to the allylic alcohol may precede migration of the double bond, although isomerization of an allylic alcohol to the homoallylic isomer has not yet been effected chemically. In view of the fact that 3β-hydroxyandrosten-4-en-17-one was formed in appreciable amount in the present study, the possibility that it is an intermediate in the conversion of androst-4-ene-3,17-dione to 3β-hydroxyandrosten-5-ene-17-one must be entertained.

REFERENCES

Isolation of a Glycolipid Containing Fucose, Galactose, Glucose, and Glucosamine from Human Cancerous Tissue*

Sen-Itiro Hako-Mori and Roger W. Jeanlou
From the Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts
(Received for publication, July 22, 1964)

Glycolipids have been shown to bear the cell-bound species or tissue antigenicity and immunological “tumor specificity” as has been reported by Rapport, Graf, and Alonzo (1, 2) and by Kobayashi (3). It was consequently of interest to investigate the glycolipids of human adenocarcinomatous tissue. From a gastric adenocarcinoma (Case 26628), a new spingolipoglycolipid containing fucose, galactose, glucose, and glucosamine was isolated. A substance with the same carbohydrate composition was also isolated in slightly higher yield from bronchogenic adenocarcinoma (Case 26626). It is characterized by a high content of fucose and glucosamine, and by the absence of galactosamine and neuraminic acid. This contrasts with the constitution of the known glycolipids of human tissue, which contain galactosamine, sialic acid, or both.

The acetone powder of 400 g of wet tissue was extracted with hot mixtures of chloroform-methanol, 2:1 and 4:1. The water-soluble glycolipid fraction was separated from the extract by partition dialysis (4) and then fractionated by chromatography on a Florisil column (5). Two major fractions, in addition to minor fractions, were eluted with chloroform-methanol, 1:1, and chloroform-methanol-water, 4:4:1. The first fraction (40 to 50 mg) is similar to a ganglioside since it contains sialic acid, but glucosamine replaces galactosamine. The second fraction (60 to 70 mg) is a glycolipid that contained fucose and a hexosamine still contaminated with phospholipids, sulfatides, and peptides. Further purification was obtained by chromatography on a Florisil column (6) in ethylene dichloride solution of the fully acetylated product obtained by reaction with pyridine and acetic anhydride. A sharp peak was eluted with ethylene dichloride-methanol, 9:1. The substance was saponified with 0.2 N sodium hydroxide in 50% ethanol. After neutralization, followed by dialysis, the solution was extracted with chloroform-methanol-petroleum ether (7). The upper phase was evaporated under reduced pressure, and the residue, dissolved in methanol-chloroform, was precipitated with a large excess of acetone to give 30 to 40 mg of a white powder. This glycolipid represents, therefore, a major constituent of the water-soluble glycolipids of human adenocarcinoma.

The substance was essentially homogeneous as shown by thin layer chromatography on silicic acid developed with chloroform-methanol-water, 65:30:8, and propanol-water, 4:1. The homogeneity of the carbohydrate components was ascertained by extraction, with chloroform-methanol, of the upper and the

<table>
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<td>Hexasse</td>
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<td>Fucose</td>
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lower part of the spot obtained by thin layer chromatography. Visual comparison of the carbohydrate components of these two parts after hydrolysis showed the same ratios of fucose, galactose, glucose, and glucosamine. The glycolipid was weakly levorotatory ($\alpha$ $-9.5^\circ$) in chloroform-methanol, 1:1). Its infrared spectra showed the typical pattern of sphingolipids (absorption peaks at 1650, 1550, and 1450 cm$^{-1}$); the presence of phosphatide and sulfatide was excluded by the absence of absorption peaks at 1750 and 1240 cm$^{-1}$, whereas the presence of a $\beta$-glycosidic linkage in the carbohydrate chain was assumed by an absorption peak in the 890 to 900 cm$^{-1}$ region.

