BBA 76061

# SELECTIVE SOLUBILIZATION WITH TWEEN 20 OF MEMBRANE PROTEINS FROM *ACHOLEPLASMA LAIDLAWII*

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(Received May 8th, 1972)

SUMMARY

1. Approx. 70 % of the plasma membrane from *Acholeplasma laidlawii* can be extracted with 2.5 % Tween 20. The residue contained a higher proportion of hydrophobic amino acids than the extract. Glucosamine and galactosamine were found only in the extract. The extraction with Tween 20 is unusually selective: polyacrylamide-gel electrophoresis in sodium dodecyl sulfate of the extract and of the residue showed that the extract contained several membrane proteins that were absent from the residue and *vice versa*.

2. The proteins in the Tween extract were fractionated by analytical polyacrylamide-gel electrophoresis in the presence of Tween 20 into three major and two minor zones (see Fig. 6).

3. *A. laidlawii* membranes, completely solubilized in sodium dodecyl sulfate, were submitted to polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate. Five major and seven minor zones were obtained. This technique also permitted a rough estimate of the molecular weights of the proteins in these twelve zones; values from 15000 to 140000 were obtained.

4. The five protein zones obtained by electrophoresis in Tween 20 (see point 2 above) were further fractionated in sodium dodecyl sulfate by a two-dimensional electrophoresis procedure (see Fig. 7).

5. The yellow color of the *A. laidlawii* membrane is due to the presence of both flavoprotein(s) and carotenoid material, the latter pigment being either in free form or in association with lipids.

6. An approach to the determination of the geometric localization of components in a membrane is discussed (although not utilized in the present studies).

7. The risk of misinterpretations in membrane studies due to the possible appearance of artificial complexes is discussed.

## INTRODUCTION

This paper is the first in a series of articles from our Institute on the structure of cell membranes, a research field which we have recently entered.

For these studies we have chosen membranes from human erythrocytes and

Abbreviation: TEMED, *N,N,N',N'*-tetramethylethylenediamine.

from *Acholeplasma laidlawii*[1-3], chiefly because these two cells lack both cell wall and internal membrane systems, which facilitates enormously the preparation of "pure membranes"*. In addition, they can easily be lysed by osmotic shock[4], which is a very gentle method for the disruption of cells. As compared to human erythrocytes, *A. laidlawii* has the advantage of permitting greater flexibility in the design of experiments, since radioactive compounds can easily be incorporated[5] and appropriate mutants and viruses[6] can be utilized.

This paper deals with the use of Tween 20 as a detergent for partial, selective solubilization of membrane proteins from *A. laidlawii*. Selective solubilization greatly facilitates the purification of the membrane components, and in addition might also provide some information about their spatial distribution in the membrane (see Discussion).

## MATERIALS AND METHODS

### *Culturing of A. laidlawii and preparation of the plasma membranes*

For inoculation, a sample of *A. laidlawii* (Strain B) was kindly supplied by Prof. S. Razin (Department of Clinical Microbiology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel). Both the culturing of the cells and the preparation of the plasma membranes were performed essentially as described by Razin *et al.*[7]. The small pellet of non-lysable cells[8] was discarded after centrifugation at 9000 × *g*.

### *Membrane solubilization*

*Solubilization with the non-ionic detergent Tween 20.* One vol. of a 0.10 M Tris–HCl buffer (pH 8.0), containing 5.0% Tween 20 (quality SD, from Atlas Chemie GmbH, Essen, Germany) was added to 1 vol. of the membrane suspension. After 2 h at room temperature the still turbid mixture, which contained 30 mg membrane material per ml, was submitted to centrifugation (130000 × *g*, 2 h, rotor SW-50). The yellow supernatant (Tween supernatant) was collected and its absorbance at 275 nm determined. The pellet was suspended in 5 ml of a 0.05 M Tris–HCl buffer (pH 8.0), containing 2.5% Tween 20. After centrifugation the absorbance in the supernatant was measured. Six such extractions with Tween 20 were performed. The result is shown in Fig. 1, where the protein contents in mg was obtained by using the conversion factor 1.3 (see *Relation between dry weight and absorbance*). The phosphate contents of the supernatants were also determined and are included in Fig. 1. The insoluble residue (Tween residue) remaining after six Tween extractions was treated with 1 ml of 0.10 M Tris–HCl buffer (pH 8.0), containing 0.20 M sodium dodecyl sulfate. After centrifugation at 130000 × *g* for 2 h the protein and phosphate content of the supernatant was determined (see supernatant No. 7 in Fig. 1). The very small amount of insoluble and sedimentable material was not further analysed. Fig. 1 shows that most of the membrane material that could be solubilized by Tween 20 was found in the first extract; as judged from a polyacrylamide-gel electrophoresis in Tween the following Tween extracts contained the same proteins (although in different proportions) as the first. For these reasons, only one Tween

* From thorough literature studies it seems doubtful that "pure" membranes (*i.e.* membranes free from contamination of whole or fragmented foreign membranes) have been prepared from any other cells.

20 extraction was performed to obtain material for the investigations described in this paper. The need for using a detergent concentration as high as 2.5% throughout the extraction procedure is evident from Fig. 2.

*Solubilization with the anionic detergent sodium dodecyl sulfate.* One volume of 0.20 M sodium dodecyl sulfate (Schuchardt, München, Germany) in 0.10 M Tris–HCl buffer (pH 8.0) was mixed with 1 vol. of membranes. The completely clear solution obtained contained 30 mg membrane material per ml.

