Biosynthesis of Chlorosulfolipids in *Ochromonas danica*. Assembly of the Docosane-1,14-diol Structure *in Vivo*†

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ABSTRACT: It has been proposed (C. L. Mooney, E. M. Mahoney, M. Pousada, and T. H. Haines (1972), *Biochemistry 11*, 4839) that formation of the diol backbone in the chlorosulfolipids of *Ochromonas danica* includes hydration of an ω-9 unsaturated intermediate. Such a mechanism is excluded by experiments using ^{18}O *in vivo* (J. Elovson (1974), *Biochemistry 13*, 2105), and we report here results on the incorporation of labeled precursors which establish instead the following biosynthetic pathway in *O. danica:* octadecanoic acid → docosanoic acid → 14-hydroxydocosanoic acid → docosane-1,14-diol. Since chlorination occurs after completion of the diol structure, this sequence accounts for the assembly of the diol backbones in all C_{22}-sulfolipid subspecies of different degrees of chlorination.

The unique chlorosulfolipids found in *Ochromonas danica* are predominantly chlorinated (Elovson and Vagelos, 1969, 1970) disulfates of docosane-1,14-diol (Mayers and Haines, 1967). A mechanism of the assembly of this docosane-1,14-diol backbone from the usual products of the fatty acid synthetic machinery has to account for elongation to the C_{22} chain length, introduction of a secondary hydroxyl at C-14, and reduction of a carboxyl group to the primary alcohol at C-1. Mooney *et al.* (1972) reported that oleic acid, as well as palmitic and stearic acids, was incorporated into these diols, and on this evidence concluded that hydroxylation occurred by hydration of an ω-9 unsaturated intermediate. In contrast, we have recently shown (Elovson, 1974) that the secondary hydroxyl group in the diols is derived solely from molecular oxygen, not from water, excluding any mechanism such as that proposed by Mooney *et al.* (1972). We have now examined the incorporation of various fatty acids as possible precursors of the chlorosulfolipid diols *in vivo,* and present results which show that formation of the C_{22} diols occurs by elongation of saturated intermediates to the C_{22} fatty acid level, followed by direct hydroxylation and reduction to docosane-1,14-diol.

Experimental Section

Materials

The following fatty acids nominally labeled with tritium in the indicated positions were obtained commercially: [9,10-^{3}H]stearic and -oleic acids (490 and 750 Ci/mol, respectively) from Amersham-Searle; [9,10-^{3}H]palmitic acid, [11,12-^{3}H]-eicosanoic acid, and [13,14-^{3}H]docosanoic acid (all 50 Ci/mmol) from Dhom Products, North Hollywood, Calif. The radiochemical purity of the saturated fatty acids was better than 99%. The tritiated oleic acid was freed of substantial amounts of contaminants which migrated as saturated and trans-unsaturated species by preparative thin-layer chromatography (tlc) of the methyl esters on $AgNO_3$ impregnated plates. The purified material was better than 99% cis unsaturated on rechromatography. [1-^{14}C]Docosanoic acid was prepared by a nitrite synthesis essentially as described (Elovson, 1964); it was better than 99% radiopure by glc after purification by tlc. Racemic [14-^{3}H]-14-hydroxydocosanoic acid was prepared by [^{3}H]$NaBH_4$ reduction of 14-ketodocosanoic acid, obtained by Jones oxidation of 1,14-docosanediol: 0.0017 ml of Jones reagent (26.72 g of CrO_3, 23 ml of H_2SO_4, distilled water to 100 ml) and 0.07 ml of acetone/μmol of diol, 5 min at 15°. The pure keto acid was obtained by methylation with diazomethane, preparative tlc, and saponification; 4.3 μmol of keto acid in 0.4 ml of methanol–2-propanol, 1:1, was added to 0.02 ml of 2 N NaOH in a screw-capped tube. About 0.5 mg of [^{3}H]$NaBH_4$ (200 Ci/mol) was added, and the tube was incubated at room temperature for 2 hr with occasional heating and shaking. The mixture was carefully acidified in the fume hood and extracted with ether; after methylation, preparative thin-layer chromatography and saponification, pure [14-^{3}H]-14-hydroxydocosanoic was obtained in about 90% yield calculated from the keto acid, assuming a specific activity ¼ of that of the borohydride used. The same series of reactions was used to prepare racemic [10-^{3}H]-10-hydroxystearic acid, starting with 10-hydroxystearic acid, which was a generous gift of Dr. George Schroepfer, Rice University, Houston, Texas. Racemic [14-^{3}H]docosane-1,14-diol was prepared by $LiAlH_4$ reduction of the acid. H^{36}Cl was obtained from New England Nuclear, [^{3}H]$NaBH_4$ (200 Ci/mol), and [^{14}C]NaCN (55.5 Ci/mol) from International Chemical and Nuclear Co., Irvine, Calif. *Ochromonas danica* is American Type Culture Collection 30004.

