GENETICAL AND BIOCHEMICAL STUDIES OF PTERIDINE GRANULE MEMBRANE IN LARVAL HYPODERMAL CELLS OF THE SILKWORM.

1. PURIFICATION AND CHARACTERIZATION OF THE MEMBRANE PROTEIN FROM PTERIDINE GRANULES OF A NORMAL STRAIN

SUSUMU SAKURAI¹⁾ AND MITSUO TSUJITA

National Institute of Genetics, Misima 411

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There are at present three models for the ultrastructure of biological membrane systems. One is the Davson-Danielli model which is a bimolecular leaflet of lipid covered on both sides by protein (Davson and Danielli 1952). This concept of membrane structure was developed further by Robertson who, in his "unit membrane hypothesis", proposed that all biological membranes have basically the same construction, with the lipid in a bimolecular leaflet (Robertson 1964). Other two models, which are respectively called the "repeating units model" and "the fluid mosaic model", have been postulated on the basis of observations with the electron microscope (Sjöstrand 1963; Stoeckenius 1962; Benedetti and Emmelot 1965; Green and Perdue 1966), freeze-etching experiments (Singer and Nicolson, 1972) and physicochemical properties (Terry *et al.*, 1967; Salton and Netschey 1965; Engelman *et al.* 1967).

In our previous reports (Tsujiata and Sakurai 1966), pteridine granules have been shown to be abundantly formed immediately after each moulting stage. A number of small vesicles which are surrounded by ribosome particles, 150-200 Å in diameter, appear and then gradually increase in size, and change their shapes, developing into the mature pteridine granules. During the growth of these granules, pteridine polypeptides and uric acid peptide complexes are secreted from the vesicular membrane and stored inside the vesicles. Owing to the appearance of a large amount of these pteridine granules the skin of the larvae of normal silkworm become opaque. However, in the hypodermal cells of the oily mutant larvae, a majority of their pteridine granules are very irregular in size and shape and the number of the granules with normal size and shape is very small so that the larval skin appears transparent due to this defective granule formation. Thus, the maturation of the granule membrane seems to play some important roles in the secretion and storage of their products such as pteridine polypeptides and uric acid peptide complexes. In order to clarify the relationship between the development of granule membrane and the formation of pteridine granules, we have carried out genetic and biochemical studies on the pteridine granule

1) Present address: Department of Microbiology, The Jikei University, School of Medicine, Minato-ku, Tokyo 105.

membrane in larval hypodermal cells of the silkworm. In this paper we describe the experiments on the membrane protein from pteridine granules in the larval hypodermis of the normal silkworm strain; I) A procedure for isolating the membrane proteins from pteridine granules in the larval skin of the normal strain and from abnormal pteridine granules of mutant strains with transparent larval skin. II) Composition of the granule membrane and amino acid composition of the membrane protein. III) Solubility of the membrane protein, the presence of membrane protein subunits and the electrophoretical and ultracentrifugal homogeneity of the solubilized membrane subunits. IV) Molecular weight of the membrane protein.

MATERIALS AND METHODS

The silkworm strains W-C, C-124 E-lem with non-transparent larval skin were used as normal strains.

Procedure for isolating pteridine granules. Pteridine granules were isolated from the hypodermal cells of normal larvae and mutant larvae on the 4th day of the 5th instar. The cuticular layer-free cells of the hypodermis containing the granules were homogenized with a Teflon-glass homogenizer in 8.56% sucrose containing 1 mM NaHCO_3 and 5 mM EDTA, pH 7.5 (0–4°C) (NaHCO_3 -EDTA-sucrose). After homogenization in NaHCO_3 -EDTA-sucrose solution, pH 7.5, unbroken cells and floating cell components of hypodermis were removed by filtration with nylon cloth. The resultant filtrate was layered over discontinuous density gradients consisting of 20, 25, 30, 35, 40, 45, 50 and 80% (w/v) sucrose, and centrifuged on the Tominaga No. 7 SW rotor at 3,000 r.p.m. for 2 hr at 2–4°C. Discrete bands of material were not obtained. There was a region of maximum turbidity at the 50%/80% interface. The 30%/35% interface was also turbid and there was slight but noticeable turbidity in the 8.56%/20% interface. The pteridine granules were collected from a compact zone at the 50%/80% interface and the same procedure repeated twice. A small and hazy zone at the 30%/35% interface consisted of mitochondria, as shown by phase contrast microscopy and by measurement of respiratory enzymes.