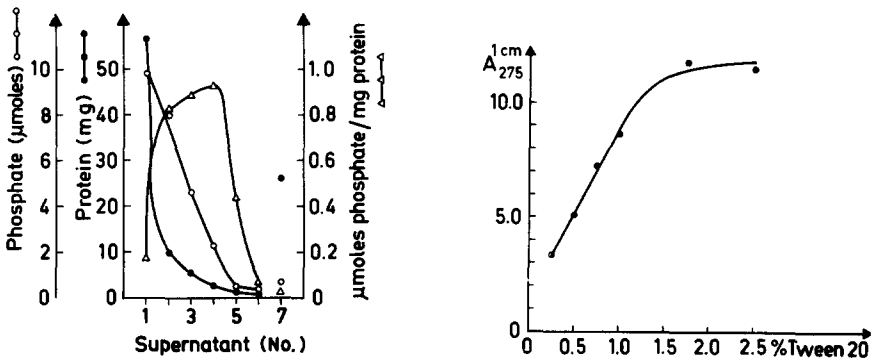


Fig. 1. The amount of protein and phosphate released by repeated extractions with Tween 20. Starting material: 116 mg of membranes containing 28 μmoles of phosphate (probably as phospholipids). The pellet obtained after six Tween-extractions was treated with 0.10 M Tris–HCl buffer (pH 8.0), containing 0.20 M sodium dodecyl sulfate; as is evident from the figure (see 7th supernatant) all phospholipids are extractable with Tween 20, but not all protein material. The figure also shows that proteins are released more rapidly than the phospholipids.

Fig. 2. The solubility of the membranes as a function of the concentration of Tween 20. A 300-μl portion of a membrane preparation, suspended in the diluted β-buffer (0.05 M Tris–HCl buffer (pH 7.4) + 0.156 M NaCl + 0.01 M 2-mercaptoethanol) was mixed with 300 μl of a 0.10 M Tris–HCl buffer (pH 8.0), containing 0.5% Tween 20. The membrane concentration in the suspension obtained was 30 mg/ml. After centrifugation at 130000 × *g* for 2 h the absorbance of the supernatant was measured at 275 nm. The experiment was repeated with another 300-μl aliquot of the same membrane preparation and a 300-μl portion of the Tris–HCl buffer, now containing 1.0% Tween 20, *etc.* The concentrations in the figure thus refer to the final Tween 20 concentration. The figure shows that for maximum solubilization the detergent concentration must be at least 2.0–2.5%.

*Free zone electrophoresis*[9]

A characteristic feature of this method is the way in which convection is supressed: by slow rotation of the horizontal electrophoresis tube an experiment can be carried out in buffer alone without the use of any stabilizing agent.

*One-dimensional analytical polyacrylamide-gel electrophoresis*

The gel slab used for electrophoresis was 10 cm high and had a rectangular cross-section (0.5 cm × 10 cm). On the top of the gel there were nine wells, permitting accurate comparative analysis of up to nine samples in one run. A detailed description of the electrophoresis apparatus (commercially available from Stålprodukter, P.O. Box 12036, S-705 12 Uppsala, Sweden) is found in ref. 10. Amido Black 10B was used as a staining agent for the membrane proteins.

*Two-dimensional analytical polyacrylamide-gel electrophoresis*

Membranes solubilized in Tween 20 can without difficulty be analyzed (without removal of Tween 20) by electrophoresis in a gel containing sodium dodecyl sulfate (it is an advantage, however, if sodium dodecyl sulfate is added to the membrane solution so that the sodium dodecyl sulfate concentration in the sample will be the same as that in the gel). On the other hand, if the membranes are first solubilized in sodium dodecyl sulfate and then submitted to electrophoresis in a gel slab containing only Tween, the zones are very extended and blurred. These findings show that in a two-dimensional electrophoresis with Tween 20 and sodium dodecyl sulfate as the two variables the electrophoresis in Tween 20 must precede that in sodium dodecyl sulfate (and not *vice versa*).

The two-dimensional electrophoresis was performed as follows, most experimental conditions being similar to those described in the preceding section. Two identical samples were applied in separate wells on top of the Tween-containing gel. Following the electrophoresis, two vertical slices, corresponding to the two sample wells, were cut from the gel. One slice was used for staining to localize the protein zones. The other gel slice was transferred to a solution containing all the chemicals (except for TEMED) required to prepare the sodium dodecyl sulfate-containing gel to be used for the subsequent electrophoresis in the second direction. The acrylamide monomers were allowed to diffuse into a thin surface layer of the gel slice. After 30 min, TEMED was added and the monomer solution was slowly poured into the gel chamber. The gel slice (which had swelled slightly) was pressed down to the bottom of the gel chamber. Polymerization provided good contact between the gel slice and the gel to be used for the electrophoresis owing to the previous diffusion of monomers into the gel slice. The electrophoresis was conducted with the anode in the upper electrode vessel.

*Scanning of gels for localization of the zones and direct measurement of their spectra*

For photometric examination of polyacrylamide gels prior to staining we have constructed a scanning device which can be attached to a Zeiss' spectrophotometer monochromator M4 Q11, connected to a recorder. This device has been used not only for localization of zones in the gel during and after electrophoresis, but also for identification of the zones by measuring their spectra without recovering the zones from the gel. For the latter purpose the gel (in a quartz tube) was scanned at a series of different wavelengths, and for each zone the peak height was plotted against the wavelength.