Methods

O. danica was cultured in the dark as described (Brown and Elovson, 1974). Radioactive fatty acids dissolved in a small volume of 70% ethanol were added to 500 ml of a logarithmically growing culture, usually at a density of 3×10^6 cells/ml; final ethanol concentration less than 1%. At the indicated times, cells were harvested by centrifugation and washed once with water. Extraction and recovery of total chloroform-soluble lipids, total chlorosulfolipids, and total and individual chlorodiol fractions have been described (Elovson, 1974). The distribution of radioactivity in the chloroform fraction was determined by tlc on silica gel G, developed with ethyl ether–petroleum ether–MeOH–acetic acid, 100:40:5:1.5, along with appropriate standards of triglycerides and free fatty acids. The polar lipids remaining at the origin were recovered for radioactivity measurements by elution with chloroform–methanol–

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TABLE I: Incorporation of Labeled Fatty Acids into Chlorosulfolipid Diols and Ester Lipids *in Vivo*.

	Diols (%)[c]	Esters (%)[c]	[Diols/(Diols + Esters)]100
14-OH 22:0[a,b]	3.8	2.90	57
10-OH 18:0	0.050[d]	8.90	0.56
16:0	4.2	20.30	17
18:0	1.6	10.60	13
20:0	1.6	2.90	36
22:0	0.53	1.33	28
*cis*Δ9 18:1	0.008	44	0.018

[a] Chain length and number of unsaturated bonds. [b] Concentration of fatty acids in medium: 16:0, 0.2 mCi, 0.008 μM; 18:0, 0.68 mCi, 2.8 μM; 20:0, 0.18 mCi, 0.007 μM; 22:0, 1.1 mCi, 0.04 μM; 10-OH 18:0, 0.32 mCi, 13 μM; 14-OH 22:0, 0.18 mCi, 7 μM; 18:1, 0.32 mCi, 0.9 μM. [c] % of precursor radioactivity recovered in diols and esters. [d] Corrected for "nonspecific labeling;" see Table III.

TABLE II: Incorporation of C_{22} Precursors into Chlorosulfolipid Diol Subspecies of Different Degree of Chlorination.

	Subfraction[a] (%)			
	0	1	2–5	6
[14-^{3}H]Docosane-1,14-diol[c]	33[b]	26	16	25
[14-^{3}H]-14-Hydroxydocosanoic acid[d]	33	30	16	21
[13,14-^{3}H]docosanoic acid[d]	24	26	32	17

[a] Number of chlorine atoms/diol in respective subfraction. [b] Per cent of total diol radioactivity in each subfraction. [c] 6.25×10^6 dpm, incubated as described for fatty acids in Table I; 0.49×10^6 dpm recovered in chlorosulfolipid diols. [d] From Table I.