The size and shape of pteridine granules produced in hypodermal cells of oily larval mutants are abnormal and the amount of mature granules is reduced. Furthermore, these granules always have a lower density than those of normal larvae (Tsujiita and Sakurai 1967). Consequently, the method described for normal pteridine granules could not be used to isolate the abnormal pteridine granules of mutant larvae. The abnormal pteridine granules of w^{oh} and w^{ol} mutants were collected at the 40%/45% interface, and those of $w-3$, w^{b} , oc and oa mutants, at the 45%/50% interface. In the case of w^{a} mutant, the 8.56% sucrose layer was corrected. The nuclei were sedimented at the 80% sucrose layer.

The granule bands were collected with a pipette, diluted with distilled water to give 5% sucrose and centrifuged at 30,000 r.p.m. for 40 min at 2–4°C in a Spinco No. 30 rotor. After washing twice with 0.01 M sodium phosphate buffer, pH 7.0, containing 5 mM EDTA, 0.1 M NaCl and 5 mM 2-mercaptoethanol, the precipitate was suspended

in distilled water. The suspension was dialysed against the same buffer overnight at 4–10°C. The suspension was again centrifuged at 30,000 r.p.m. for 40 min in a Spinco No.30 rotor, and the pellet was collected, washed with distilled water and stored at 0° or –20°C.

Preparation of membrane proteins. The pellet was suspended in a solution containing 1% sodium dodecyl sulfate, 0.025 M Tris, 0.3 M NaCl, 1 mM EDTA and 1 mM 2-mercaptoethanol (pH 8.5) (SDS-Tris-NaCl-EDTA-2-mercaptoethanol). The suspension was incubated at 37°C for 2 hr to solubilize the membranes. Any insoluble material was removed by centrifugation at 40,000 r.p.m. for 60 min in a Spinco No. 40 rotor. The sample was dialysed overnight at room temperature against 0.025 M Tris buffer containing 0.1% sodium dodecyl sulfate, 0.1 M NaCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, pH 8.5 (Tris-SDS-NaCl-EDTA-2-mercaptoethanol, pH 8.5), and the dialysis solution was chromatographed on a 2.4×50 cm column of Sephadex G-75 (Pharmacia, Uppsala-Sweden) equilibrated with Tris-SDS-NaCl-EDTA-2-mercaptoethanol (pH 8.5). The protein eluted at the void volume (Fig. 1) was then applied to a 4.0×55 cm column of Sephadex G-200 (Pharmacia, Uppsala-Sweden) equilibrated with the same buffer at room temperature. The effluent protein peak (Fig. 2) was dialysed overnight against 0.025 M Tris, 0.1 M NaCl, 1 mM 2-mercaptoethanol (pH 8.5). The contents of the bag were precipitated by lowering the pH to 5.5–5.7 through the addition of 1% acetic acid, and after standing overnight at 4°C the precipitate was collected by centrifugation at 30,000 r.p.m. in a spinco No. 30 rotor. The sediment was again dissolved in a solution containing 1% sodium dodecyl sulfate, 0.025 M Tris, 0.3 M NaCl, 1 mM EDTA and 1 mM 2-mercaptoethanol (pH 8.5), dialysed and precipitated by lowering the pH as described above. The final sediment was freeze-dried and stored at –20°C.

Chemical analysis. Chemical analysis were carried out using the lyophilized mem-

Fig. 1. Gel filtration chromatography of pteridine granule membrane protein on Sephadex G-57 in 0.025 M Tris buffer containing 0.1% sodium dodecyl sulfate, 0.1 M NaCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, pH 8.5.

