GLYCEROL DIPHOSPHATE DISACCHARIDE-PENTAPEPTIDE: A FUNCTIONAL GROUP OF THE LIPID INTERMEDIATE IN CELL WALL GLYCOPEPTIDE SYNTHESIS*

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The cell wall glycopeptide of Staphylococcus aureus and Micrococcus lysodeikticus is synthesized in a complex reaction sequence involving two uridine nucleotides: uridine diphosphate acetylglucosamine (UDP-GlcNAc) and uridine diphosphate acetylmuramyl-pentapeptide (UDP-MurNAc-pentapeptide) as well as glycine, ATP and, in the case of S. aureus, sRNA (Chatterjee and Park, 1964; Meadow, Anderson and Strominger, 1964; Struve and Neuhaus, 1965; Anderson, Matsuhashi, Haskin and Strominger, 1965; Matsuhashi, Dietrich and Strominger, 1965). The acetamido sugars and amino acids are first attached to a membrane-bound lipid intermediate before utilization for glycopeptide synthesis. This presumably serves as a means by which the intracellular intermediates are transported through the membrane and utilized for the synthesis of an extracellular product, the cell wall. The sequence of reactions involves formation of the following lipid intermediates: MurNAc(-pentapeptide)-P-lipid (in which the phosphate is derived from the uridine nucleotide precursor), GlcNAc-MurNAc(-pentapeptide)-P-lipid and finally in S. aureus, GlcNAc-MurNAc(-pentapeptide-pentaglycine)-P-lipid or, in M. lysodeikticus, a similar intermediate containing only a single glycine residue (Anderson et al., 1965;

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Matsuhashi et al., 1965; Matsuhashi, Dietrich and Gilbert, 1965). Each of these lipid intermediates has been isolated and studies of their structures have indicated that the lipid carrier is itself a phospholipid - that is, it contains phosphate residues (probably four) in addition to that derived from the uridine nucleotide (Anderson and Strominger, 1965). The purpose of the present paper is to report that glycerol diphosphate disaccharide(-pentapeptide) has been isolated as a degradation product of GlcNAc-MurNAc(-pentapeptide)-P-phospholipid from both S. aureus and M. lysodeikticus. Therefore, the substituted sugar phosphate fragment is attached to the phospholipid through a pyrophosphate bridge.

Disaccharide(-pentapeptide)-P-phospholipid, labeled with ^{14}C-lysine, was isolated by chromatography on DEAE-cellulose (Anderson and Strominger, 1965). Heating this material at pH 4.2 for 20 minutes at 100° resulted in decomposition to a number of products which may represent a sequential degradation. Our purpose is to focus on the properties of compound A (R_f in isobutyric acid:1 N ammonia (5:3)=0.13). On treatment with a wheat germ enzyme preparation (Sigma Chemical Co.) which contains a phosphodiesterase as well as a phosphomonoesterase, a second compound (B, R_f=0.21) was formed. By the use of ^{32}P-labeled compound A (prepared from ^{32}P-labeled cells) it was established that compound B contained organic phosphate as well as disaccharide-pentapeptide and that in addition to compound B ^{32}P-inorganic phosphate was formed during hydrolysis of compound A catalyzed by the wheat germ enzyme. The phosphomonoesterase in the wheat germ enzyme catalyzes the hydrolysis of primary alcohol phosphates, but not of sugar 1-phosphates. The phosphodiesterase splits UDP-sugar compounds at the pyrophosphate linkage.

On treatment of compound B with E. coli phosphomonoesterase, which does catalyze hydrolysis of sugar 1-phosphates, a third compound (C, R_f=0.43) was formed. Compound C is disaccharide-pentapeptide and could also be formed from compound A by treatment with 0.1 N HCl at 100° for 15 minutes, or by hydrolysis with an acetylmur-

amidase of the glycopeptide product formed enzymatically from the lipid intermediate. Although this enzymatic degradation sequence suggested the presence of a pyrophosphate linkage in compound A, this structure could not be unambiguously deduced from the hydrolysis of a previously unknown substrate by partially purified enzymes.

A large scale preparation of compound A was carried out from both S. aureus and M. lysodeikticus. The data which follow were obtained with the preparation from M. lysodeikticus. All of the analyses and degradations were also carried out with compound A from S. aureus and yielded the same results. The compound was purified by chromatography on Dowex 1-formate and filtration on Sephadex G-25. The purified material yielded the following analyses, expressed as moles per mole of disaccharide-pentapeptide: total phosphate, 2.05; glycerophosphate (determined enzymatically with α-glycerophosphate dehydrogenase (Sigma Chemical Co.) after hydrolysis in 1 N HCl at 100° for 7 minutes), 1.03; periodate consumption, 1.03 moles after 1 hour and 2.05 moles after 14 hours. The fast periodate consumption was due to oxidation of glycerol and was accompanied by formation of 1.04 moles of formaldehyde. The second slow mole of periodate consumed was due to oxidation of GlcNAc in the disaccharide. Only traces of fatty acids were present in compound A. Compound B contained only 1 of the 2 phosphate residues present in compound A and compound C contained no phosphate.

