A glycolipid which was expressed during a differentiation from haploid myxamoebae to diploid plasmodia of a true slime mold, *Physarum polycephalum*, has been examined. In the amoeboid stage, cells did not contain this glycolipid, but after conjugation of the haploid cells, this substance appeared and increased in its amount.

From structural studies of the purified glycolipid, it has been identified as poriferasterol monoglucoside.

In the life cycle of a true slime mold, *Physarum polycephalum*, it has haploid and diploid stages (1–3). Haploid myxamoebae are germinated from spores under moist conditions, and they conjugate in pairs to form diploid plasmodia. At the amoeboid stage, cells recognize different mating types and fuse to form zygotes. Zygotes grow and fuse each other and differentiate into young plasmodia. These steps are genetically regulated; zygote formation occurs only between myxamoebae carrying different alleles of a multiallelic locus (4–8), and differentiation of the zygotes to plasmodia is also genetically determined (4, 9, 10). Tiny young plasmodia grow up as multinuclear plasmodia, and they fuse each other without any artificial treatments. During the course of differentiation from haploid myxamoebae to diploid plasmodia, the change of membrane characteristics might occur.

We examined lipid composition of the membranes of the cells at both stages and found an expression of a novel glycolipid which was correlated with a process of differentiation. This glycolipid was purified and characterized and was identified as poriferasterol monoglucoside.

**EXPERIMENTAL PROCEDURES AND RESULTS**

Expression of a Glycolipid during the Differentiation of *P. polycephalum* from Haploid Amoebae to Diploid Plasmodia—TLC analysis of crude lipid fractions (see “Experimental Procedures”) showed the pattern of nonpolar glycoconjugates of haploid cells differed from that of diploid cells. Fig. 1 shows the TLC patterns of the nonpolar glycoconjugates from haploid amoebae, strain J, or diploid plasmodia of *P. polycephalum*. There was no difference between two different mating types of myxamoebae, strains J and F. Four major carbohydrate-containing bands from haploid cells were designated as A to D according to their mobility. Five major sugar-containing bands were obtained in the extract from diploid cells: three of them were corresponding to A, C, and D from haploid cells, and other two bands designated as E and F were characteristics for diploid stage. Band D showed blue color and others showed purple colors.

Among these nonpolar glycoconjugates, substance F was expressed after conjugation of haploid amoebae and increased in its amount during the differentiation into diploid plasmodia (Fig. 2). A content of this substance was maintained constantly under different culture conditions either on rolled oats or in semidefined culture media. Substance E appeared after the differentiated plasmodia were transferred into culture media. When the crude membrane fraction was extracted, bands A and B were not detected, but other glycoconjugates were also extracted and almost the same results were obtained.

Purification and structural analysis of glycolipid F are presented in the Miniprint Supplement.

From the evidence described in the Miniprint Supplement, the structure of substance F was designated as that shown in Fig. 10 (poriferasterol monoglucoside).

**DISCUSSION**

In this work it is shown that a novel glycolipid was expressed during a differentiation of haploid amoebae of *P. polycephalum* into diploid plasmodia. The glycolipid was purified and identified as poriferasterol monoglucoside.

Poriferasterol was a major sterol component in haploid cells of *P. polycephalum* (34) as well as in diploid cells (25). But in a haploid stage, the poriferasterol monoglucoside was completely absent, and during the differentiation into diploid plasmodia it appeared and increased in its amount. Other steryl-glucosides and their derivatives could not be detected in both stages.

The steryl-monoglucosides and their 6′-O-acyl derivatives are known as common constituents of higher plants (27, 28), and their biological functions have been suggested to be metabolically active components of plant membrane structure (29), intercellular transporters of sterols (30), or glucose carriers through cell membranes (31, 32).

Amoeboid cells are uni-nuclear and behave like protozoan soil-amoebae on solid substrata or amoeba-flagellate in non-
Fig. 1. Thin layer chromatogram of nonpolar glycoconjugates. Plate a, developed in solvent I; plate b, developed in solvent II. Lane 1, mixture of glycolipids from rat brain (SCMH, galactosylsulfatide; CDH, lactosylceramide; Gm1, GM1, GD1a, gangliosides having different length of sugar chains); lane 2, crude extract from myxamoebae; lane 3, crude extract from plasmodia. Bands were visualized by spraying with orcinol-H2SO4.