Fig. 2. Gel filtration chromatography of pteridine granule membrane protein on Sephadex G-200 in 0.025 M Tris buffer containing 0.1% sodium dodecyl sulfate, 0.1 M NaCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, pH 8.5.

brane proteins. Samples were mixed vigorously with a mixture of chloroform-methanol (2:1) at room temperature. After 3 hr, the precipitate was collected by centrifugation for 30 min at 10,000 r.p.m. in the Tominaga No. 5 angle rotor at 4°C. The precipitate was resuspended in chloroform-methanol as above, and the extraction repeated two more times. The residue was collected by centrifugation, dried in vacuo and weighed. The hydrolysate with 6N HCl at 110°C of this residue was analysed on a Hitachi KLA-3B amino acid analyzer. The protein content was calculated from the total amino acid content. The chloroform-methanol solution obtained in the above procedure was evaporated under reduced pressure and dried in vacuo. This fraction was weighed and the lipid content of the preparation was calculated. Total carbohydrate was determined according to Dubois *et al.* (1956) with glucose as standard. RNA was determined by the method of Drury (1948).

Amino acid analysis. Aliquots of the lipid-free membrane protein were suspended in 6N HCl, evacuated and sealed, and hydrolysed for 22 hr at 110°C. The hydrolysate was dried under vacuum and analysed on a Hitachi KLA-3B amino acid analyzer.

SDS-urea-polyacrylamide gel electrophoresis. The method of Davis (1964) was used to prepare 7.5% polyacrylamide gels, but without spacer gel. The gels contained 5 M urea, 1 mM 2-mercaptoethanol, 1 mM EDTA and 0.1% sodium dodecyl sulfate. The buffer was Tris-glycine (pH 8.8) (Davis 1964). The electrolyte also contained 1 mM EDTA, 1 mM 2-mercaptoethanol and 0.1% sodium dodecyl sulfate. Protein samples of 150–300 µg to be electrophoresed in sodium dodecyl sulfate gels were first incubated in 1% sodium dodecyl sulfate in 0.05 M Tris–0.38 M glycine (pH 8.8) containing 8 M urea, 10 mM EDTA and 10 mM 2-mercaptoethanol for 1 hr at 37°C, and then applied directly to the top of the gel. Electrophoresis was carried out at 5 ma/gel at 5–10°C. The gels were stained with 1% Amido-Black 10B in 7.5% acetic acid for 60 min and destained by

electrophoresis or washing in 7% acetic acid. Bromophenol blue was used as tracking dye.

Column chromatography. Freeze-dried membrane protein was dissolved in 1% sodium dodecyl sulfate in 0.05 M Tris-0.38 M glycine buffer (pH 8.8) containing 8 M urea, 10 mM EDTA and 10 mM 2-mercaptoethanol, and incubated at 37°C for 1 hr. The solution was dialysed against 1% sodium dodecyl sulfate, 2 M urea, 0.025 M Tris, 0.1 M NaCl, 1 mM EDTA and 5 mM 2-mercaptoethanol (pH 8.5). The dialysis solution was chromatographed at room temperature on a 1.9×100 cm column of Sepharose 6B (Pharmacia, Uppsala-Sweden) equilibrated with 1% SDS, 2 M urea, 0.025 M Tris, 0.1 M NaCl, 1 mM EDTA and 5 mM 2-mercaptoethanol (pH 8.5) (Fig. 4).

Pure molecular weight markers including human immunoglobulin G (IgG) (mol wt 160,000), bovine serum albumin (mol wt 67,000), beef pancreas chymotrypsinogen A (mol wt 25,000), and horse cytochrome C (mol wt 12,400) were chromatographed using the same columns equilibrated with 1% SDS, 0.025 M Tris, 0.1 M NaCl, 1 mM EDTA, 2 M urea and 5 mM 2-mercaptoethanol (pH 8.5), and eluted with the same buffer.