After methanolation followed by hydrolysis, the carbohydrate components fucose, galactose, glucose, and glucosamine were identified qualitatively by paper chromatography in the following solvent systems: 1-butanol-acetic acid-water, 4:1:5; ethyl acetate-pyridine-water, 10:4:3; and ethyl acetate-pyridine-acetic acid-water, 5:5:1:3. The presence of fucose was also indicated by the periodate-piperazine-sodium nitroprusside reaction, and by the characteristic greenish fluorescence under ultraviolet light after reaction with aniline-phthalate. In addition, the hydrolysate was separated on Dowex 50 into neutral sugars and amino sugars. The amino sugar fraction obtained by elution with hydrochloric acid gave on paper chromatography a spot corresponding to glucosamine. Ninhydrin degradation of the amino sugar gave a pentose corresponding to arabinose. The amino sugar fraction obtained by thin layer chromatography in the following solvent systems: 1-butanol-acetic acid-water, 4:1:5; ethyl acetate-pyridine-water, 10:4:3; and ethyl acetate-pyridine-acetic acid-water, 5:5:1:3. The presence of fucose was also indicated by the periodate-piperazine-sodium nitroprusside reaction, and by the characteristic greenish fluorescence under ultraviolet light after reaction with aniline-phthalate. In addition, the hydrolysate was separated on Dowex 50 into neutral sugars and amino sugars. The amino sugar fraction obtained by elution with hydrochloric acid gave on paper chromatography a spot corresponding to glucosamine. Ninhydrin degradation of the amino sugar gave a pentose corresponding to arabinose. The components of the neutral sugar fraction gave, by chromatography on Dowex 1-borate, complex peaks which had the same retention volume as fucose, galactose, and glucose. In the phenol-sulfuric acid reaction, the fucose peak gave a golden yellow color absorbing at 480 m$\mu$, whereas the galactose and glucose peaks gave a reddish color absorbing at 490 m$\mu$. The carbohydrate component were determined directly or after methanolation followed by hydrolysis (Table I). These results indicate that the molar ratio of fucose to galactose to glucose to glucosamine was 1:2:1:1. Fatty base and fatty acids were separated from the methanolyse by chromatography on silicic acid. The former product was analyzed by thin layer chromatography (Table I), and the latter by gas chromatography. The hydroxylated fatty acids could not be determined because of scarcity of the material, but seemed to represent a minor part. Palmitic, stearic, arachidic, and lignoceric acids accounted for the largest part of the nonhydroxylated fatty acids.

This is the first isolation of a glycolipid with a large proportion of fucose. Glycolipids with blood group A activity were reported to contain a small amount of fucose (0.5 to 2%) (6, 8, 9), but not the ones with blood group B activity (6). It is possible that these glycolipids were contaminated by a glycolipid containing a large proportion of fucose, since isolation of glycolipids devoid of fucose and possessing blood group A activity has been reported (10). Whereas the glycolipid described here represents a large part of the water-soluble glycolipid isolated from adenocarcinomatous tissue, attempts to isolate a similar component from normal tissue were not successful; fractionation of the glycolipids of normal gastric mucosa gave, as water-soluble glycolipid, a ganglioside type of substance containing glucosamine and galactosamine, but no detectable fucose.

The authors are indebted to Dr. E. B. Taft for supplying cancerous tissue. They are also very grateful to Dr. P. J. Stoffyn for the determination of the fatty acids.

Enzymatic Phosphorylation of D-Glucose with Acetyl Phosphate

MAMDOH Y. KAMEL† and RICHARD L. ANDERSON

From the Department of Biochemistry, Michigan State University, East Lansing, Michigan 48823

(Received for publication, July 27, 1964)

Most enzyme-catalyzed phosphorylations of sugars involve nucleoside triphosphates as the phosphorylating agents. Exceptions to these are the phosphorimidate-dependent phosphorylation of hexoses by an enzyme from Escherichia coli (1), which phosphorylates d-glucose at carbon atom 1 (2), and the complex phosphoenolpyruvate-dependent system, also recently purified from E. coli (3), which phosphorylates hexoses at carbon atom 6. The present communication presents evidence for an enzyme in Aerobacter aerogenes PRL-R3 which phosphorylates d-glucose with acetyl phosphate, but not with adenosine triphosphate or phosphoenolpyruvate, to yield d-glucose 6-phosphate.

A. aerogenes PRL-R3 was grown and extracts were prepared as described previously (4). Crude extracts containing about 30 mg of protein per ml were chromatographed on Sephadex G-75 at 4°C. The first fractions, which contained acetyl phosphate:glucose 6-phosphotransferase activity devoid of ATP:glucose 6-phosphotransferase activity, were purified further by fractionation with protamine sulfate and ammonium sulfate. In a typical fractionation, a Sephadex G-75 fraction was made 0.1 M with ammonium sulfate, and 0.2 volume of 2% protamine sulfate was added slowly to stirring at 0°C. The precipitate that formed was centrifuged and discarded, and the supernatant solution was fractionated with ammonium sulfate. The protein fraction precipitating between 55 and 90% of saturation (at 0°C) contained all of the activity. The increase in specific activity was 5- to 6-fold over the Sephadex G-75 fraction.

The enzyme was assayed by measuring NADP reduction with a Gilford recording spectrophotometer in a glucose 6-phosphate

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* This research was supported by Grant AI-04890 from the National Institute of Allergy and Infectious Diseases, United States Public Health Service. Journal Article 3439 of the Michigan Agricultural Experiment Station.

† Predoctoral Fellow of the Egyptian government.
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