LIPIDS OF DICTYOSTELIUM DISCOIDEUM: PHOSPHOLIPID COMPOSITION AND THE PRESENCE OF TWO NEW FATTY ACIDS: cis,cis-5,11-OCTADECADIENOIC AND cis,cis-5,9-HEXADECADIENOIC ACIDS

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An aggregateless mutant (Agg 204, Sussman (1954)) of the cellular slime mold, D. discoideum, was grown in submersion culture on lipid-free, autoclaved E. coli, suspended in 0.04 molar phosphate buffer, pH 6.0, in the presence of 60 mg. of penicillin/1. After incubation at room temperature for 48 hours, the amebae were harvested and washed three times by centrifugation at 60 x g.

Phospholipid Composition: Washed amebae were blended in 20 volumes of $CHCl_3$: CH_3OH, 2:1, filtered and the lipid extract taken repeatedly to dryness from $CHCl_3$ at 38° under reduced pressure. The yield of total lipid was 180 mg. per gram of lipid-free dry weight. The residue was dissolved in $CHCl_3$, applied to a column of silicic acid and the neutral lipids eluted with $CHCl_3$. The neutral lipids accounted for 40% of the total lipids by weight. The phospholipids were sub-fractionated by silicic acid column chromatography using a linear gradient of methanol in chloroform. The elution pattern was followed by phosphorus analysis and silicic acid paper chromatography (Marinetti, 1962).

Four major fractions were obtained: cardiolipin, 6% of total phosphorus; phosphatidyl serine, 4.5% plus phosphatidyl ethanolamine, 39%; monophosphoinositide, 14%; and phosphatidyl choline, 37%. The phosphatides were identified by their mobilities and staining characteristics on silicic acid impregnated paper (Marinetti, 1962); ester, phosphorus, glycerol, inositol, ethanolamine and serine analyses; and electrophoresis and chromatography of ethanolamine, serine, and inositol phosphate derived from phospholipids.

Fatty Acid Composition: Lipid samples were transesterified overnight at 65° in anhydrous 9% H_2SO_4 in methanol. The methyl esters of the fatty acids were analyzed by gas-liquid chromatography (GLC) on ethylene glycol adipate polyester, SE 30, and Apiezon M (which gave the best separations). The major components[1] of the fatty acids of the total lipids were: 16:0, 14%; 16:1, 7%; 16:2, 10%; 18:0, 2%; 18:1, 23%; 18:2, 41%. The identification of these acids is described below. In addition, there were ten minor components, as yet unidentified.

The fatty acids of the neutral lipid fraction were composed of approximately 60% saturated acids, 18:0 predominating, and 40% unsaturated acids. The total phospholipids contained a remarkably low percentage of saturated fatty acids. Individual phosphatides varied in fatty acid composition, the most extreme case being phosphatidyl choline, which contained only 6% of 16:0, and less than 10% total saturated fatty acids. The unsaturated fatty acid, 18:2, accounted for 50% of the total fatty acids of phosphatidyl choline.

Identification of Fatty Acids 16:0 and 18:0: As expected for saturated fatty acids, neither 16:0 nor 18:0 formed mercuric acetate adducts, nor was their GLC behavior changed by hydrogenation. They were identified as palmitic and stearic acids, respectively, by the retention times of their methyl esters relative to known standards in three GLC systems.

Identification of Fatty Acid Fractions 16:1 and 18:1: Chain length was established by hydrogenation followed by GLC of the methyl esters of the saturated products. The degree of unsaturation was suggested by the relative retention times of the fatty acids methyl esters in two GLC systems, and by the chromatographic behavior on silicic acid of the mercuric acetate adducts.

The 16:1 and 18:1 fatty acid fractions were isolated as their methyl esters by silicic acid column chromatography (Jantzen and Andreas, 1961) of the mercuric acetate adducts, followed by chromatography of the regenerated olefinic

[1]The first number refers to the number of carbon atoms in the fatty acid chain; the second to the number of double bonds in the molecule, and the numbers in parentheses to the positions of the double bonds, counting from the carboxyl end of the molecule.

esters on a column of powdered rubber (Hirsch, 1961). Purity was assessed by GLC. The number and position of the double bonds of each fraction was then precisely established by GLC identification of the methyl esters of the products of periodate-permanganate oxidative degradation (von Rudloff, 1956).

