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MOLECULAR ORGANIZATION IN BACTERIAL CELL MEMBRANES

IV. ISOLATION BY PREPARATIVE ELECTROPHORESIS IN SODIUM DODECYLSULPHATE AND PROPERTIES OF THE TWO MAJOR POLYPEPTIDE GROUPS OF A "SOLUBLE" FRACTION FROM *STREPTOMYCES ALBUS* MEMBRANES*

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SUMMARY

Two polypeptide fractions have been purified from a "soluble" fraction of *n*-butanol-extracted *Streptomyces albus* membranes by preparative electrophoresis in sodium dodecylsulphate. They accounted for approx. 80 % of the protein of the fraction (i.e. 20 % of the total membrane protein). The ultraviolet spectrum of Group 1 (relative mobility 1.0) revealed the presence of nucleotide material, while that of Group 3 (relative mobility 0.65 ± 0.05) showed the presence of a possibly aggregated protein-like material. About 100 and 30 % of the protein contents (Lowry method) of Groups 3 and 1, respectively, were recovered as amino acid residues. These results confirm the protein nature of both fractions and suggest an overestimation of the protein value in Group 1. Both polypeptide groups can be classified as "extrinsic" membrane proteins on the basis of their similar amino acid composition (Vanderkooi, G. and Capaldi, R. A. (1972) Ann. N.Y. Acad. Sci. 195, 135–138). Three N-terminal amino acids were found in each fraction: one common (alanine), methionine, leucine or isoleucine (Group 3) and glutamic acid, lysine (Group 1). The sedimentation coefficients calculated were 2.46 S for Group 3 and 1.54 S for Group 1. Analysis of the isolated groups by gel electrophoresis under non-dissociating conditions or with Triton X-100, gave aggregate-like patterns.

Sodium dodecylsulphate electrophoresis revealed an anomalous staining behaviour of Group 3 depending upon the dissociating conditions. The whole "soluble" fraction bound 0.40 mg dodecylsulphate /mg protein (0.55 mg detergent/mg protein corrected for overestimation). After dialysis, the fraction retained 10 % of the bound dodecylsulphate. Circular dichroism of the isolated groups after exhaustive dialysis showed similar spectra, although of lower dichroism, to those obtained by other authors on soluble enzymes treated with sodium dodecylsulphate. Strong acid conditions were required to change the CD spectra of the polypeptides.

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INTRODUCTION

In a previous paper [1] we described the isolation of a "soluble" fraction by *n*-butanol extraction of *Streptomyces albus* membranes. This fraction represented about 25 % of the whole membrane protein and showed three main groups of protein components in sodium dodecylsulphate-polyacrylamide gel electrophoresis which corresponded to the major polypeptide groups of the membrane [1]. Some of them might be glycoproteins [1]. We then studied the molecular status of the fraction by gel filtration [1]. These studies showed that the components of the fraction were in various states of aggregation. The apparent anomalous behaviour of the proteins of the fraction with regard to detection and to their susceptibility to dissociation by sodium dodecylsulphate was also described [1].

In a subsequent step, we attempted to isolate the polypeptide components of the fraction. In this report, we describe the separation of the two predominant protein groups of the "soluble" fraction (i.e. Groups 3 and 1) by dodecylsulphate preparative gel electrophoresis. We also report their chemical characterization as well as some of their optical properties. These studies provide a possible explanation for the response of the isolated proteins to further dissociation with sodium dodecylsulphate.

MATERIALS AND METHODS

"Soluble" fractions were obtained by *n*-butanol extraction of *S. albus* membranes solubilised by sodium dodecylsulphate as reported previously [1]. Prior to their fractionation by preparative electrophoresis, the fractions were freeze-dried and subsequently dialysed overnight at 4 °C against 50 mM Tris–HCl (pH 7.5).

Chemicals

Triton X-100, bovine serum albumin, *p*-toluenesulphonic acid and tryptamine · HCl [3-(2-aminoethyl)-indole-HCl] were purchased from Sigma Chemical Co. (St. Louis, Mi., U.S.A.). ATP disodium salt, was obtained from PL Biochemicals (Milwaukee, Wisc., U.S.A.). *N,N'*-Methylene-bisacrylamide and *N,N,N',N'*-tetramethylethylenediamine were from Fluka and Buchs (Switzerland). Sodium dodecylsulphate was from Fisher Scientific Co. (Fair Lawn, N.J., U.S.A.), or specially pure from B.D.H. Chemicals Ltd (Poole, England). Coomassie brilliant blue was obtained from Schwarz/Mann (Orangeburg, N.Y.). 1-fluoro-2,4-dinitro-[^{3}H]benzene (200 Ci/mole) and sodium dodecyl-[^{35}S]sulphate (16.6 Ci/mole) were from Amersham, the Radiochemical Centre (London, England). Tris-(hydroxymethyl)aminomethane, bromophenol blue, glycine, silica gel G, 1-fluoro-2,4-dinitrobenzene and all other reagents were of the highest quality available from Merck (Darmstad, Germany).