acetic acid–water, 50:39:1:10; less polar lipids were scraped directly into counting vials. Methyl esters of normal fatty acids were chromatographed on silica gel plates in benzene; methyl esters of hydroxy and keto acids were similarly separated in a system of benzene–chloroform–methanol, 110:40:3. Methyl esters of different degrees of unsaturation were separated by chromatography on silica gel G plates prepared in 11% $AgNO_3$. The plates were activated 30 min at 120° immediately prior to use, and developed in toluene at −17°. Radioactivity in esterified fatty acids was also determined after their conversion to hydroxamic acids. Lipid mixtures were treated with alkaline hydroxylamine as described by Skidmore and Entenman (1962); the residues were taken up in 2 ml of hexane and the hydroxamic acids were extracted with three 1-ml portions of the ethanolic acid–ferric perchlorate reagent diluted 1:1 with water; the aqueous phases were sequentially backwashed with 1 ml of hexane, and radioactivity was determined in the pooled aqueous extracts and pooled hexane extract plus wash. Control experiments showed better than 95% recovery of radioactivity from esterified and free fatty acids in the aqueous and hexane fractions, respectively. The amount of tritium specifically located on the secondary hydroxyl carbon in precursor hydroxy acids and product diols was determined by Jones oxidation to the respective keto acids; the latter were purified by tlc and identified by glc–mass spectrometry, and their specific activity was compared to that of the original hydroxy compound as a measure of "nonspecific" tritium activity. The sterically highly hindered hexachlorodocosanediol was oxidized in CrO_3–acetic acid–H_2O as described (Elovson and Vagelos, 1970). Schmidt decarboxylation was performed as described (Elovson, 1964).

Results and Discussion

In order to obtain a meaningful comparison between different fatty acids as precursors for the (chloro)diols *in vivo* a correction for trivial differences in their assimilation had to be made. It is not adequate to normalize the data to total "uptake" of precursor, since the fatty acids adsorbed extensively to the cells as isolated, and were recovered in the chloroform-soluble lipids as unchanged free fatty acids. A better index is obtained when comparing diol synthesis to the formation of fatty acid esters, since it is reasonable to assume that sulfolipid diols and esterified fatty acids both derive from fatty acyl CoA esters (Elovson, 1974). Thus, a comparison of the relative rate of appearance of label from different radioactive fatty acids in the diol *vs.* ester fractions should give an indication of the relative effectiveness of these acids as diol precursors, relatively independently of differences in their rate of uptake and activation by the cells.

Table I summarizes the results when *O. danica* was pulsed for 4 hr with trace amounts of different precursor fatty acids. The absolute recoveries of each fatty acid in sulfolipid diols and in total fatty acid esters are shown as per cent of added radioactivity; the last column shows the relative amount in the diols as per cent of total metabolized activity, *i.e.*, diols plus esters.

14-Hydroxydocosanoic acid is the expected immediate diol precursor, and as seen in Table I it was in fact efficiently and relatively specifically incorporated into the chlorosulfolipid diols: about 4% of the added radioactivity was recovered in the diol fraction at 4 hr, accounting for over one-half of the total radioactivity metabolized by the cells; since the racemic acid was used this represents a minimum figure. The cells were cultured in chloride-containing media and it is seen in Table II that the radioactivity from 14-hydroxydocosanoic acid was found in all (chloro)diol subspecies. Furthermore, when cells were incubated with synthetic [14-^{3}H]docosane-1,14-diol under the same conditions the (chloro)diol subspecies recovered after hydrolysis of the chlorosulfolipids also displayed the same labeling pattern (Table II). Since chlorination therefore occurs after the diol structure is finished, the same pathway will account for the formation of the backbone structure in all the docosane(chloro)diols. The tritium distribution in the a-, mono-, and hexachlorodiol species, as well as that in the [14-^{3}H]-14-hydroxydocosanoic acid starting material, was further established by CrO_3 oxidation; it is seen in Table III that the diols retained the specific C-14 ^{3}H labeling found in the precursor, demonstrating a direct conversion of the acid to diols, rather than label recirculation *via* acetate.

If the ω-9 secondary hydroxyl group were introduced at the C_{18} level, followed by elongation to the C_{22} species, 10-hydroxystearic acid should also be a reasonably efficient and specific precursor for the chlorosulfolipid diols. This, however, was not the case. As shown in Table I less than one-tenth of a per cent of the [10-^{3}H]-10-hydroxystearic acid added to the medium was incorporated intact into chlorosulfolipid diols, as determined by retention of tritium on the secondary hydroxyl carbon. About twice as much tritium activity was incorporated into diols by some other pathway which resulted in labeling of

TABLE III: Direct Conversion of [^{3}H]Hydroxy Acids[a] to Chlorosulfolipid Diols.