When the 16:1 fraction was oxidized, more than 90% of the dicarboxylic and monocarboxylic acids obtained were azelaic and heptanoic acids, respectively. This fraction is, therefore, composed almost entirely of palmitoleic acid, 16:1(9). Very small quantities of pimelic and pelargonic acids were also observed, indicating the presence of some 16:1(7).

Upon oxidative degradation of the 18:1 fraction, 75% of the dicarboxylic and monocarboxylic acids obtained were undecanedioic and heptanoic acids, respectively. Thus the major fatty acid in this fraction is vaccenic acid, 18:1(11). Approximately 20% of the di- and mono-carboxylic acids were azelaic and pelargonic acids, respectively, establishing the presence of oleic acid, 18:1(9), in the 18:1 fraction. Sebacic and octanoic acids were present in the oxidation products to the extent of 5%, indicating that the 18:1 fraction also contains some 18:1(10).

Identification of Fatty Acid Fractions 18:2 and 16:2: Chain lengths and the number of double bonds were established as described above for fractions 16:1 and 18:1. The 18:2 acid was readily distinguishable from linoleic acid, 18:2(9,12), by GLC on ethylene glycol adipate polyester, the former having a significantly shorter retention time than the latter. The only products obtained in significant quantities after oxidative degradation of fraction 18:2 were the dicarboxylic acids, glutaric and adipic, and the monocarboxylic acid, heptanoic. This established the presence of one double bond at position 11, and another at either position 5 or 6. To distinguish between these alternatives, the ethyl ester of 18:2 was prepared and oxidized in the usual manner, except for the omission of the saponification step of the original procedure. In a control experiment, it was found that the ethyl ester linkage is retained under these conditions. The free carboxyl groups of the oxidation products were then converted to methyl esters by reaction with diazomethane, and the products identified by GLC. The

products obtained by this method were methyl heptanoate, dimethyl adipate, and methyl ethyl glutarate. Thus the glutaric acid was derived from the carboxyl end of the original 18:2 fatty acid, and its structure is therefore established as 5,11-octadecadienoic acid, 18:2(5,11).

Upon oxidation of fraction 16:2, heptanoate and glutarate were obtained in good yield. Succinate was the only other compound observed, but it was in smaller quantity than the other two acids. As with the 18:2 fraction, methyl ethyl glutarate was identified in the oxidation products of the ethyl ester of 16:2. The structure of fatty acid 16:2 is therefore tentatively established as 5,9-hexadecadienoic acid, 16:2(5,9), although the poor yield of succinic acid remains to be explained.

Infrared Spectra: The C-H deformation band at 965 cm^{-1}, characteristic of the trans-configuration (Ahlers, 1953) was absent from the infrared spectra of the purified fractions 16:1, 16:2, 18:1 and 18:2, indicating that all the double bonds of all four acids are of the cis configuration.

DISCUSSION

The lipids of D. discoideum are unique in several respects. For one, the phospholipids contain an overwhelming proportion of unsaturated fatty acids whereas most other phospholipids that have been described contain very nearly equal amounts of saturated and unsaturated fatty acids. This must mean that the composition of the fatty acids esterified at the β-position, which in other phospholipids are almost all saturated, must in these phospholipids be predominantly unsaturated.

Perhaps even more unusual than the relatively high percentage of unsaturated fatty acids is the structure of these acids. The major fatty acid of the amebae, accounting for 40% of the total fatty acids, is the heretofore unknown cis,cis-5,11-octadecadienoic acid, and the equally unique cis,cis-5,9-hexadecadienoic acid accounts for another 10% of the fatty acids. The more usual type of polyunsaturated acids, with methylene interrupted double bonds, are not present. It will be of great interest to compare the fatty acid

compositions of phylogenetically related organisms. In this regard, we have found the composition of the fatty acids of the aggregating wild type D. discoideum to be identical to that of the mutant described here.

Studies have been initiated in the biosynthesis of the unsaturated fatty acids described in this paper and a preliminary report is presented in an accompanying communication (Davidoff and Korn, 1962).

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