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EX AEDIBVS ACADEMICIS IN CIVITATE VATICANA

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SVMMARIVM — Auctores describunt novam biochemicam reactionem, quae in iecore fit propter polyprenol-phosphati-glycosii actionem in lipidos, et glycolipidos.

After uridine diphosphate glucose was discovered as a co-factor in the transformation of galactose-1-phosphate into glucose-1-phosphate, it was found that it also acts as glucose donor for the formation of trehalose-phosphate, sucrose, and sucrose-phosphate.

Many other nucleotide sugars and also transfer reactions have since been detected in cells of different origins and at present the growth of most polysaccharides is generally believed to consist of a direct transfer reaction from a nucleotide sugar. However, recent work has shown that at least in some cases lipids are involved in the transfer reactions. A lipid intermediate was first found in studies on the synthesis of the 0 antigen in *Salmonella* (1) and of the cell wall in *Staphylococ-*

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cus (2) and was shown to be a phosphorylated polyprenol of eleven isoprene units. Its action in the formation of the 0 antigen may be summarized by the following equations:

$$\text{BMP} + \text{UDP-gal} \longrightarrow \text{BDP-gal} + \text{UMP} \quad (1)$$

$$\text{BDP-gal} + \text{TDP-rham} \longrightarrow \text{BDP-gal-rham} + \text{TDP} \quad (2)$$

$$\text{BDP-gal-rham} + \text{GDP-man} \rightarrow \text{BDP-gal-rham-man} + \text{GDP} \quad (3)$$

$$n\ \text{BDP-gal-rham-man} \longrightarrow n\ \text{BDP} + (\text{gal-rham-man})_n \quad (4)$$

$$\text{BDP} \longrightarrow \text{BMP} + \text{P} \quad (5)$$

In these equations B stands for Bactoprenol and BMP and BDP for its mono- and diphosphates respectively. WRIGHT et al. (1) called the compound antigen carrier lipid (ACL), whilst SCHER et al. (3) referred to it as undecaprenol which is a good name but unfortunately begins with U and its abbreviation turns out to be the same as that of uridine. The name bactoprenol avoids this ambiguity.

In equation (1) galactose is transferred to the monophosphorylated lipid so that bactoprenol diphosphate-galactose is formed. In the next steps a rhamnose (equation 2) and a mannose (equation 3) are transferred so that a bactoprenol diphosphate-trisaccharide is formed. The latter can give rise to long chains of a polysaccharide containing the repetitive sequence galactose-rhamnose-mannose.

In bacterial cell wall formation similar events occur so that alternating units of acetyl glucosamine and acetyl muramyl peptide become linked (2).

The formation of mannan in *Micrococcus lysodeikticus* has been found to take place with the intermediate formation of bactoprenol monophosphate-mannose (3) All these studies were carried out with bacteria and there was no information on the role of lipid intermediates in mammalian tissues. A mannolipid

has been found to be formed from GDP-mannose with enzymes from various tissues and endogenous acceptors but its role and structure remained uncertain (4). Independent studies on unsaponifiable lipids by a group of organic chemists at the University of Liverpool (5) had shown that polyprenols are present in many organisms. The compound found in mammalian liver was named dolichol on account of the length of the molecule which contains about 20 isoprene units. It has been detected in the free form and esterified with fatty acids but there were no indications of its physiological role. In our laboratory we have investigated the formation of a lipid intermediate in rat liver (6). Incubation of radioactive uridine diphosphate glucose (UDPG) was found to lead to the formation of a radioactive compound soluble in lipid solvents. Further work showed that the following transformations are catalyzed by liver enzymes:

$$\text{UDPG} + \text{L} \longrightarrow \text{GL} + \text{UDP} \qquad (6)$$

$$\text{GL} + \text{Protein} \longrightarrow \text{G-Protein} + \text{L} \qquad (7)$$

$$\text{G-Protein} \longrightarrow \text{G} + \text{Protein} \qquad (8)$$

In these equations G stands for glucose and L for a lipid which acts as acceptor of glucose.

The formation and properties of the glucosylated lipid

Incubation of radioactive UDPG with the microsomal fraction of liver and magnesium ions plus a detergent (either deoxycholate or Triton X100 were effective), led to the formation of a radioactive compound which was soluble in chloroform-methanol mixtures. Addition of certain lipid containing extracts which may be referred to as acceptor lipid led to an enhanced formation of the compound. Since the reaction was proportional

to the amount of acceptor lipid added the latter could be estimated and a purification procedure was developed.

The glucosylated lipid was found to be more acid labile than any of the glycolipids described in the literature. Thus at 18° in 0.1N acid it was nearly completely decomposed in 40 minutes. Under these conditions the radioactive compound liberated was identified as glucose by paper chromatography.

Alkaline treatment also decomposed the glucosylated lipid and in this case the product was 1-6-anhydroglucosan, a compound which is known to be formed by alkaline degradation of β phenylglucosides.

Natural and synthetic acceptor lipid

The acceptor lipid was extracted from liver and could be purified by a procedure involving the destruction of contaminants by acid and alkaline treatment, DEAE cellulose chromatography in chloroform methanol and thin layer chromatography in various solvents.

The acceptor lipid had the properties of an acid and the infrared spectrum was similar to that of polyprenols. However, completely pure preparations were not obtained due in part to the decomposition that took place during purification. For this reason the approach was changed. Pure dolichol was prepared by the procedure developed by BURGOS *et al.* (5) and it was chemically phosphorylated. The resulting products turned out to behave the same as the purified preparations extracted from liver. The products were compared before and after enzymic glucosylation in respect to acid and alkaline stability, and migration during thin layer chromatography. It was therefore concluded that the acceptor lipid is dolichol monophospate.

It may be mentioned that bactoprenol monophosphate is

labile to acid while dolichol monophosphate is stable. The difference is attributed to the fact that in dolichol the first isoprene residue is saturated.

Utilization of the glucosylated lipid

Incubation of the glucosylated acceptor lipid, which will now be referred to as DMPG (dolichol monophosphate-glucose), with a liver enzyme preparation was found to lead to a transfer of radioactive glucose to a product believed to be a protein. The product could be precipitated with trichloroacetic acid and gave rise to glucose by acid hydrolysis.

One of the few proteins which contain glucose is collagen. Some of its hydroxylysine residues are substituted by galactose which in turn is substituted at position 2 by a glucose residue. The sugar-containing residue of collagen is alkali stable so that it can be easily separated from the rest of the molecule. Preliminary experiments with the protein glucosylated by DMPG and hydrolyzed with alkali did not lead to the identification of the product expected from collagen. However, more work will have to be carried out on this point as well as on Reaction 8 in which the glucosylated protein is hydrolyzed to glucose.

Perspectives

Many of the reactions in which nucleotide sugars act as donors may be mediated by lipid intermediates. In the case of DMPG, glycogen formation is a possibility but experiments designed to test the hypothesis have been negative. Another glucose containing compound found in tissue is collagen but as mentioned before, it is doubtful whether this is the glucoprotein formed by transfer from DMPG.

Preliminary experiments have shown that a glycolipid is formed from GDP-mannose. The latter reaction had already been described by CACCAM *et al.* (4) but the identity of the compound was not established. It seems likely that many dolichol monophosphate sugars will be found and that they may turn out to be involved in the biosynthesis of glycoproteins and proteoglycans such as hyaluronic acid and chondroitin sulfate. There is a whole new field open to investigation.

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