	10-OH-18:0 (%)	14-OH-22:0 (%)
OH acid precursor[b]	98.3[d]	98.2
Docosane-1,14-diol[c]	42	98.5
Monochlorodocosane-1,14-diol[c]	33	98.8
Hexachlorodocosane-1,14-diol[c]	29	97.8

[a] Proportion of ^{3}H-label in precursor hydroxy acids and diol products located on the secondary alcohol carbon (see Methods). [b] Added to medium; see Table I. [c] Recovered from chlorosulfolipids; see Tables I and II. [d] Fraction of total ^{3}H removed by CrO_3 oxidation of the secondary hydroxyl; see text and Methods.

TABLE IV: Direct Conversion of Docosanoic Acid into Docosane 1,14-Disulfate.

	$^3H/^{14}C$	Carboxyl-$^{14}CO_2$ (%)
[1-^{14}C;13,14(n)[a]-^{3}H]Docosanoic acid[b]	1.00[f]	88[e]
Docosane-1,14-diol[c]	0.95	
14-Ketodocosanoic acid[d]	0.59	86

[a] Nominal. [b] Added to medium: 45 μCi of ^{14}C, 90 μCi of ^{3}H; final concentration 1.45 μM. [c] Isolated from sulfolipids. [d] Obtained by CrO_3 oxidation of diol. [e] % of ^{14}C recovered as $^{14}CO_2$ in the Smidt degradation; see Methods. [f] Normalized.

the remainder of the backbone (Table III), most likely by total synthesis *via* labeled acetate. This poor utilization of 10-hydroxystearic acid for diol synthesis was not due to a general inability of the cells to activate and metabolize this acid, since it was quite efficiently converted into lipid ester form; thus, as seen in the last column of Table I the C_{18} hydroxy acid had a 100-fold lower diol/(diol + ester) ratio than did the C_{24} species. The nature of the esterified hydroxy acids recovered from the cells was not established in detail; the greater part presumably was present in mixed triglyceride form, since it migrated somewhat more slowly than standard normal triglyceride on tlc; less than one-tenth of the esterified activity was found in the polar lipid fraction as previously defined (Brown and Elovson, 1974). After treatment with alkaline hydroxylamine the radioactivity in both these ester fractions was recovered in the hydroxamic acid fraction, showing that the hydroxystearic acid residues indeed were derivatized at their carboxyl groups. After transmethylation, over 95% of esterified radioactivity in the cells pulsed with the 10-hydroxystearic acid migrated with the authentic methyl ester on tlc.