*Amino acid analysis*

The amino acid content was determined with a Biocal BC 200 amino acid analyzer, as described by Eaker[11].

*The relation between dry weight and absorbance*

The relation between dry weight and absorbance was estimated gravimetrically. For unfractionated membranes dissolved in sodium dodecyl sulfate the absorbance at 275 nm in a 1-cm cuvette multiplied by a factor 1.3 gives the dry weight of the membranes in mg/ml. For practical reasons the same factor has been used for membrane fractions dissolved in sodium dodecyl sulfate and Tween 20, although we

are aware of the fact that other conversion factors should be used for these fractions, particularly because they differ in lipid content[12] and amino acid composition.

*Determination of phosphate*

The phospholipids were determined as inorganic phosphate by the method of Bartlett[13]. To localize the phospholipids after a polyacrylamide-gel electrophoresis the gel was cut parallel to the direction of the electrophoretic migration into slices corresponding to the different sample wells. Each slice, which contained all the components in the sample was cut into 0.5-cm segments. Each segment was eluted twice with a total of 1.0 ml of buffer. The phosphate concentrations were determined in the combined extracts.

## RESULTS

*Free zone electrophoresis*

Fig. 3 shows the result of a free zone electrophoresis of the washed membranes. After electrophoresis, the material corresponding to the two peaks was withdrawn from the rotating electrophoresis tube for analysis by electron microscopy. For this purpose the material was negatively stained with 2 % ammonium molybdate (pH 7.1); no difference was observed in the appearance of the membranes from the two electrophoresis fractions, which were not further analyzed.

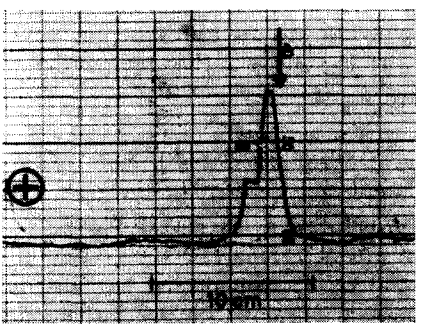

Fig. 3. Free zone electrophoresis of *Acholeplasma laidlawii* membranes. Buffer: β-buffer (0.05 M Tris–HCl buffer (pH 7.4) + 0.156 M NaCl + 0.01 M 2-mercaptoethanol). Sample: 25 μg in 5 μl of buffer. Inner diameter of the revolving electrophoresis tube, 3 mm; current, 10 mA; voltage, 300 V. Temperature of cooling water, 21 °C. The arrow indicates the position of the starting zone. The scan was made after an electrophoresis of 1 h. The fractions corresponding to the two peaks were examined by electron microscopy, which revealed that both fractions contained membranes; no differences in structure were observed.

*Polyacrylamide-gel electrophoresis in sodium dodecyl sulfate*

*Starting material: unfractionated membranes solubilized in sodium dodecyl sulfate.* By an analytical polyacrylamide-gel electrophoresis the membrane proteins were resolved into 12 zones (see the slices denoted u.m. in Fig. 4). The designations introduced in Fig. 4 for these zones will be used throughout the paper in order to facilitate the discussion (d and D stand for dodecyl sulfate; D refers to heavily stained zones which we assume to represent the main components, and d for the weakly

stained zones (the minor components)). The yellow color of the applied membranes became distributed over two zones, which were analyzed photometrically as described below in *Scanning of polyacrylamide gels*. After staining, an opalescent zone was observed in front of the protein zones. This zone coincided with the part of the gel that contained phosphate, and probably represents the phospholipids.

