prior activation. Freezing and thawing a mixture of am^1 and am^3 enzymes where am^1 is in excess will produce a preponderance of am^1 -rich hybrids that are active in system *A*. A similar mixture of enzymes in which am^3 is in excess will produce hybrids rich in am^3 . Such hybrids would not be expected to be as active in system *A*. The experimental results quoted above tend to support this explanation.

Such an argument presupposes a similarity between wild-type and mutant sub-units and hybridization might be expected to occur between them. This is clearly shown between am^3 and wild type and am^2 and wild type (see Table 1). The enzyme from am^2 is similar to that from am^3 in that it can be activated by glutamate. In both cases increasing the proportion of mutant to wild-type enzyme in the starting mixture causes, after freezing and thawing, a progressive decrease in *A* activity. This must be due to the formation of increasing amounts of hybrids rich in mutant sub-units that are not as active in system *A* as was the wild-type enzyme alone. These hybrids do, however, approach wild-type activity in system *C*. It would seem that the am^2 and am^3 sub-units can impose their faulty conformations on to the wild-type sub-units with which they are hybridized.

With am^1 and wild-type mixtures there appeared to be no significant change in either the *A* or *C* specific activity after freezing and thawing. This could either be due to a complete lack of hybridization between the two or to the fact that the presence of the am^1 sub-unit together with that of wild type in a hybrid does not alter the conformation of the wild-type sub-unit in any way. The undoubted involvement of am^1 with am^3 (Coddington & Fincham, 1965) and with am^{19} (Coddington, Fincham & Sundaram, 1966) enzyme in hybrid formation suggests that the latter explanation is the correct one.

I thank Dr J. R. S. Fincham for his interest and for many stimulating discussions.

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Biochem. J. (1966) **99**, 11c

The Linkage between Teichoic Acid and Glycosaminopeptide in the Walls of a Strain of *Staphylococcus lactis*

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(Received 25 February 1966)

One of the major unsolved problems in the chemistry of bacterial cell walls is the way in which the various component polymers are held together. The ribitol teichoic acid of *Staphylococcus aureus* has been shown to be held in the wall by linkage to the glycosaminopeptide through the terminal phosphate residue of the teichoic acid chain (Strominger & Ghuyssen, 1963; Ghuyssen, Tipper & Strominger, 1965). Moreover, the polysaccharide in walls of *Lactobacillus casei* N.I.R.D. RO 94 is believed to be held in the wall by a sugar 1-phosphate linkage between the reducing terminal amino sugar residue of the polysaccharide and the glycosaminopeptide (Knox & Hall, 1965); acid hydrolysis of the glycosaminopeptide gave an

organic phosphate, probably that of muramic acid, and it seems likely that the polysaccharide is attached to the glycosaminopeptide by a phosphodiester linkage between position 1 of the reducing terminal *N*-acetylglucosamine residue of the polysaccharide and a hydroxyl group of a muramic acid residue in the glycosaminopeptide (Hall & Knox, 1965).

Although suggestions have been made (Hay, Archibald & Baddiley, 1965; Archibald & Baddiley, 1965), the chemical nature of the linkage between teichoic acid and glycosaminopeptide has not been established so far, and in particular the identity of the residue in the mucopeptide involved in such a linkage is unknown. We now present evidence

Scheme 1. Ac, acetyl; Ala, D-alanine; R, OH or peptide

showing that the novel teichoic acid from a strain of *Staphylococcus lactis* (Archibald, Baddiley & Button, 1965) is attached to the wall through its terminal phosphate and that in this case the linkage is a phosphodiester bond between glycerol and muramic acid. The wall teichoic acid of this strain of *S. lactis* is atypical and is characterized by the presence of *N*-acetylglucosamine 1-phosphate residues as an integral part of the polymer chain (I). Such linkages are particularly labile to acid and under controlled conditions it is possible to hydrolyse the polymer almost quantitatively into a sub-unit with the structure (II) (D. Button, A. R. Archibald & J. Baddiley, unpublished work).

