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Methylation studies on ox-brain mucolipid

A large proportion (nearly 60 %) of the mucolipids consists of sugars (hexoses, hexosamine) and sialic acid[1]. In order to gain an insight into the backbone of these complicated structures it is, therefore, of interest to study the types of linkage supporting the various constituents. Preliminary information on the mode of attachment of sialic acid is presented in the preceding note[2]. Here, we discuss the results of methylation experiments designed to provide an understanding of the types of linkage involving the hexose moieties.

Purified mucolipid preparations from ox brain[1] were employed. For exhaustive methylation it was found convenient to use the methyl iodide–silver oxide arrangement with dimethylformamide[3] as the solvent. Methylation was repeated 6 to 8 times, with agitation of the mixture in the dark, but with decreasing quantities of the methylating reagent. The first treatment was performed in the cold (3°) for 40 h, the subsequent ones at room temperature, each time for 16 h. After each treatment the precipitate was washed with dimethylformamide and 4 times with chloroform; the combined extracts were, after dilution with more chloroform, clarified by filtration and concentrated to a small volume *in vacuo* at 35°. After every second or third methylation treatment the concentrated solution was first subjected to dialysis against running and distilled water (for 24 h each) and lyophilized.

The progress of the methylation was followed both by methoxyl determinations and by the infrared spectra (KBr plate) of the purified reaction products. The initially strong OH peak around 3600 cm^{-1} of the untreated mucolipid disappeared gradually until it reached the level of the control KBr plate. The spectroscopic test is more sensitive than the analytical one: even before the final stage the methoxyl content was already at a plateau of 25.3 %.

Measurements of osmotic pressure[4] indicated a molecular weight of 180,000 for the untreated mucolipid, in agreement with the value obtained previously by means of the ultracentrifuge[1], and of 200,000 for the fully methylated product. It is obvious that no appreciable degradation of the polymer had occurred during methylation.

The fully methylated mucolipid was methanolysed with 4 % methanolic HCl for 20 h at 105° in a sealed tube and then treated with 6 % aq. HCl for 100 min in a boiling-water bath. The solution was neutralized with Ag_2CO_3 and the filtrate, after being treated with H_2S and clarified, was passed through a short Amberlite IR-4B column and lyophilized. The residue, dissolved in little water, was examined chromatographically. First, separation on Whatman No. 1 filter paper was employed with several solvent systems and spraying reagents. *n*-Butanol-ethanol–water (4:1:5, v/v/v, top layer) and an aniline *p*-diodosalicylate spray reagent[1] were used routinely. After the preliminary identification of the spots by paper chromatography, a cellulose column and elution by ligroin–*n*-butanol–water (60:39:1, v/v/v) served for the separation of larger amounts of the methylated sugars for a more adequate identification.

Among the criteria of identification were the melting point, methoxyl content, infrared absorption, periodate oxidation and, when reference samples were available, chromatographic mobility. The following products of the hydrolytic cleavage of the methylated mucolipid thus were identified: (a) 2,3,4,6-tetra-O-methyl-D-galactopyranose; (b) 2,4,6-tri-O-methyl-D-galactopyranose; (c) 2,6-di-O-methyl-D-galactopyranose; and—but not yet fully identified—(d) 2,4,6-tri-O-methyl-D-glucopyranose.

The quantitative estimation of the methylated sugars utilized two methods employing separation by paper chromatography and colorimetry of the eluates. The compounds were estimated by means of the benzidine reaction[5] or of a reaction with aniline *p*-diiodosalicylate similar to a previously described procedure using aniline hydrogen phthalate[6]. It was found that (a) the recovery of the total hexoses of the intact mucolipid in form of their methyl derivatives was nearly quantitative; (b) the molar ratios of di- to tri- to tetramethylgalactose to trimethylglucose were 7:3:2:2. These figures represent the averages (corrected to the nearest integer) of seven separate determinations on three different preparations.

Before these observations as well as those outlined in the preceding note[2] can be applied unambiguously to a definition of the structure of the mucolipid molecule, more information will be needed concerning the other components of this polymer. We are continuing our efforts in this direction. A few general remarks may, however, be made provisionally. (1) The high proportion of dimethyl — and therefore triply linked — hexose indicates a high degree of branching. The low proportion, on the other hand, of tetramethyl hexose suggests that the side chains cannot consist only of hexose moieties. (2) Ether linkages between the sugar would have withstood the hydrolysis conditions employed. Since the existence of such linkages in the polymer seems unlikely, disaccharides not having been found in the hydrolysates, the only principal constituent capable of interconnecting the fatty acid–sphingosine–hexose units is sialic acid. To some extent, certain amino acids could also engage in crosslinking. (3) No simple repeating unit is apparent at present that would account for our observations.

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