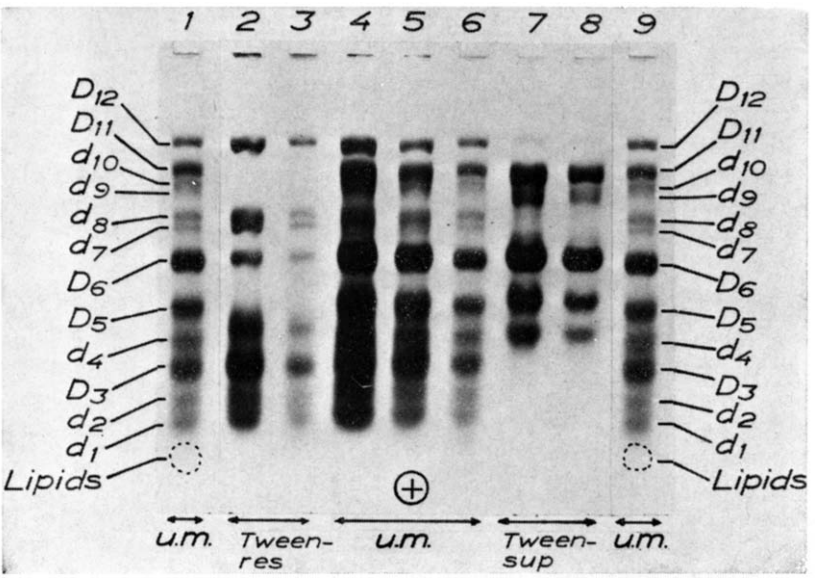


Fig. 4. Polyacrylamide-gel electrophoresis in sodium dodecyl sulfate of unfractionated membranes (u.m.), the Tween 20-soluble material (Tween supernatant), and the non-Tween 20-soluble material (Tween residue). Dimensions of the gel slab: 0.5 cm × 10 cm × 10 cm. Buffer: 0.1 M Tris–HCl buffer (pH 8.0), containing 0.02 M sodium dodecyl sulfate. Composition of the gel: T = 6%; C = 5% (for definition of T and C, see ref. 27). Voltage: 50 V. Current: 100 mA. Duration of the run: 5 h. The amount of material applied to sections 2 and 3 were 1 and 0.5 mg, respectively; to sections 4, 5, and 6: 3, 2, and 1 mg, respectively; to sections 7 and 8: 2 and 1 mg, respectively. To facilitate a comparison between unfractionated membranes (u.m.), Tween residue and Tween supernatant, Section 6 has been cut out from two copies of this photograph and inserted as Sections 1 and 9. Proteins absent in Tween supernatant (zones $d_1$, $d_2$, $D_3$, $d_7$, and $d_8$) are present in Tween residue, while proteins absent in Tween residue (zones $D_5$, $d_9$, $d_{10}$, and $D_{11}$) are present in Tween supernatant. Some zones are present both in Tween residue and Tween supernatant ($d_4$, $D_6$ and $D_{12}$).

All migration distances were independent of the amount of starting material applied (*cf.* Fig. 6).

*Starting material*: *Tween 20-treated membranes*. The electrophoretic pattern of the Tween supernatant (= membrane material that can be solubilized in Tween 20; see *Solubilization with the non-ionic detergent Tween 20*) and the Tween residue (= membrane material that cannot be solubilized in Tween 20) are also shown in Fig. 4. Comparison with the pattern of unfractionated material (u.m.) indicates that the Tween supernatant is completely devoid of the three fastest migrating protein zones $d_1$, $d_2$, and $D_3$; the two intermediate zones $d_7$ and $d_8$ are also absent. As expected, the Tween residue contained the zones that are lacking in the Tween supernatant, *i.e.* the zones $d_1$, $d_2$, $D_3$, $d_7$, $d_8$, and $D_{12}$ (which is present only in very low concentrations also in the Tween supernatant). The Tween residue is completely devoid of

$D_5$, $d_9$, $d_{10}$, and $D_{11}$, which are all found in the Tween supernatant. However, the zones $d_4$ and $D_6$ are present both in the Tween supernatant and in the Tween residue.

*Estimation of the molecular weights of the membrane proteins*[14,15]

Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulfate can provide fairly reliable estimates of the molecular weights of proteins although exceptions have been reported, particularly for strongly basic or acidic proteins[16] and for glycoproteins[17]. As markers we have used some common water-soluble proteins and the less hydrophilic coat protein from tobacco mosaic virus (Fig. 5a). In a plot of the logarithm of the molecular weights *versus* the migration distances, all of the points, including that corresponding to the tobacco mosaic virus protein, fall on a straight line (Fig. 5b). Hopefully this calibration curve should be applicable also for the hydrophobic membrane proteins. The molecular weights estimated by the use of this calibration curve are listed in Table I.

*Polyacrylamide-gel electrophoresis in Tween 20 of the Tween 20-soluble membrane material (Tween supernatant)*

The result is given in Fig. 6. The separated zones have been denoted in a way analogous to that used in Fig. 4 (the letters t and T stand for Tween 20). Fig. 6