To test the alternative sequence, *i.e.*, chain elongation followed by hydroxylation at the C_{22} level, the incorporation of labeled C_{16}, C_{18}, C_{20}, and C_{22} saturated acids into chlorosulfolipid diols was determined. As seen in Table I, and in agreement with the results of Mooney *et al.* (1972), palmitic and stearic acid were fairly efficient precursors of the diols; more to the point, however, the same is also seen to be the case for arachidic and behenic acid. The absolute amounts of each saturated acid recovered in both diols and ester lipids decreased with increasing chain length, but the proportion in the diols if anything increased, suggesting that the rate of an initial assimilation step, presumably a thiokinase reaction, decreased with increasing chain length; however, once activated, the C_{20} and C_{22} acids are efficient substrates for diol formation. The rate of diol formation from the saturated acids was fairly linear over the 4-hr pulse, but the rate of esterification tended to drop off about equally for all the precursor acids. The relative distribution of radioactivity between the different docosane(chloro)diol subspecies was similar for the four saturated fatty acids and essentially the same as that for 14-hydroxydocosanoic acid; the data for docosanoic acid are included in Table II. Mooney *et al.* (1972) obtained results with ^{14}C-labeled palmitic and stearic acids which were consistent with their being incorporated into the monochloro species as intact units, rather than by recirculation *via* the acetate pool. The data in Table I were obtained with saturated acids nominally labeled with tritium at the ω-9,10 positions, but since it is difficult to establish the actual distribution of tritium in these starting materials the products were not further analyzed. Instead, to prove unambiguously that the key intermediate behenic acid was indeed incorporated directly into the diols a separate experiment was performed using the doubly labeled [1-^{14}C;13,14-^{3}H(nominal)] acid. The incubation was performed for 8 hr in chloride-free medium to optimize the yield of a single product, docosane 1,14-disulfate. The docosane-1,14-diol obtained in this experiment accounted for 1.2% of the added ^{14}C acid, which compares well with the value in Table I. It is seen in Table IV that it had a $^3H/^{14}C$ ratio similar to that of the precursor acid. This excluded any significant contribution by label recirculation *via* the acetate pool, since this results in over a tenfold depression of this ratio, due to labilization and loss of hydrogens in the β oxidation/fatty acid synthesis reaction (Elovson, 1964). The docosane-1,14-diol product was also oxidized to 14-ketodocosanoic acid and the latter subjected to Schmidt decarboxylation, along with the original [1-^{14}C]docosanoic acid. As seen in Table IV, about 85% of the ^{14}C in both compounds was released as $^{14}CO_2$, the somewhat low recovery in both cases presumably being due to incomplete trapping of the carbon dioxide liberated; that is, docosanoic acid was directly converted to docosane-1,14-diol, without randomization of the C-1 ^{14}C label. Thus, the data in Tables I and III unequivocally show that in the biosynthesis of docosane-1,14-diol elongation occurs prior to hydroxylation. Although, as mentioned above, 10-hydroxystearic acid also was poorly but significantly incorporated into diols, this may most simply be accounted for as nonspecific elongation of an unnatural compound, comparable to its incorporation into ester lipids, which normally do not contain this acid.

It should be mentioned that the docosane-1,14-diol isotope ratio in Table III was much higher than expected, since direct substitution of a C-14 hydroxyl group should remove one of the four labeled hydrogens from [13,14-^{3}H]docosanoic acid. Although a significant proportion of the label in this precursor in fact was located at positions other than C-13,14 (since the $^3H/^{14}C$ ratio in the 14-ketodocosanoic acid derivative was 0.59 rather than 0.50), this is clearly not sufficient to account for the high $^3H/^{14}C$ ratio in the docosane-1,14-diol, and it must be concluded that introduction of the secondary hydroxyl at C-14 proceeds with a very pronounced isotope effect. The high ratio is also incompatible with an ω-9 unsaturated intermediate where a loss of 2/4 hydrogens would occur.

In sharp contrast to the saturated fatty acids, oleic acid was very efficiently assimilated by *O. danica* and converted into normal fatty acid esters, but it was completely ineffective as a precursor for sulfolipid diols (Table I). The diol label shown

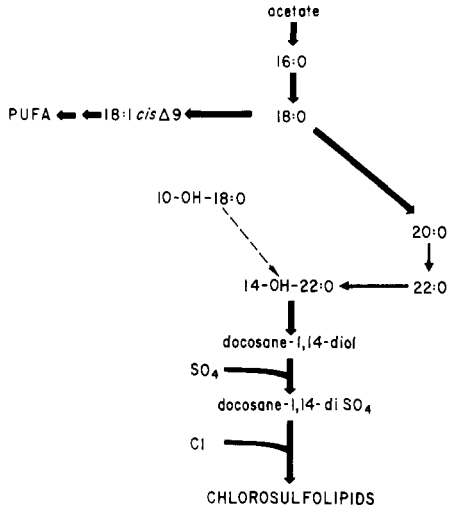

FIGURE 1: Pathway for biosynthesis of sulfolipid diols in *O. danica*.

could result from less than 1% residual contamination with stearic acid in the starting material; however, no such contamination was detected after the purification described in Methods, and a more likely explanation is a small amount of label recirculation *via* acetate. The crude chlorosulfolipid fraction obtained by solvent partitioning contained about 0.1% of the added labeled oleic acid, that is, ten times more than the activity accounted for by the chromatographically purified diols obtained from that fraction. However, when the crude material was treated with 0.1 M sodium hydroxide in methanol at room temperature, this excess radioactivity was completely released as free oleic acid, while sulfolipids were completely unaffected by this procedure. A similar small amount of radioactivity was also released by alkaline treatment of the chlorosulfolipids in the stearic acid experiment, but in that instance it represented only a small fraction of the label recovered in the purified diols. In both cases the contaminating radioactivity was present in a form other than the free acid, since it could not be removed by repeating the solvent partitioning procedure without prior alkaline treatment; the nature of this polar lipid was not further investigated.