Treatment of walls (10mg.) with *N*-HCl (10ml.) at 37° resulted in the release of organic phosphate, mainly compound (II), at a rate comparable with the rate of hydrolysis of *N*-acetylglucosamine 1-phosphate under the same conditions (Leloir & Cardini, 1956). About 12% of the phosphorus was not extracted under these conditions even after prolonged (12hr.) incubation, but hydrolysis of the residual walls in 2*N*-HCl (2ml.) at 100° for 5hr. gave glycerol diphosphates and no other organic phosphates. This indicated that all the sugar 1-phosphate linkages were hydrolysed during the initial treatment with acid, leaving only the terminal glycerol diphosphate residues attached to the wall.

Further information has come from a study of the products of autolysis of walls. When walls (200mg.) were kept at 37° for 24hr. in 0.01*M*-ammonium formate buffer, pH 7.4, 90% of the original weight was converted into soluble products and 96% of the organic phosphate was now in solution as a non-diffusible material associated with mucopeptide. A sample (7mg. of P) of this material was treated with 0.2*N*-HCl (25ml.) at 100° for 10min. and then dialysed, whereupon 88.4% of the phosphorus diffused through the membrane leaving 11.6% of the phosphorus associated with the soluble non-diffusible glycosaminopeptide. Hydrolysis of this non-diffusible material (14μg. of P) in 2*N*-HCl (1ml.) for 3hr. at 100° gave glycerol diphosphates; no other organic phosphates were detected on chromatography. Incubation of a further sample (510μg. of P) in 0.01*M*-ammonium carbonate buffer, pH 9.4 (40ml.), containing calf intestinal phosphomonoesterase (0.1mg./ml.) and MgCl₂ (5mm) converted 50% of the phosphorus into inorganic phosphate; acid hydrolysis of a portion of the resulting material gave glycerol monophosphates. Oxidation of a sample of the phosphatase digest (380μg. of P) in 2mm-NaIO₄ solution (6ml.) gave 1.06mol.prop. of formaldehyde for every 2mol. prop. of phosphate in solution (i.e. 1mol.prop. of organic phosphate). Acid hydrolysis of the oxidized material gave a single organic phosphate with the chromatographic

mobility of a sugar phosphate giving a positive reaction with both ninhydrin and AgNO₃ spray reagents. The remainder of the hydrolysate was run as a band on Whatman no. 4 paper in *m*-ammonium acetate (pH 3.5)-ethanol (3:7.5, v/v) (Paladini & Leloir, 1952) and the organic phosphate was eluted with water. The eluate (about 15μg. of P) was incubated in 0.01*M*-ammonium carbonate buffer, pH 9.4 (0.5ml.), with intestinal phosphomonoesterase (0.1mg./ml.). Chromatographic examination of the digest revealed that dephosphorylation of the muramic acid phosphate was complete. The muramic acid was identified by comparison of its chromatographic mobility with an authentic sample in three solvents, and by paper electrophoresis at pH 8.9.

The above sequence of degradations is outlined in Scheme 1 where the soluble polymer complex (I) is hydrolysed in acid to the unit (II) and the glycosaminopeptide compound (III). The products of acid hydrolysis of compounds (III), (IV) and (V) are in accord with the structures shown. Hydrolysis of compounds (III) and (IV) will take place through five-membered cyclic phosphate intermediates involving the secondary hydroxyl groups of the glycerol residues giving glycerol di- and mono-phosphate respectively. This mechanism is less likely in structure (V) where elimination of the glycolaldehyde residue (Fleury & Courtois, 1939) presumably occurs, leaving the phosphate group attached to the muramic acid.

These results establish the presence of a phosphodiester linkage between the terminal glycerol of the teichoic acid and muramic acid in the glycosaminopeptide. It is not yet known whether this linkage involves the 4- or the 6-hydroxyl group of the muramic acid; either of these positions is possible if the muramic acid is at the non-reducing end of the glycosaminopeptide chain. The teichoic acid from this *S. lactis* has several major structural differences from both the ribitol and the glycerol teichoic acids that have been described previously. Consequently the linkages between glycosaminopeptides and the latter teichoic acids may well be of a different type, as suggested by Archibald & Baddiley (1965).