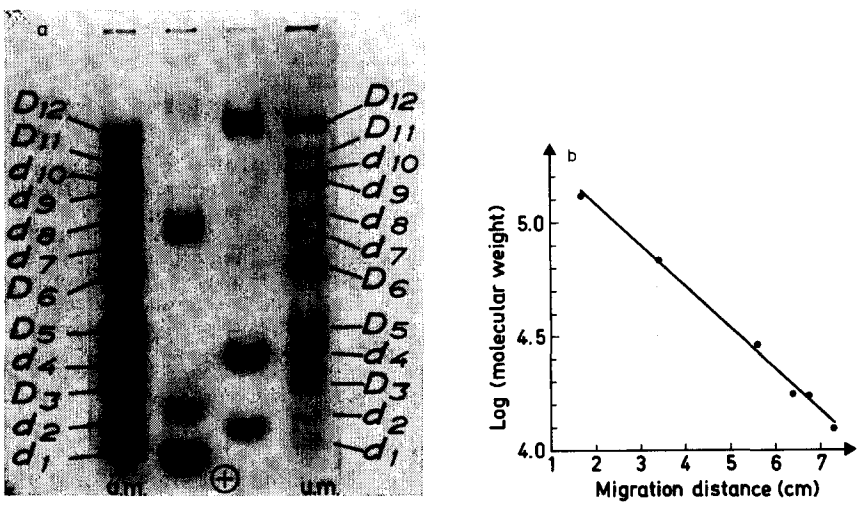


Fig. 5. Molecular weight estimation of membrane proteins by polyacrylamide-gel electrophoresis in sodium dodecyl sulfate[14,15]. (a) Dimensions of the gel slab: 0.5 cm × 10 cm × 10 cm (only half the gel is shown in the figure). Composition of the gel: T = 6%; C = 5%. Buffer: 0.05 M sodium phosphate buffer (pH 7), containing 0.02 M sodium dodecyl sulfate and 0.05 M 2-mercaptoethanol. Voltage, 50 V; current, 100 mA; duration of the run, 4 h. The electrophoretic patterns refer to unfractionated membranes (u.m.) and the following marker proteins: β-galactosidase (mol. wt, 130000), human serum albumin (mol. wt, 68000; KABI, Stockholm, Sweden), human carbonic anhydrase C (mol. wt, 29000), the coat protein from tobacco mosaic virus (mol. wt, 17500), myoglobin from sperm whale (mol. wt, 17200; Koch-Light Laboratories, England) and cytochrome *c* from horse heart (mol. wt, 12300; Sigma Chemical Co., U.S.A.). (b) A plot of the logarithms of the molecular weights *vs* the migration distances of the marker proteins. The migration distances are obtained from (a). This calibration curve, and a measurement of the migration distances of the membrane proteins in the experiment shown in (a), permitted an estimate of their molecular weights (see Table I).

indicates that the migration distances in Tween are dependent on the amount of sample applied. (No such a concentration dependence was observed in the sodium dodecyl sulfate electrophoresis: Fig. 4.) After staining, an opalescent zone was observed behind the protein zones. Phosphate analysis, performed as described under *Determination of phosphate*, indicated that the phospholipids were localized in this opalescent zone. Two yellow zones were observed, and were further analyzed photometrically as described in *Scanning of gels for localization of the zones and direct measurement of their spectra.*

TABLE I

TENTATIVE MOLECULAR WEIGHTS OF THE MEMBRANE PROTEINS IN *A. laidlawii*

The molecular weights have been estimated by polyacrylamide-gel electrophoresis in sodium dodecyl sulfate[14,15]. The gel is shown in Fig. 5a and the calibration curve in Fig. 5b.

| *Protein zone* | *Mol. wt* | *Protein zone* | *Mol. wt* |
|---|---|---|---|
| $d_1$ | 15 000 | $d_7$ | 66 000 |
| $d_2$ | 18 000 | $d_8$ | 74 000 |
| $D_3$ | 23 000 | $d_9$ | 93 000 |
| $d_4$ | 28 000 | $d_{10}$ | 97 000 |
| $D_5$ | 34 000 | $D_{11}$ | 110 000 |
| $D_6$ | 52 000 | $D_{12}$ | 140 000 |

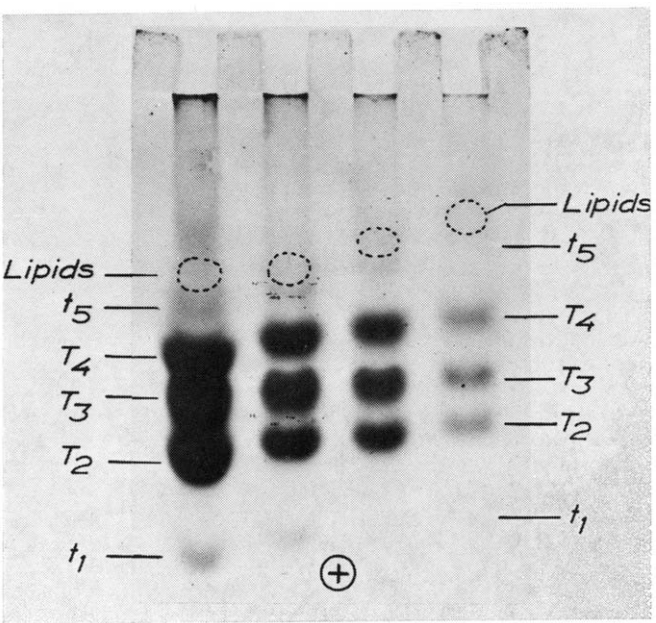


Fig. 6. Polyacrylamide-gel electrophoresis in Tween 20 of Tween 20-soluble membrane material (Tween supernatant). Dimensions of the gel slab: 0.5 cm × 10 cm × 10 cm (only half the gel is shown in the figure). Composition of the gel: T = 4%; C = 2%. Buffer: 0.1 M Tris–HCl buffer (pH 8.0), containing 0.5% Tween 20. Voltage, 50 V; current, 75 mA; duration of the run, 14 h. The amount of material applied: (from the left well to the right well) 2, 1, 0.5, and 0.2 mg, respectively. The lipids appeared as opalescent zones, which could not be reproduced in the photograph. Observe that the migration distances are dependent on the amount of material applied.

*Two-dimensional analytical polyacrylamide-gel electrophoresis*

A photograph of the electrophoretic pattern is given in Fig. 7. The splitting of the zone $T_4$ into two heavily stained spots ($D_5$ and $D_6$) is the most striking result, although the two other main zones ($T_2$ and $T_3$) also give multiple spots.