Fig. 2. A, thin layer chromatogram of crude lipids from Physarum at various developing stages. Lane 1, mixture of glycolipids from rat brain (see Fig. 1); lane 2, 0 days after mating of the haploid cells; lane 3, 2 days after mating; lane 4, 4 days after mating; lane 5, 6 days after mating. Plate a, developed in solvent I; plate b, developed in solvent II. Bands were visualized by spraying with orcinol-H2SO4. B, content of substance F during differentiation of Physarum from haploid amoebae into diploid plasmodia. The content of substance F was expressed as the amount of lipid-bound glucose. O—O, percent of conjugated cells; •—•, content of glycolipid F.
nutrient liquid. Diploid cells grow as multinucleated plasmodia in which an intranuclear mitosis occurs, and they fuse each other very easily without any artificial treatments. The cell membrane of plasmodia seems to have higher fluidity than that of haploid amoebae, and when the cell membrane has been injured the repair of it is completed immediately. Plasmodia are capable of growth in liquid or on agar media, but amoebae may be injured the repair of it is completed immediately. Plasmodia are capable of growth in liquid or on agar media, but amoebae capable of utilizing them as nutrients. Poriferasterol monoglucoside may have some active functions in membranes of plasmodial membrane seems to have higher fluidity than amoeboid membrane. It is also important to examine the regulation of the enzyme, UDP-glucose:poriferasterol glucosyltransferase, which synthesizes substance F, during the process of differentiation of P. polycephalum.

REFERENCES
1. Alexopoulos, C. J. (1982) in Cell Biology of Physarum and Didymium (Aldrich, H. C., and Daniel, J. W., eds) Vol. I, pp. 3-23, Academic Press, New York
2. Wick, R. J., and Sauer, H. W. (1982) in Cell Biology of Physarum and Didymium (Aldrich, H. C., and Daniel, J. W., eds) Vol. II, pp. 3-20, Academic Press, New York
3. Raut, T. J., and Aldrich, H. C. (1982) in Cell Biology of Physarum and Didymium (Aldrich, H. C., and Daniel, J. W., eds) Vol. II, pp. 21-75, Academic Press, New York
4. Dee, J. (1982) in Cell Biology of Physarum and Didymium (Aldrich, H. C., and Daniel, J. W., eds) Vol. I, pp. 3-23, Academic Press, New York
Poriferasterol Monoglucoside: Differentiation of Physarum

Gas liquid chromatography (GLC). - For the detection of components of the glycolipid, homogeneous substance was methanolyzed in 3% HCl/methanol, and the resulting methyloximate and sterol were trimethylsilylized and analyzed by GLC on a column of 3% OV-101.

Gas liquid chromatography-mass spectrometry (GC/MS). - GC/MS was carried out on JMS-HX50A with MS-30 spectroscopic ionization voltage, 70 eV. GC conditions for free sterols were as follows: Column, 9.4 m window column DB-1; flow rate of He carrier gas was 20 ml/min; column temperature, from 284°C (4'C/min) to 300°C (2°C/min). For other components, 80 m window column DB-1; flow rate was 1 ml/mm; column temperature, 230°C isothermally.

Fast atom bombardment mass spectrometry (FAB/MS). - FAB/MS for an intact glycolipid was performed on JMS-HX50A under the following conditions: Xe gas gun, volt, 3 kV; emission, 10 nA; matrix, diorthocarbonic acid.

Determination of NMR spectra. - The 400 MHz NMR spectra were measured on JNM-GX-400 (Jasco, Ltd). For an intact glycolipid, room temperature was 27°C and the compound was dissolved in DMSO. The acetylated moiety of the glycolipid and standard sterols were dissolved in CDCl₃, and NMR spectra were measured using TMS as an internal standard.

Acetylation of sterols. - The sterol was dissolved in acetic anhydride containing 4 NaOAc, 2% p-bromobenzyl alcohol acid. The mixture was allowed to stand at room temperature overnight, ethyl acetate was added and washed with aqueous sodium bicarbonate. Then, the ethyl acetate solution was dried over anhydrous sodium sulfate and evaporated to give white crystals.

Enzymatic hydrolysis. - 8-Glucosidase treatment was done as follows. The glycolipid preparation (180 μg) was dissolved in 450 μl of 50 mM sodium citrate buffer, pH 5.0, and 0.6 unit of 8-glucosidase was added. The mixture was incubated at 37°C for 6 hrs and 0.6 unit of the enzyme (20 μl of the buffer was added to each sample). The reaction was stopped by the addition of 2.5 ml of n-hexane and centrifuged. The lower phase was dried, redissolved in 0.5 ml of 1% heptane and analyzed by TLC developed in chloroform and GC. 8-Glucosidase treatment was done essentially as described above, but potassium phosphate buffer, pH 7.0, was used instead of sodium citrate buffer.