In order to isolate glycerol pyrophosphate, compound A was subjected to hydrolysis in 1 N formic acid at 100° for 10 minutes. The glycerol pyrophosphate formed was separated from disaccharide-pentapeptide and from glycerol phosphate and inorganic phosphate formed simultaneously by chromatography on Dowex 1-formate. Analysis of this material yielded ratios of glycerophosphate:total phosphate:acid - labile phosphate of 1:2.06:1.00. The compound took up 1.16 moles of periodate rapidly.

Finally, in order to provide an unequivocal demonstration of the presence of the pyrophosphate bridge in compound A, inorganic pyrophosphate was isolated by the following degradation sequence. Mild acid hydrolysis converted compound A to glycerol

pyrophosphate. Oxidation with periodate was then carried out. This procedure should yield glycolaldehyde pyrophosphate which on treatment with an amine should undergo an elimination reaction (Neu and Heppel, 1964). On treatment of the periodate oxidation mixture with lysine, inorganic pyrophosphate was formed, and was provisionally identified by paper chromatography in two solvents (Ebel, 1953) and by paper electrophoresis. A definitive identification was provided by the susceptibility of the compound to crystalline yeast inorganic pyrophosphatase. After degradation followed by treatment with inorganic pyrophosphatase 59% of the total phosphate was liberated as inorganic phosphate, compared to a recovery of 65% from inorganic pyrophosphate treated in the same manner. The same degradation procedure also led to formation of inorganic pyrophosphate from UDP-MurNAc-pentapeptide with a similar yield when measured after enzymatic hydrolysis to inorganic phosphate.

These experiments thus provide unambiguous evidence that GlcNAc-MurNAc-pentapeptide is attached to a glycerol residue in the phospholipid carrier in cell wall synthesis through a pyrophosphate bridge, one phosphate of which is derived from the phospholipid and the other from UDP-MurNAc-pentapeptide. The presence of some high energy linkage of MurNAc(-pentapeptide)-P to the lipid had been suggested by the finding that the formation of the first lipid intermediate, MurNAc(-pentapeptide)-P-lipid, was readily reversible on addition of UMP and had an equilibrium constant near 1 (Struve and Neuhaus, 1965a, b). Struve and Neuhaus (1965b) also reported orally that potato apyrase hydrolyzed the lipid intermediate. In our experience the intact lipid intermediate was not hydrolyzed by the potato apyrase preparation (Sigma Chemical Co.) employed, but one of its decomposition products, compound A, was readily hydrolyzed by this enzyme or more effectively by the wheat germ enzyme, as described.

SUMMARY

Glycerol diphosphate disaccharide-pentapeptide has been isolated as a degradation product of a phospholipid intermediate in cell wall glycopeptide synthesis. Thus, the

disaccharide-pentapeptide is linked to a glycerol residue in the phospholipid through a pyrophosphate bridge.

REFERENCES

Anderson, J. S., Matsuhashi, M., Haskin, M. A. and Strominger, J. L., Proc. Natl. Acad. Sci. U.S. 53, 881 (1965).

Anderson, J. S., and Strominger, J. L., Biochem. Biophys. Res. Commun. 21, preceding paper (1965).

Chatterjee, A. N., and Park, J. T., Proc. Natl. Acad. Sci. U.S. 51, 9 (1964).

Ebel, J. P., Bull. Soc. Chim., France 10, 991 (1953).

Matsuhashi, M., Dietrich, C. P., and Gilbert, J. M., Fed. Proc. 24, 607 (1965).

Matsuhashi, M., Dietrich, C. P., and Strominger, J. L., Proc. Natl. Acad. Sci. U.S. 54, 587 (1965).

Meadow, P. M., Anderson, J. S., and Strominger, J. L., Biochem. Biophys. Res. Commun. 14, 382 (1964).

Neu, H. C., and Heppel, L. A., J. Biol. Chem. 239, 2927 (1964).

Struve, W. G., and Neuhaus, F. C., Biochem. Biophys. Res. Commun. 18, 6 (1965a).

Struve, W. G., and Neuhaus, F. C., Fed. Proc. 24, 607 (1965b) and orally communicated.