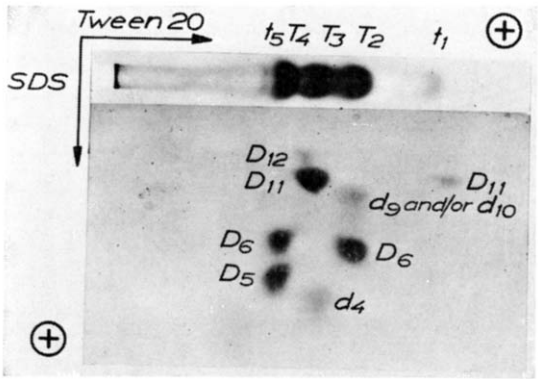


Fig. 7. Two-dimensional polyacrylamide-gel electrophoresis. Starting material: Tween supernatant containing 2 mg membrane material. The first electrophoresis was performed in Tween 20 (*cf.* Fig. 6) and the second in sodium dodecyl sulfate (SDS). To facilitate for the reader to interpret the electrophoretic pattern a photograph of a stained section of the run in Tween has been inserted on top of the sodium dodecyl sulfate gel. For identification of the spots in the sodium dodecyl sulfate gel Fig. 4 must also be consulted. The figure shows that all of the main zones obtained in Tween electrophoresis ($T_2$, $T_3$, $T_4$) are heterogeneous in sodium dodecyl sulfate electrophoresis: $T_4$ is resolved into $D_5$ and $D_6$; $T_3$ into $d_4$, $D_{11}$ and $D_{12}$; $T_2$ into $D_6$ and $d_9$ or/and $d_{10}$. The photo was inverted to facilitate comparison with Figs 4 and 5a.

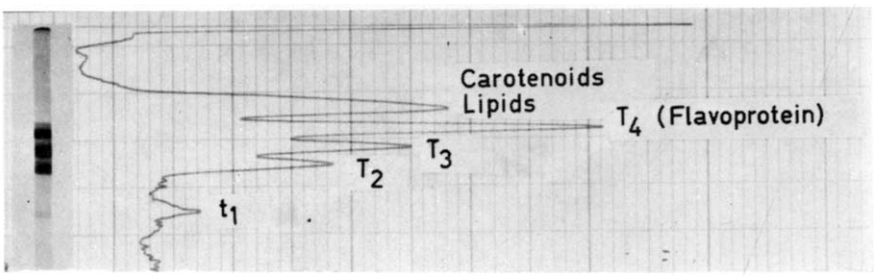


Fig. 8. Scanning of a polyacrylamide gel at 280 nm following an electrophoresis in Tween 20 of Tween soluble membrane material (Tween supernatant). The gel composition and the buffer were the same as described in the legend to Fig. 6. The run was made in a quartz tube with an inner diameter of 0.45 cm and a length of 10 cm. Voltage, 40 V; current, 5 mA/tube; duration of the run, 10 h. Following scanning, the gel was stained and photographed (insert). The notations for the zones are the same as in Fig. 6.

*Scanning of polyacrylamide gels*

When the membranes were submitted to electrophoresis in polyacrylamide gels two yellow zones were observed, as mentioned above. A direct scan of such a gel at 280 nm is shown in Fig. 8, which should be compared with the staining pattern shown in Fig. 6. The spectrum of each zone was determined as described in Materials and Methods. To judge from these spectra the yellow color of the protein zone $T_4$ corresponds to a flavin molecule (maxima at 370 and 450 nm), while the other yellow

zone, which is located at the front part of the lipid zone, seems to contain carotenoid material. Similarly, the two yellow zones appearing in polyacrylamide-gel electrophoresis in sodium dodecyl sulfate, coincident with zone $D_5$ and the lipid zone, were found to contain flavin and carotenoid, respectively (Fig. 9). In a forthcoming paper, dealing with the preparative isolation of these yellow zones, more details of the spectra will be presented: the yellow color does not disappear upon dialysis.

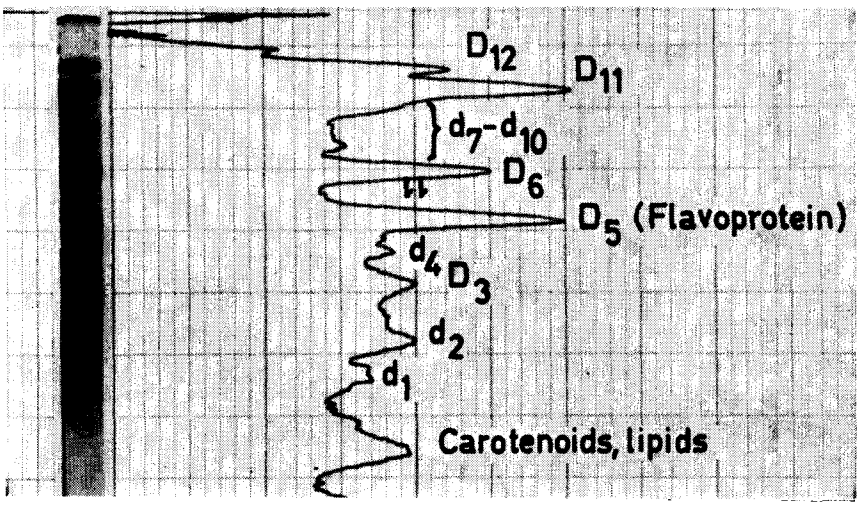


Fig. 9. Scanning of a polyacrylamide gel at 280 nm following an electrophoresis in sodium dodecyl sulfate of the membrane solubilized in sodium dodecyl sulfate. The composition of the gel: T = 8%; C = 3%; a gel of this composition is not opalescent and is therefore more suitable for ultraviolet scanning than the gel employed in the experiment corresponding to Fig. 4. The buffer was the same as that described in the legend to Fig. 4. The run was made in a quartz tube with an inner diameter of 0.45 cm and a length of 10 cm. Voltage, 22 V; current, 3 mA/tube; duration of the run, 7 h. Following scanning, the gel was stained and photographed (insert). The notations for the zones are the same as in Fig. 4.