Ultracentrifugation. Analytical ultracentrifugation was performed on a Spinco model E ultracentrifuge, equipped with electric speed control and on RITC temperature control unit in a synthetic boundary cell, at 59,780 r.p.m. at 20°C. Freeze-dried membrane protein was dissolved in 1% sodium dodecyl sulfate in 0.05 M Tris-glycine buffer (pH 8.8) containing 8 M urea, 10 mM EDTA and 10 mM 2-mercaptoethanol, and incubated at 37°C for one hr. The solution was dialysed against 1% sodium dodecyl sulfate, 0.1 M NaCl, 5 mM 2-mercaptoethanol and 10 mM EDTA at pH 10.0 at room temperature. The protein concentration used in this analytical ultracentrifugation was 3.8-4.0 mg/ml.

RESULTS

1) *Composition of the membrane protein*

As shown in Table 1, the membrane protein isolated from pteridine granules was composed almost entirely of protein and lipid which made up about 90-99% of its dry weight. The protein content was 65-68% and the lipid content by weight of the chloroform-methanol-soluble fraction was 31-33% of the dry weight. The carbohydrate content seemed to be low, accounting for 1.1-1.4% of the dry weight, and traces of nucleic acids were also present. The protein, lipid and carbohydrate contents of the

Table 1. Chemical composition of membrane protein obtained from pteridine granules of normal sliworm strains

Components Strain	Protein	Lipid	Carbohydrate	RNA
	(Percentage of total dry weight of membrane protein)			
C-124	66.2	30.8	1.21	trace
W-C	65.3	31.9	1.35	—
E-lem	67.7	32.7	1.11	trace

Table 2. Amino acid composition of membrane protein from normal granules

Amino acid	Strain	W-C	E-lem
		(μ mole)	
Asp		0.254	0.273
Thr		0.115	0.122
Ser		0.106	0.114
Pro		0.154	0.220
Glu		0.390	0.426
Gly		0.178	0.186
Ala		0.218	0.234
Val		0.110	0.122
Cys		0.016	0.020
Met		0.039	0.043
ILeu		0.125	0.134
Leu		0.216	0.240
Tyr		0.069	0.082
Phe		0.080	0.089
NH ₃		0.325	0.329
Lys		0.189	0.202
His		0.044	0.044
Arg		0.139	0.150

Table 3. Types of amino acids composing the membrane protein from normal granules

Strain	Amino acid	Acidic amino acids Glu, Asp	Basic amino acids His, Lys, Arg	Non-polar amino acids Pro, Gly, Ala, Val, Met, ILeu, Leu, Tyr, Phe	Cystine
		(μ mole)			
W-C		0.644	0.372	1.189	0.016
E-lem		0.699	0.396	1.350	0.020

membrane protein obtained from pteridine granules of the three normal strains are very similar (Table 1).

Analysis of the amino acid composition shows that all the membrane protein isolated from the two normal strains includes relatively large amounts of hydrophobic amino acids, such as alanine, leucine, phenylalanine and valine. The cystine content was the lowest and the content of histidine, methionine and tyrosine was low in comparison with that of the other amino acids (Table 2). As shown in Table 3, the content of acidic amino acids, aspartic and glutamic acid, was high in comparison with that of basic amino acids and the membrane protein of pteridine granules is hydrophobic in nature. From these results it seems clear that the amino acid composition of the granule membrane protein is surprisingly similar to that of other structural proteins of isolable membrane systems as postulated by Benson (1968): e.g., red cell, mito-

Table 4. Solubility of granule membrane protein

Solvent	Per cent protein solubilized
0.8% sodium dodecyl sulfate (SDS)	80-85
95% formic acid	70-75
80% 2-chloroethanol (pH 2)	63-70
1% sodium deoxycholate (DOC)	75-78
8 M urea in 0.05% SDS	65-70
0.05 M phosphate buffer containing 0.15 M NaCl (pH 7)	5- 8
0.05 M Tris-glycine buffer (pH 8.8) containing 1% SDS, 10mM EDTA, 10 mM 2-mercaptoethanol and 8 M urea	95-97

chondrial and chloroplast membranes, on algal (*Dunaliella*) membrane and halobacterium envelope.