Other methods. - Protein determination was done by the method of Lowry et al. [22] with orcinol-H₂SO₄. Bands were visualized by spraying with orcinol-H₂SO₄. The melting points were measured according to Bullock and Dawson [25].

RESULTS

Purification and structural studies of a glycolipid F. - Glycolipid F was purified by the method described in Experimental Procedures, and the purity of this substance was analyzed by TLC in two different solvent systems I and II and some different color-developing reagents for the detection of some biochemical components. Glycolipid F showed a single band in each solvent system used, and was identified with orcinol-H₂SO₄ and ferric chloride solution. No other reagents tested (orcinol-H₂SO₄, amino acid, L-DOPA, and ferric chloride solution) showed the same color of this substance. Other substances contained heptane and heptane as the components. Fig. 3 shows the TLC of the purified glycolipid F.

Fig. 3. Thin layer chromatogram of purified non-polar mono- and diglucosides. - The plate was developed in solvent 1. Lane 1, crude lipids from myxobacteria; lane 2, crude lipids from myxobacteria after a single cycle of 10% ethanol; lane 3, purified A; 4, purified B; 5, purified C; 6, purified D; 7, purified E; 8, purified F. Bands were visualized by spraying with orcinol-H₂SO₄.

Fig. 4. Fast atom bombardment mass spectra of substance F. - FAB/MS was carried out on JMS-HX50A mass spectrometer. When the substance F was methanolyzed and trimethylsilylized derivatives of methyl glucoside and sterol were analyzed by GLC. Fig. 5A shows GC patterns of methylglucoside (TMS) derivatives of some known sugars: glucose, galactose, glucosamine, N-acetyl glucosamine, N-acetyl neuraminic acid. The patterns of TMS-glucosides and TMS-sugars corresponded to the glucose shown in Fig. 5A. No other sugars were detected in the glycolipid. Fig. 6A shows the GC patterns of the derivatives of standard sterols and Fig. 4A is the pattern of TMS derivatives of Physarum sterol. The mass pattern of the glycolipid F was considered to be a stigmasterol. Other components were detected by GLC analysis under some different conditions with some different columns.

Fig. 5. Gas liquid chromatograph of O-trimethylsilyl derivatives of methyl glucosides from 3 standard sugars and 3 glycolipids. - Peaks correspond to (1) glucose, (2) fructose, (3) sucrose, (4) N-acetyl glucosamine, (5) N-acetyl neuraminic acid, (6) glucosamine, (7) purine, (8) guanine, (9) 4-methyl-3-carboxyphenanthrene, and (10) N-acetyl neuraminic acid; 12. GC analysis was done on a column of 3% OV-101 at 150-250°C.

Fig. 6. Gas liquid chromatograph of O-trimethylsilyl derivatives of methyl glucosides from 3 standard sugars and 3 glycolipids. - Peaks correspond to (A) standard sugars and (B) non-polar moiety of substance F. - Peaks correspond to the following sugars: glucose, galactose, mannose, p-glucose, r-glucose, 1, 2, mannose, 3, 4, 5-trimethyluracil, and 6. GC analysis was done on a column of 3% OV-101 at 250°C.

The sterol moiety of the substance F was subjected to GC/MS, and a molecular ion at m/e 412 and fragmentation ion at m/e 394 (m-H₂O) was detected together with number of other ions (Fig. 7A). The mass pattern of the sterol of substance F was quite similar to that of standard stigmasterol (Fig. 7A). The sterol moiety was acetylated and also subjected to GC/MS (Fig. 6C). The sterol moiety of substance F is represented by a molecular ion at m/e 414, which corresponds to the loss of acetate from the expected molecular ion. The mass pattern of the sterol of the substance F is also quite similar to that observed for stigmasterol acetate (Fig. 6B), and that of poriferasterol acetate (Fig. 6B).

Fig. 7. Mass spectra of (A) stigmasterol and (B) non-polar moiety of glycolipid F. - GC/MS analysis was done on JMS-HX50A.
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From above results, the sterol moiety could not be identified as stigmas-entrant or poriferasterol. Either one is a C-24 epimer of the former one. The 400 MHz NMR spectra of the sterol moiety of the substance showed the presence of a stigmasterol but a poriferasterol judging from the methyl group chemical shifts which were reported by Rublstein et al. This value also confirmed that the sterol was not stigmasterol (m.p., 170°C) but a poriferasterol (m.p., 156°C) as described by Bullock and Dawson.

![Fig. 1. Mass spectra of acetates of (A) stigmasterol, (B) poriferasterol and (C) non-polar moiety of substance F. GC/MS was performed on JEOL SX-102 mass spectrometer.](image)