*Some studies of the possible formation of artificial complexes between membrane proteins*

As is pointed out in Discussion it is quite conceivable that artificial protein complexes might form in connection with the solubilization of a membrane. If such complexes arise one might also expect proteins from two different membranes to interact to give artificial complexes. Therefore a comparison was made between the protein patterns obtained when a membrane $M_1$, a membrane $M_2$, and a mixture of $M_1$ and $M_2$ were submitted to analysis by polyacrylamide-gel electrophoresis in the same electrophoresis apparatus as was used as for the experiments illustrated in Figs 4–7. The membrane systems used for this investigation were: (1) $M_1$ = a suspension in diluted $\beta$-buffer of membranes from *A. laidlawii*; and $M_2$ = a suspension in water of membranes from ethythrocytes. $M_1$, $M_2$, and the mixture $M_1 + M_2$ were dissolved in 0.10 M Tris–HCl buffer containing 0.20 M sodium dodecyl sulfate. The same buffer with a 10-fold lower sodium dodecyl sulfate concentration was used for the polyacrylamide-gel electrophoresis. The composition of the polyacrylamide gel was T = 5%; C = 3%. (2) The same membrane system as in (1) with the exception that the membranes $M_1$ and $M_2$ were solubilized in 0.10 M Tris–HCl buffer +

0.20 M sodium dodecyl sulfate before they were mixed. The electrophoresis was performed as in (1). (3) $M_1$ = Tween supernatant, *i.e.* the supernatant obtained after treatment of the *A. laidlawii* membranes with Tween 20 and centrifugation; $M_2$ = the Tween 20 soluble fraction of the proteins of 30-S ribosomes from *E. coli*. The buffer used for the electrophoresis was that described in the legend to Fig. 6. (4) $M_1$ = erythrocyte membranes, dissolved in 0.10 M Tris–HCl buffer + 0.20 M sodium dodecyl sulfate; $M_2$ = tobacco mosaic virus dissolved in the same buffer. The electrophoresis was performed in the same buffer with a 10-fold lower sodium dodecyl sulfate concentration. (5) The same system as in (4), with the exception that $M_1$ consisted of membranes from *A. laidlawii* instead of erythrocyte membranes.

In all the Expts 1–5 the electrophoretic pattern of the mixture $M_1 + M_2$ was virtually identical to a superimposition of the electrophoretic pattern of $M_1$ upon that of $M_2$: there was no evidence for the formation of hybrid complexes.

TABLE II

THE AMINO ACID COMPOSITIONS OF UNFRACTIONATED MEMBRANE MATERIAL, OF MEMBRANE MATERIAL SOLUBLE IN TWEEN 20 (TWEEN SUPERNATANT), AND OF MEMBRANE MATERIAL INSOLUBLE IN TWEEN 20 (TWEEN RESIDUE)

Corrections for losses during hydrolysis were taken as 5% for threonine, 11% for serine and 32% for the amino sugars. The amount of amino sugar is expressed as moles per 100 moles of amino acids. The tryptophan content was not determined. Compare our data with those of Engelman and Morowitz[28]. $\Sigma\Delta f_t$ is a measure of the degree of the hydrophobicity[18,19] of unfractionated membranes, the Tween supernatant and the Tween residue.

| *Amino acid* | $\Delta f_t$ (*kcal/mole*) | *Unfractionated membranes* (*mole %*) | $\Delta f_t$ (*kcal*) | *Tween supernatant* (*mole %*) | $\Delta f_t$ (*kcal*) | *Tween residue* (*mole %*) | $\Delta f_t$ (*kcal*) |
|---|---|---|---|---|---|---|---|
| Lys | 1.5 | 6.15 | 9.23 | 5.68 | 8.52 | 5.85 | 8.78 |
| His | 0.5 | 1.20 | 0.60 | 0.97 | 0.49 | 1.16 | 0.58 |
| Arg | 0.73 | 2.57 | 1.88 | 2.06 | 1.50 | 2.54 | 1.85 |
| Cys | | 0.18 * | | 0.24 * | | Traces | |
| Asp | | 12.15 | | 13.68 | | 10.61 | |
| Met | 1.3 | 2.00 | 2.60 | 1.42 | 1.85 | 2.37 | 3.08 |
| Thr | 0.4 | 7.29 | 2.92 | 8.53 | 3.41 | 6.37 | 2.55 |
| Ser | −0.3 | 6.82 | −2.05 | 6.78 | −2.03 | 7.05 | −2.12 |
| Glu | | 8.75 | | 8.95 | | 7.98 | |
| Pro | 2.6 | 3.32 | 8.63 | 3.53 | 9.18 | 3.23 | 8.40 |
| Gly | | 7.44 | | 7.47 | | 7.52 | |
| Ala | 0.5 | 8.56 | 4.28 | 9.32 | 4.66 | 7.77 | 3.89 |
| Val | 1.5 | 6.95 | 10.43 | 7.13 | 10.70 | 7.22 | 10.83 |
| Ile | 2.97 | 7.04 | 20.91 | 5.75 | 17.08 | 8.68 | 24.78 |
| Leu | 1.8 | 9.72 | 17.50 | 8.41 | 15.14 | 11.37 | 20.47 |
| Tyr | 2.3 | 4.54 | 10.44 | 5.17 | 11.89 | 4.24 | 9.75 |
| Phe | 2.5 | 5.32 | 13.30 | 4.91 | 12.28 | 6.05 | 15.13 |
| GlcN | | 2.15 | | 2.86 | | Traces | |
| GalN | | 5.64 | | 5.16 | | Traces | |
| $\Sigma\Delta f_t$ | | | 101 | | 95 | | 108 |

* Determined as cysteic acid after performic acid oxidation according to Moore[29].