2) Solubility of the membrane protein

The solubility of the membrane protein in different solvents was tested. 5 mg of freeze-dried delipidated membrane protein was solubilized in 3 ml of the solvent to be tested by mixing and stirring vigorously at room temperature.

Fig. 3. Effect of pH on solubility of the membrane protein. Solubility is expressed as the percentage of protein (at 280 nm absorption) remaining in solution after reduction of the pH from 8.5.*

Symbols: ● : membrane protein solubilized by sodium deoxycholate.

: ---: membrane protein solubilized by sodium dodecyl sulfate.

* Granule membrane protein was dissolved in 0.025 M Tris buffer containing 0.8% sodium dodecyl sulfate or 1% sodium deoxycholate, 0.1 M NaCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, pH 8.5.

Protein that did not sediment at $104,000\times g$ for 45 min was considered soluble. The relative solubility of the protein in different buffer systems is shown in Table 4. The membrane protein was scarcely dissolved in a neutral salt solution. In 1% sodium deoxycholate solution and 8M urea in 0.05% sodium dodecyl sulfate solution, 65-78% of the membrane protein was dissolved. 63-75% of the membrane protein was soluble in 80% 2-chloroethanol (pH2) or 95% formic acid. The solubility of the granule membrane protein is markedly dependent on pH. As shown in Fig. 3, the protein is precipitated between pH4.0 and 5.7, indicating a low isoelectric point typical of membrane proteins.

3) *Presence of membrane subunits*

Freeze-dried membrane protein obtained from a normal strain was dissolved in 1% sodium dodecyl sulfate in 0.05M glycine (pH8.8) containing 8M urea, 10mM EDTA and 10mM 2-mercaptoethanol, and incubated at 37°C for one hr. The solution was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described above. A clear-cut single band was observed on electrophoresis (Fig. 5).

When freeze-dried membrane protein was dissolved in 1% sodium dodecylsulfate, 0.1M NaCl, 5mM 2-mercaptoethanol, 8M urea and 10mM EDTA at pH10.0, the protein concentration was found to be 3.8-4.0mg/ml. Analysis by ultracentrifugation at 59,780 r.p.m. in a synthetic boundary cell gave the Shlieren pattern shown in Fig. 6; the membrane protein appears to be monodisperse. The experimental results described above indicate that the membrane protein of normal pteridine granules dissociates into electrophoretically and centrifugally homogeneous subunits.

4) *Molecular weight approximation of the membrane protein isolated from normal pteridine granules*

The result of gel filtration chromatography of the membrane protein is shown in

Fig. 4. Gel filtration chromatography of the granule membrane protein obtained from E-lem normal strain on Sepharose 6 B in 1% sodium dodecyl sulfate, 2 M urea, 0.025 M Tris, 0.1 M NaCl, 1 mM EDTA, and 5 mM 2-mercaptoethanol (pH 8.5). The membrane protein was eluted with the same buffer (pH 8.5). Run was performed at a flow rate of 20 ml/hour and fractions of 3 ml collected.

(A) (B)

Fig. 5. Polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate. Protein samples of 150-300 μ g were applied to 7.5% acrylamide -0.1% sodium dodecyl sulfate gels.

(A): C-124; (B): W-C

Fig. 6. Sedimentation velocity ultracentrifugation of granule membrane protein obtained from C-124 strain. Direction of sedimentation from left to right. Photograph taken after 96 minutes at 52680 rev./minute.

Fig. 4. One major protein peak and two minor protein peaks were observed. The major protein peak was separated in the molecular weight range between 25,000 (beef pancreas chymotrypsinogen A) and 12,400 (horse cytochrome C). The dispersion seen on gel filtration is presumably due to polymerization of a single monomeric unit.

The molecular weight of the membrane protein were estimated to be about 20,000 when calibrated with these standard markers.