Analytical procedures

Protein was determined by the method of Lowry et al. [2] using bovine serum albumin as standard and by ultraviolet spectrophotometry in a Gilford 2400 spectrophotometer. Phosphorus was determined by a modification of the method described by Lowry et al. [3], with ATP as standard.

Gel electrophoresis

(a) Analytical polyacrylamide gels (7 %) were prepared and the electrophoresis performed as described elsewhere [4], except that longer gels (11 cm) were used. Gel electrophoresis was also carried out in the presence of the non-ionic detergent Triton X-100 (0.5 % in the buffer and gels). Samples were previously treated with 0.5 % Triton X-100 [5]. Proteins were stained with coomassie brilliant blue as described by Fairbanks et al. [6]. After staining, the gels were scanned at 575 nm (for coomassie stain) with a Gilford 2400 spectrophotometer equipped with a model 2410-S linear transport.

(b) Preparative electrophoresis was carried out in a Poly-Prep 200 apparatus from Büchler Co. No stacking gels were used. Separating gels with 7 % acrylamide and 0.17 % *N,N'*-methylenebisacrylamide containing 0.1 % dodecylsulphate were employed. All percentages are given in weight over volume. Gels were polymerised in 0.38 M Tris–0.06 M HCl (pH 8.8–9) at room temperature for 3 h in the dark. Buffers were as follows: (a) Lower chamber: 0.4 M Tris–HCl (pH 8.0)–0.1 % dodecylsulphate, (b) Upper chamber: 54 mM glycine in 0.1 M Tris, giving a pH 8.0–0.1 % dodecylsulphate and (c) Elution buffer: 0.1 M Tris–HCl (pH 8.0)–0.1 % dodecylsulphate.

Samples pretreated with 1 % dodecylsulphate at 85 °C for 5 min, were added to sucrose to reach a final concentration of 20 %, and layered in the top of the gel. Electrophoresis was carried out at 40 mA for the first 30 min and then at 90 mA for the next 20 h. Temperature was kept constant at 4 °C with a circulating bath from Forma Scientific Inc. model 2095. The effluent was continuously monitored at 260 and 280 nm with a UA-4 absorptiometer from Isco. Fractions were collected in a Fractiomette 200 fraction collector from Büchler Co.

Amino acid analysis

All samples were exhaustively dialysed against water, freeze-dried, and then hydrolysed with 3 N *p*-toluenesulphonic acid as described by Liu and Chang [7]. Hydrolysis of the protein samples (1 mg/ml 0.2 % tryptamine-*p*-toluenesulphonic acid) was carried out under N_2 at 110 °C for 18, 24 and 48 h. Amino acid analysis was carried out on a Jeol JLC-5 AH amino acid analyser. The use of *p*-toluenesulphonic acid as catalyst permits the quantitative estimation of tryptophan [7].

Determination of N-terminal amino acids

Samples (1–2 mg depending of the fraction, see Table II) isolated by preparative gel electrophoresis were exhaustively dialysed against water prior to their analysis. N-Terminal amino acids were determined by dinitrophenylation [8], but 1-fluoro-2,4-dinitro-[^{3}H]benzene (1 μCi) was added to each reaction mixture. Dinitrophenyl derivatives were separated by two-dimensional thin-layer chromatography on silica gel G using as first solvent the upper phase obtained at room temperature after mixing equal volumes of *n*-butanol and 0.1 % NH_4OH (v/v) and then methanol–acetic acid–water (95 : 5 : 1, by vol.) as second solvent. After the runs, the spots were eluted (with 0.04 M NH_4OH in ethanol), and their absorption at 360 nm measured. The dinitrophenyl derivatives were then rechromatographed in the second solvent with known standards, and identified. The derivatives were eluted again from the plates and their radioactivity counted in a scintillation counter (Mark II, Nuclear Chicago). This procedure permitted a good identification of the dinitrophenyl derivatives as well as their approximate quantitation by two different methods.

Analytical ultracentrifugation

The sedimentation velocity experiments were carried out in a Spinco model E ultracentrifuge equipped with schlieren optics and a RTIC temperature control unit. All runs were performed at 20 °C in a single sector cell. Sedimentation coefficients were calculated by measuring the position of the sedimenting boundary at various intervals of time, using speeds of 52 000 rev./min and taking photographs at intervals of 8 min. Samples for all runs (at different concentrations of protein) were equilibrated in 50 mM Tris (pH 7.5). Sedimentation coefficients were calculated by the least squares method.