Muramic acid phosphate has previously been obtained from *L. casei* (Ågren & De Verdier, 1955) and from the products of acid hydrolysis of a C-polysaccharide preparation from a rough strain of pneumococcus R36A (Liu & Gotschlich, 1963). In the latter case the C-polysaccharide appears to have been associated with some glycosaminopeptide and this association may also involve linkage through muramic acid phosphate, as probably does the linkage to the glycosaminopeptide of the polysaccharide in walls of *L. casei* (Hall & Knox, 1965).

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Biochem. J. (1966) **99**, 14c

An Extended Amino Acid Sequence around the Reactive Cysteine Residues of Glyceraldehyde 3-Phosphate Dehydrogenase

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(Received 25 February 1966)

As part of a continuing investigation of the amino acid sequence of glyceraldehyde 3-phosphate dehydrogenase [D-glyceraldehyde 3-phosphate-NAD oxidoreductase (phosphorylating), EC 1.2.1.12], a study has been made of the peptides produced by pepsin digestion of the enzyme from ox skeletal muscle. The catalytic role of the reactive cysteine residues in the activity of the enzyme isolated from a number of organisms has been the subject of much recent attention (Harris, Meriwether & Park, 1963; Perham & Harris, 1963, and unpublished work; Allison & Harris, 1965). Also, each enzyme has been shown to consist of four identical polypeptide chains (Harris & Perham, 1965). Other experiments have shown that each polypeptide chain in the enzyme from both pig and rabbit skeletal muscle contains a uniquely reactive lysine residue as indicated by its ability to become acetylated during the enzyme reaction with *p*-nitrophenyl acetate (Harris & Polgar, 1965) and acetyl phosphate (Mathew, Agnello & Park, 1965). It is the purpose of the present paper to report an extension of the amino acid sequence around the reactive cysteine residues of the ox enzyme that enables the relative positions of the reactive cysteine and lysine residues in the primary structure of the pig enzyme to be deduced.

Glyceraldehyde 3-phosphate dehydrogenase was isolated from ox skeletal muscle by the method of Elödi & Szörényi (1956) and oxidized with performic acid (Hirs, 1956). The oxidized protein (120 mg.) was dissolved in 12 ml. of 5% (v/v) formic acid and digested with 0.5% pepsin for 1 hr. at 37°. The resulting peptides were recovered by freeze-drying and applied to a column (22 cm. × 2 cm.) of DEAE-

Sephadex A-25 equilibrated with 0.05N-acetic acid adjusted to pH 8.2 with 5N-NH₃. The column was developed at a flow rate of 36 ml./hr. and 6 ml. fractions were collected. After 204 ml. of effluent had been collected, a linear gradient was started, consisting of 200 ml. of starting buffer into which flowed 200 ml. of 0.5N-acetic acid adjusted to pH 5.3 with 5N-NH₃. Peptides in the column effluent were detected by means of the Folin-Ciocalteu reaction (Lowry, Rosebrough, Farr & Randall, 1951) and were freeze-dried. The peptides were further fractionated by high-voltage paper electrophoresis in pyridine-acetic acid buffers as described by Ryle, Sanger, Smith & Kitai (1955). Three peptides are of particular interest to us here. These are peptides P11c, which emerged in fractions 64–67 and was obtained pure on ionophoresis at pH 6.5, P12a, which emerged in fractions 68–73 and was obtained pure on successive ionophoresis at pH 6.5 and pH 3.5, and P14d2, which emerged in fractions 80–84 and was also obtained pure on successive ionophoresis at pH 6.5 and pH 3.5. Peptide yields were 11, 10 and 7% respectively.

Peptide P11c. Lysine was shown to be the *N*-terminal amino acid by means of the 'dansyl' method of Gray & Hartley (1963a). On digestion with trypsin four peptide fragments (P11cT1, P11cT2, P11cT3 and P11cT4) were obtained, which were separated by paper electrophoresis at pH 6.5. Peptide P11cT1 had the following amino acid composition: Lys (1), Asp (2), Ser (2), Thr (2), Pro (1), Ala (3), Val (1), Ile (1), Leu (2), CySO₃H* (2). The *N*-terminal residue was found to be

* Abbreviations: CySO₃H, cysteic acid; MetSO₂, methionine sulphone.