DISCUSSION

The present work was designed to characterize the physicochemical properties of the membrane protein of pteridine granules obtained from the larval hypodermis of a normal silkworm strain. Electron micrographs of sectioned granules isolated from hypodermal cells of normal larvae showed that they have a single membrane of 60-80 Å in thickness (Tsujita and Sakurai 1966). According to our electron microscopical studies of hypodermal cells of normal larvae, pteridine granules at first appear as small vesicles. They increase in size as secretion products consisting of pteridine polypeptide and uric acid polypeptide complexes are secreted and stored, finally becoming large mature granules. Two types of granule formation are distinguished. In one of them, ribosomes, 150-200 Å in diameter arrange themselves uniformly in a layer on the outer surface of the growing vesicles (sometimes the vesicles are surrounded

by polysomes) and secretion products are secreted through the vesicular membrane into the vesicles and stored. In the other type, the vesicles are connected with coarse tubular endoplasmic reticulum from which secretion products are transported into the vesicles; these grow into large mature granules as the secretion products are gradually stored. Because the mature pteridine granules resisted sonication or homogenization by a Teflon homogenizer, it was difficult to isolate the granule membrane fraction. When isolated pteridine granules were dissolved in 1% sodium dodecyl sulfate in 0.025 M Tris, 0.3 M NaCl, 1 mM EDTA and 1 mM 2-mercaptoethanol (pH 8.5), the secretion products, pteridine polypeptide and uric acid peptide complexes (mol wt 8,000), were removed by dialysis in visking dialysis tubing. Phenol oxidase has been shown by us to be localized in small vesicles sedimented at $78,000\times g$ and $104,000\times g$ (Tsujita and Sakurai 1971). However, the enzyme activity could not be detected in the mature granules (unpublished data).

Table 3 compares the amino acid composition of the membrane structural protein fractions of pteridine granules obtained from two normal strains, W-C and E-lem. Essentially the amino acid composition of the granule membrane protein was high in acidic and hydrophobic amino acid contents. Thus we confirmed that pteridine granule membrane protein is very similar to that of structural membrane proteins from other species (Benson 1968). A relatively high content of non-polar amino acids in the membrane protein of pteridine granules could give rise to extensive hydrophobic regions which might account for the stability of granule membrane protein. As shown in Table 4, granule membrane protein revealed a low solubility in neutral buffer solution, indicating a hydrophobic nature typical of high non-polar amino acid content.

Benson (1968) suggests that in the absence of disulfide groups and in view of the high content of glutamic acid and aspartic acid in membrane protein, carboxylic acid hydrogen bonding may take place between adjacent helical regions of the membrane structural protein in the absence of detergent. The granule membrane protein has a tendency to aggregate unless SDS or DOC is present and the aggregate eluted on Sepharose 6 B ranges between 480,000 and 160,000 in molecular weight.

It has been found that the lipid component also varies widely from membrane to membrane, constituting up to 80% of myelin, but only 15% of skeletal muscle membrane, and that not only the total lipid component but also the chemical nature of the lipids varies considerably (Cereijido and Rotunno 1970; McMurray 1973). However, studies reported so far on bacterial cell membrane (Salton 1967), human platelet membrane (Nachman and Ferris 1970) and the membrane of *Mycoplasma laidlawii* (Razin *et al.* 1963) and *Dictyostelium discoideum* (Yanagida and Noda 1969) have revealed that the lipid content is between about 20 and 30%, the protein content is between about 50 and 70% and the carbohydrate content is between about 1 and 5%.