Binding of sodium dodecylsulphate to the "soluble" fraction

The binding of sodium dodecylsulphate by the "soluble" fraction was measured by equilibrium dialysis. The experiments were carried out as follows: dialysis bags containing the "soluble" fraction (22 mg) in 50 mM Tris–HCl buffer, pH 7.5, (20 ml) were dialysed at 4 °C against 1 l of the same buffer containing 0.1 % (w/v) sodium dodecyl-[^{35}S]sulphate (spec. act. 0.23 μCi/mg). Under these conditions, the concentration of dodecylsulphate monomer must be 2.2 mM, and that of monomers in micellar form 1.27 mM, according to Reynolds and Tanford [9]. After increasing periods of time, aliquots (20 μl) were drawn from the diffusible and non-diffusible fractions and their radioactivity counted. When equilibrium was reached (after about 60–72 h), the concentration of detergent inside the dialysis bag was higher than in the diffusate, the difference in concentration being the amount of dodecylsulphate bound by the protein. To test the reversibility of detergent binding, the "soluble" fraction equilibrated with dodecyl-[^{35}S]sulphate was dialysed for 7 days against seven changes of 1 l of 50 mM Tris–HCl (pH 7.5) or water.

Ultraviolet spectroscopy

Ultraviolet spectra were determined using a Cary 16-S double beam recording spectrophotometer with automatic slit width adjustment. Prior to recording the spectra, the fractions from the preparative polyacrylamide electrophoresis (13–15 mg of protein in 5 ml of elution buffer) were dialysed at 4 °C, 38 h against three changes of 3 l of 50 mM Tris–HCl, pH 7.5, followed by 48 h, against two changes of 3 l of water. Protein concentrations and cuvette light paths are indicated in the legend of Fig. 2.

Circular dichroism

The samples dialysed as those for ultraviolet spectrophotometry were freeze-dried and redissolved in water to give a protein concentration of 1 mg/ml estimated by the method of Lowry et al. [2]. This solution or appropriate dilution in water or 10 mM sodium phosphate buffer, pH 7.4, were used for the measurement of the CD of the protein in the near and far ultraviolet. CD spectra were recorded using a Roussel–Jouan Dichrographe II. To test the effect of sodium dodecylsulphate on the CD of the dialysed fractions, the protein solutions in water (10 μl) were added to a solution of detergent (final conc., 48 %, w/v) in sodium phosphate buffer (10 mM, pH 7.41). CD spectra were recorded immediately and after the solution had stood for 90 min at room temperature. The effect of pH on the spectra was examined by adding aliquots of 2 M HCl, measuring the pH (Radiometer pH meter 26) and recording the spectrum immediately and after standing for 1 h at room temperature. The CD was corrected for the small variations in protein concentration during this experiment.

RESULTS

Preparative gel electrophoresis in sodium dodecylsulphate. Preparation of Groups 1, 2, 3

The fractionation of the "soluble" fraction by sodium dodecylsulphate preparative electrophoresis is illustrated in Fig. 1. It is worth noting the parallelism between its elution pattern, monitored by absorption at 280 nm and the protein pattern obtained by analytical gel electrophoresis. The effluent was divided into three fractions denominated Groups 1, 2 and 3 [1] according to the relative mobilities (0.98–1.0, 0.81, 0.61–0.64). The groups were pooled, freezedried and dialysep against 6 l of 50 mM Tris–HCl (pH 7.5). Their phosphorus and protein contents were 20 and 18 % for Group 1; 10.5 and 13 % for Groups 2, and 13.6 and 18.75 % for Group 3, respectively. The recovery of both components was low. We do not have any obvious explanation for this. It may be caused by difficulties of detection (see ref. 1) and/or losses during preparative electrophoresis. But in contrast with previous results [1], we now found a correlation between the protein detection in the effluent and in the pooled, concentrated fractions.

Ultraviolet spectrum

After isolation of the polypeptides, our main concern was the characterization of the two major groups: 1 and 3. Their ultraviolet spectra are shown in Fig. 2. Group 3 (B in the figure) shows a spectrum of protein-like material, possibly aggregated, with a maximum at 274 nm and shoulders at 283 and 291 nm. In the far ultraviolet, a shoulder was evident at about 226 nm, but although we reached as far as