Our previous ^{18}O labeling studies (Elovson, 1974) excluded hydration of an unsaturated intermediate as a mechanism for introduction of the secondary hydroxyl in the chlorosulfolipids, but several questions remained concerning the actual pathway for the assembly of the docosane-1,14-diol backbone. An ω-9 unsaturated intermediate such as oleic acid could be incorporated *via* some reaction other than simple hydration. Thus, a mixed function oxygenase epoxidation of such an intermediate (Hayano, 1962) followed by reduction could afford the unchlorinated diol, while nucleophilic attack by chloride on the epoxide could yield the ω-9,10-chlorohydrin structure which is found in the major chlorosulfolipids. In fact, Mooney *et al.* (1972) recently claimed that oleic acid was incorporated into sulfolipid chlorodiols, although these authors concluded on that evidence alone that the secondary hydroxyl was introduced by hydration of the unsaturated bond.

Our present results, however, are incompatible with mechanisms involving such intermediates: we find no significant conversion of oleic acid to any diol species, but do find direct conversion of the saturated C_{22} fatty acid to both the chlorinated and unchlorinated diols. Furthermore, our data, as well as those of Mooney and Haines (1973), clearly demonstrate that chlorination occurs subsequently to, and independently of, the introduction of the secondary hydroxyl group, which excludes concerted formation of the chlorohydrin structure. The reasons for the discrepancy between our results on oleic acid incorporation and those of Mooney *et al.* (1972) are not clear. These authors reported an incorporation of radioactive palmitic and stearic acid into the total (chloro)diol fraction after 3–5 days incubation which appears roughly comparable to those reported here for a 4-hr pulse, but the amount of radioactive oleic acid actually incorporated into the chlorodiols was not stated: only the amount recovered after degradation of the monochlorodiol, less than 0.001% of the radioactivity added to the growth medium, is shown, and without further information the significance of this cannot be evaluated.

The pathway for the biosynthesis of the diol backbone of the C_{22} chlorosulfolipids in *O. danica* may now be summarized as in Figure 1. The fatty acid synthetic machinery supplies the growing hydrocarbon chain, and at the C_{18} chain length level, where several desaturation steps intervene on the pathway to the usual polyunsaturated fatty acids, elongation of the saturated chain continues up to docosanoic acid. Direct hydroxylation of this normal fatty acid yields the 14-hydroxydocosanoic acid intermediate, which is reduced to the docosane-1,14-diol. Oleic acid is not on the pathway to the diols, and 10-hydroxystearic acid is not a normal intermediate. Docosane-1,14-diol is then sulfated and chlorinated to the final chlorosulfolipid products as reported very recently by Mooney and Haines (1973). The kinetics of the latter process is dealt with in a separate report (J. Elovson, manuscript in preparation). *O. danica* also elaborates a series of chlorosulfolipids derived from tetracosane-1,15-diol (Elovson and Vagelos, 1969). Since under the growth conditions used in this study the concentration of these was quite low, their labeling pattern was not investigated in these experiments; the expectation would be that these diols are formed by hydroxylation of a saturated tetracosanoic acid.

The simplest mechanism for introduction of the secondary hydroxyl group which is consistent with our previous ^{18}O data and the results presented here is direct mixed function oxygenase hydroxylation of docosanoic acid, analogous to the formation of ricinoleic acid by hydroxylation of oleic acid in castor beans (Galliard and Stumpf, 1966; Morris, 1967), the hydroxylation of fatty acids by Torulopsis, described by Heinz *et al.* (1969, 1970), and the formation of hydroxy fatty acids in plant cutins (Kolattukudy and Waldon, 1972). Evidence for such an enzymatic activity is now being sought in cell-free preparations of *O. danica*.