*Amino acid analysis*

The amino acid composition has been determined for the unfractionated membranes, the material soluble in Tween 20 (Tween supernatant), and the material insoluble in Tween 20 (Tween residue); see Table II. To estimate the difference in hydrophobicity between Tween supernatant and Tween residue we have utilized literature data[18,19] for the contributions of some amino acid chains to free energies of transfer from organic solvents to water ($\Delta f_t$). It is possible that the degree of hydrophobicity, in Table II expressed as $\Sigma\Delta f_t$, would have been significantly different if the content of tryptophan had been determined, as this amino acid might be the most hydrophobic of all amino acids.

## DISCUSSION

Many articles on the structure of biological membranes deal with the important problem of determining the number and the properties of the protein constituents. However, knowledge of the spatial relationships among these proteins is also of fundamental interest[20,21]. One way to get this information is to disrupt the membrane into a small number of large complexes which might be isolated and split further into separable subunits. The procedure is analogous to that used for the determination of the amino acid sequences of proteins[22].

We have tried to apply the above approach to the membranes of *A. laidlawii* by first treating the membranes with Tween 20, a detergent which only gives partial solubilization. If proteins are bound to each other in the native membrane by strong hydrophobic interactions or by other types of bindings, there is a possibility that native complexes might persist in the presence of this weak dissociating agent. By then treating such complexes with a strong dissociating agent (*e.g.* sodium dodecyl sulfate), it should be possible to split them and isolate the constituents. If such an experiment is successful, one could draw conclusions about the spatial arrangement of these constituents. Fig. 7 illustrates our attempt to use the above strategy, and as can be seen, there is at least a chance that $T_4$ is a complex. We have now proved, however, that $T_4$ can be separated into two protein fractions also in Tween 20, which will be published in the above forthcoming paper. We were consequently unable to employ the above method, utilizing detergents with different solubilizing power, to find proteins which are neighbours in the native membrane. Even if this technique, also in future experiments, cannot be used for the determination of spatial relationships among membrane proteins, it is still a practical advantage to start with a detergent that gives selective solubilization, thereby reducing the number of proteins that must be separated at one time. There can be several reasons why we did not succeed in finding complexes of proteins. The choice of solubilizing agents might have been unsuitable. The explanation can also be that the proteins in the native membrane are not in contact with each other but are held apart by lipids which are solubilized by both Tween 20 and sodium dodecyl sulfate, the detergents we have used.

Whether the native membrane is built up of proteins that are in contact with each other or not, one cannot rule out the possibility that proteins, following solubilization, can coalesce into more or less stable artifactual complexes. When using detergents such artefacts may arise in cases where the (hydrophobic) interaction between two proteins is greater than the interaction between the detergent and the proteins.

However, our electrophoresis experiments with mixtures of different membranes did not indicate the formation of "hybrid" complexes. This finding does not exclude the possibility that such complexes may arise under other experimental conditions, since (in the absence of detergents) membranes from different sources can form hybrides[23,24]. (One should also consider the possibility that "foreign" protein components may appear if cytoplasmic proteins are adsorbed to membrane fragments or trapped in vesicles formed during the isolation procedure[25].

It is interesting to note that the proteins that are not soluble in Tween 20. contain more hydrophobic side chains than the Tween-soluble proteins (Table II). Possibly all partially, but selectively solubilizing detergents (to which Tween 20 belongs) behave roughly in the same way, *i.e.* are unable to solubilize the most hydrophobic membrane proteins.

The yellow color of *A. laidlawii* membranes is in general attributed to the presence of carotenoid(s). However, the data of Morowitz and Terry[26] suggest that these membranes also contain another yellow pigment, a flavin chromophore associated with a protein. Our experiments support this view and indicate that this flavoprotein can be extracted with Tween 20 and purified by polyacrylamide-gel electrophoresis (it is found in zone $T_4$ in Fig. 6, together with at least one additional protein). In this connection it should be mentioned that the yellow color of the organism due to the presence of carotenoids decreased in intensity when we extended the culturing time over a period longer than 15 h, an observation which should be recalled when a high yield of carotenoids is desired.

ACKNOWLEDGMENTS

We want to thank Miss Erna Åberg and Mrs Irja Blomqvist for skilful technical assistance and for the interest they have shown in this work, and are grateful to Drs P. Lundahl and J.-C. Janson for valuable discussions.

We are very grateful to Prof. S. Razin for the supply of a sample of *A. laidlawii* for inoculation and to Dr E. Carlemalm for his advice in growing the organisms. Tobacco mosaic virus, ribosome proteins, β-galactosidase and carbonic anhydrase were generous gifts from Drs P. Oxelfelt, C. Kurland, A. Ullman and B. Strandberg, respectively. The electron microscopic examinations were kindly performed by Dr S. Höglund and Dr G. Blomkvist, and the amino acid analyses by Dr D. Eaker, Mr S. Centring and Mr R. Thorzelius.

This work was supported by the Swedish Natural Science Research Council, the Wallenberg Foundation, the Lennander Foundation, and the Foundation of Ollie and Elof Ericsson.

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