Our results showed that the lipid content in the membrane protein fraction soluble in chloroform-methanol was 31-33% of its dry weight and also the carbohydrate content was about 1.1-1.4% of the dry weight. Maddy (1966) reported that 90% of the membrane protein of the ox-erythrocyte ghost passes straight through a CM-cellulose column at pH 6.0, while a similar proportion is absorbed onto a DEAE-cellulose column and cannot be eluted; the protein is precipitated between pH 3.7 and 4.8, indicating a

low isoelectric point typical of sialoproteins. When the granule membrane protein is chromatographed on a DEAE-cellulose column, the membrane protein is strongly absorbed on the DEAE-cellulose and cannot be eluted by gradient dilution from 0 to 0.2 M NaCl. As shown in Fig. 3, the membrane protein of pteridine granules is precipitated between pH 4.0 and 5.7, indicating a low isoelectric point typical of a high acidic amino acid content. We could not detect sialic acid residues in the membrane proteins of pteridine granules.

Zwaal and Van Deenen (1968) have recently reported that n-butanol solubilized erythrocyte membrane proteins of human, rat, rabbit, pig, ox and sheep do not consist of homogeneous protein subunits, and demonstrated that the protein composition of the erythrocyte membranes from other species is markedly different. Similar observations were noted by Marchesi *et al.* (1970) working with human erythrocyte membranes. Studies reported so far on human platelet membrane proteins (Nachman and Ferris 1970), rat liver plasma membrane (Bont *et al.* 1969), bean and wheat chloroplast membrane structural protein (Mani and Zalik 1970), mitochondrial membrane structural protein (Halder 1966; Tuppy, 1968; Schatz and Salzgeber 1969) and microsomal structural protein (Ward and Pollak 1969; Schnaitman 1969) have revealed marked heterogeneity in the nature of these proteins.

On the other hand, different results have been observed with sodium dodecyl sulfate-solubilized Mycoplasma membranes. Razin *et al.* (1965) reported that Mycoplasma laidlawii membrane dissolved by sodium dodecyl sulfate consisted of ultracentrifugally homogeneous subunits having a sedimentation constant of 3.3 Svedberg units, and that these subunits were composed of lipid and protein and also could be reaggregated to form membrane-like structures in the presence of di- or multivalent cations.

Yanagida and Noda (1969) have recently reported that the cell membrane of the Dictyostelium discoideum myxamoeba contains a single homogeneous structural lipoprotein unit, the molecular weight of which is about 160,000 with a sedimentation coefficient of 7S. In agreement with this result we have found that the membrane protein of normal pteridine granules solubilized by SDS and urea contained a single homogeneous structural protein with a molecular weight of about 20,000.

This result indicates that the membrane protein of normal pteridine granules dissociates into electrophoretically homogeneous protein subunits on being subjected SDS-urea polyacrylamide gel electrophoresis.

Rosenberg and Guidotti (1968, 1969) reported that erythrocyte membrane protein has a molecular weight of about 50,000. On the other hand, Bakerman and Wasemiller (1967) have reported a lower value, closer to 20,000 and also Criddle *et al.* (1962) have reported that mitochondrial structural protein has a molecular weight of 22,500. Thus, as pointed by Nachman and Ferris (1970), it should be emphasized that the membrane proteins of several biological systems are of relatively low molecular weight.

Recently, Laico *et al.* (1970) showed that a group of peptides having a molecular weight of about 5,000 forms a major component of biological membranes, and that these "miniproteins" are found in membranes of human and bovine red blood cells, purified bovine liver mitochondria, and the rhodopsin-containing membranes of the outer segments of bovine retinal rods.

SUMMARY

The membrane protein of pteridine granules produced in the larval hypodermis of a strain of normal silkworm larvae was isolated and its chemical composition analysed. This protein is composed almost entirely of protein and lipid. The protein content makes up 65-68% of the dry weight, the lipid content, 31-33% and the carbohydrate content, 1.1-1.4%. The amino acid composition of the membrane protein of normal granules is shown. Essentially this amino acid composition is very similar to that of structural membrane proteins from other species.

Using several different solvents, the solubility of the membrane protein was tested. The membrane protein is readily soluble in sodium dodecyl sulfate urea solution. It was found that the membrane protein dissociates into electrophoretically and ultra-centrifugally homogeneous subunits and thus it is inferred that the membrane protein of normal pteridine granules consists of a single homogeneous structural protein having a molecular weight of about 20,000.

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