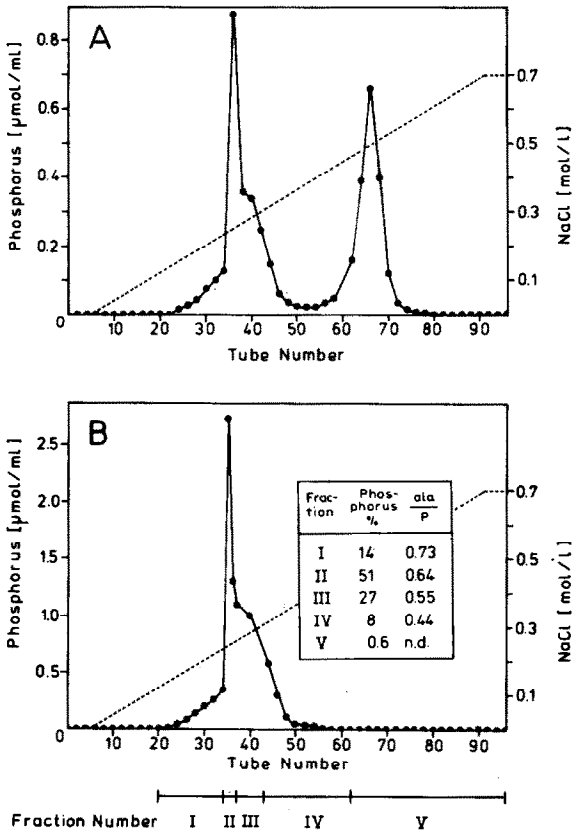

Fig.1. Separation of alanine ester-containing LTA from its alanine ester-free derivative and fractionation of native LTA according to decreasing alanine ester content. Column chromatography on DEAE–Sephacel: (A) Artificial mixture of native LTA from *S. aureus* DSM 346 and its dealanylated derivative, alanine was completely recovered in the first peak; (B) native LTA, fractions were combined as indicated, and analyzed for alanine and phosphorus. Phosphorus content and molar ratios of alanine to phosphorus are given in the inset.

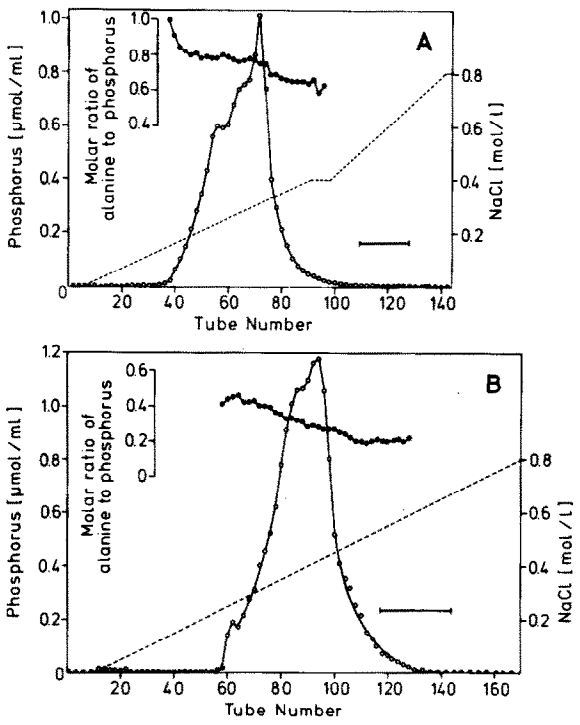

Fig.2. Range of alanine ester substitution of LTA from *S. aureus* DSM 20233 grown in 0.2% (A) and 7.5% NaCl (B). Column chromatography on DEAE–Sephacel. Markers indicate the location of the alanine-free derivative which was determined in a separate run. In the combined and concentrated fractions 106–140 of (A) no glycerol was observed after acid hydrolysis. The combined fractions 128–150 of (B) contained 1.6 µmol glycerol, but also 0.36 µmol alanine ester.

that molecular species also separate eluting in the order of decreasing alanine ester content. It decreased, as shown in the inset of fig.1B, from 0.73–0.44, but 78% of the polymer was substituted in the narrow range of 0.64–0.55.

A more detailed picture was obtained when every second fraction was analyzed (fig.2). The alanine ester content of LTA species from cells grown in 0.2% NaCl varied from 1.0–0.59, whereas the species from cells grown in 7.5% NaCl showed values of 0.49–0.21. In both polymers most values were continously distributed in a narrow range around the mean values (table 1) and no evidence for the presence of alanine-free polymer was obtained. This was confirmed for the low substituted LTA in a scaled down experiment in which substituted and non-substituted polymers separated almost completely (not shown).

4. Discussion

Whereas the stage of growth and decreasing pH values in the medium during growth had little effect on the alanine ester content of LTA in *S. aureus* cells [5], high [NaCl] in the growth medium reduced the substitution markedly (table 1). This has also been observed with wall teichoic acid of *S. aureus* [10], which suggests that alanine ester substitution of both polymers is under the same control and may serve the same function. In a study of the distribution of alanine ester along the poly(glycerophosphate), stepwise enzymic hydrolysis revealed each third of the chain to be substituted to the same extent [5]. The results described here exclude a mixture of substituted and unsubstituted chains and show in addition that in polymers of both high and low alanine ester content most molecular species are substituted within a narrow range (fig.1,2). Esterification of LTA with alanine therefore seems to be an ordered rather than a random process.

In connection with [5], the absence of alanine-free polymer from *S. aureus* cells and the high substitution of all molecular species in cells grown in physiological salt concentration (fig.2A) supports the hypothesis [5] that at least under certain conditions LTA cannot function in *S. aureus* as LTC in wall teichoic acid biosynthesis. A different situation is apparently existent in *Micrococcus varians* ATCC 29750. It seems to form as an intermediate in wall teichoic acid synthesis a LTC–wall-polymer complex [11] and, explanatory for this, possesses a LTA that bears neither alanine ester nor any other substituent (W. F., unpublished).

The documented existence of native LTAs with high, low and lacking alanine ester substitution necessitates studies on the relationship between the content of alanine ester groups and their effect on the biological properties of LTA. All molecular species desirable for such studies can now be prepared (fig.1,2).

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