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Discoidin, a Developmentally Regulated Carbohydrate-Binding Protein from *Dictyostelium discoideum*. Purification and Characterization†

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ABSTRACT: A carbohydrate-binding protein from the cellular slime mold, *Dictyostelium discoideum*, previously shown to be synthesized as these cells become cohesive, was purified by affinity chromatography. The protein was assayed by agglutination of formalinized erythrocytes, which is selectively blocked by sugars with a galactose configuration. The protein bound quantitatively to a column of Sepharose 4B and was quantitatively eluted with D-galactose. A minor contaminant was removed either by isoelectric focusing or by adsorption of the active protein to formalinized erythrocytes and elution with D-galactose. Purified agglutinin had a molecular weight of 100,000 ± 2000 determined by sedimentation equilibrium in the presence of galactose. In the absence of galactose the protein tended to aggregate. Subunit molecular weight measured under dissociating conditions by sedimentation equilibrium was 28,000 ± 2000. Determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, the subunit molecular weight was 26,000 ± 1000. The molecular weight data suggest that the native molecule is a tetramer. A single symmetrical peak of agglutination activity with an $s_{20,w}$ of 5.1 S was determined by sucrose density gradient centrifugation. The protein is rich in aspartic and glutamic acids and 3-hydroxyamino acids. Its isoelectric point was 6.10 determined in the presence of galactose. The protein contained no hexosamine and no detectable neutral sugar. The role of this developmentally regulated agglutinin in the differentiation of *Dictyostelium discoideum* is considered.

The life cycle of the cellular slime mold, *Dictyostelium discoideum*, is divided into two distinct phases (Bonner, 1967; Gerisch, 1968): the first, a single-cell vegetative state in which cells grow and multiply; and a second social stage characterized by cellular aggregation, intercellular adhesion, and further differentiation culminating in fruiting body formation and sporulation. Recently, we have reported on the presence of a developmentally regulated carbohydrate-binding protein from *Dictyostelium discoideum* (Rosen *et al.*, 1973), whose synthesis closely parallels the development of cell cohesiveness. This "lectin-like" protein agglutinates formalinized sheep erythrocytes and agglutination is specifically inhibited by sugars with a galactose configuration. A striking 400-fold increase (Rosen, 1972) in specific agglutination activity is observed in a 12-hr period after cells are deprived of food, a signal which initiates cellular aggregation. Preliminary evidence was presented indicating that this factor is present at the cell surface of cohesive slime mold cells. These observations, although circumstantial, suggest that this agglutinin may mediate intercellular adhesion in this organism (Rosen *et al.*, 1973). In this paper, we describe the purification and characterization of "discoidin,"[1] a protein from *Dictyostelium discoideum*.

Experimental Procedures

Materials. Chemicals used throughout this investigation were the best available commercially.

Cell Culture. Dictyostelium discoideum, strain A3 cells, a mutant derived from the NC-4 strain, were grown in axenic culture (Loomis, 1971) to cell densitites of 7×10^6 cells/ml. Growth-phase cells of this mutant line contain high levels of agglutination factor (Rosen *et al.*, 1973). The cell density is critical since densities substantially higher or lower than those used here have much less extractable factor.

Preparation of Extract. Slime mold cells were harvested by certrifugation at 1500 rpm in a Sorvall RC-2 refrigerated centrifuge and washed three times with cold water. The cells were resuspended at room temperature at a concentration of 10^6–10^7 cells/ml in a 0.015 M Tris–0.075 M NaCl–0.075 M KI buffer containing 1 mM EDTA (pH 7.3); this buffer (Takeuchi and Yabuno, 1970) will be subsequently referred to as ECT[2] buffer. Cells were either homogenized in a Potter-Elvejhem

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[1] The authors propose the name "discoidin" for this developmentally regulated carbohydrate-binding protein from *Dictyostelium discoideum*.

[2] Abbreviations used are: ECT, EDTA-calcium-free salt-Tris buffer; ECT-galatose, ECT buffer containing 0.3 M D-galactose; PBS, phosphate-buffered saline.