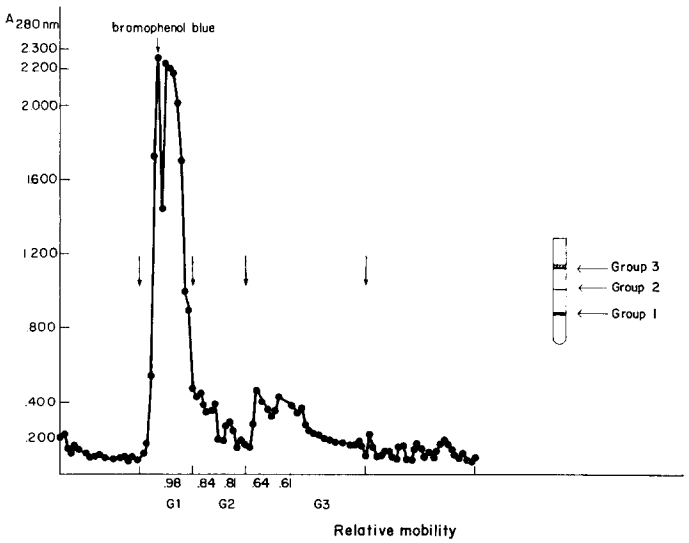

Fig. 1. Preparative electrophoresis in sodium dodecylsulphate of the "soluble" fraction from *n*-butanol-extracted *S. albus* membranes. "Soluble" fraction (85 mg/15 ml of 50 mM Tris–HCl, pH 7.5 containing 20 % (w/v) sucrose and 360 mg of sodium dodecylsulphate) was heated at 85 °C for 5 min and then electrophoresed as described under Materials and Methods. For other experimental details see the text. The arrows delimitate the three peaks or polypeptide groups: G1, G2 and G3. The insert illustrates schematically an analytical gel electrophoresis of the fraction.

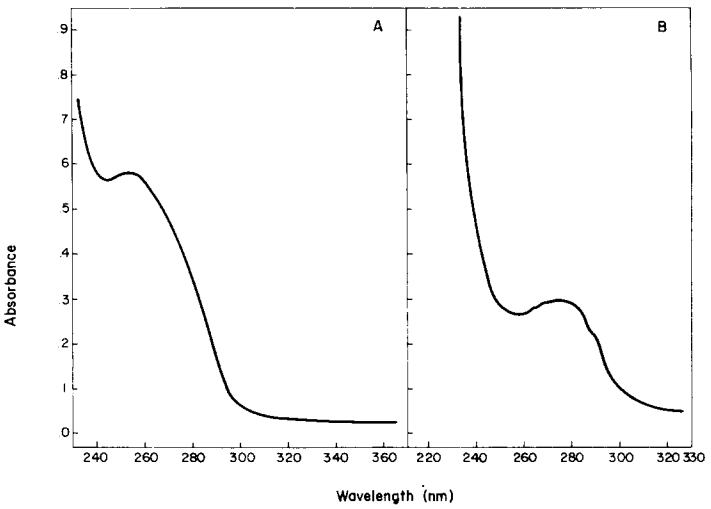

Fig. 2. Ultraviolet spectra of the polypeptide groups 1 (A) and 3 (B). The fractions were isolated by preparative electrophoresis in sodium dodecylsulphate and dialysed against 50 mM Tris and water as described in Materials and Methods. Distilled water was used as reference. Protein concentration by the method of Lowry et al. [2] was 1 mg/ml. The spectra were recorded as described under Materials and Methods using 1-mm light path cuvettes.

188 nm we could not find any maximum or indication of its approach, so confirming, that in spite of intensive dialysis against water, some detergent remained bound to the protein fraction (see below). On the other hand, the spectrum of Group 1 (A in the figure) reveals the predominance of nucleotide material. These findings may explain the high phosphorus content of Group 1, but they do not account for the relatively high percentage also found in Group 3.

Amino acid and N-terminal group composition

Hence, we could not yet conclude that Group 1 actually contained protein components, in spite of its staining with coomassie blue and its positive Lowry reaction. However, hydrolysis and amino acid analysis showed that Fraction 1 contained protein material. However, it is worth noting the low (approx. 30 %) recoveries obtained in this group as compared to the values estimated by the method of Lowry et al. [2]. Although we cannot rule out the possibility of a high destruction of amino acids during the hydrolysis, we are inclined to believe that it probably reflects an overestimation of protein by Lowry's method. This suggestion agrees with previous findings [1]. Table I shows the amino acid composition of Groups 1 and 3 compared to that of whole membrane. The composition of both polypeptide groups is very similar, although the variations in the content of some residues (e.g. glycine and glutamic acid), and in some indexes suggest they differ in their overall amino acid composition. By their polarity indexes, both polypeptide groups can be classified as "extrinsic" membrane proteins. In this regard, they differ from the whole membrane.

The determination of N-terminal amino acids allowed us to draw conclusions

TABLE I

AMINO ACID COMPOSITION OF *STREPTOMYCES ALBUS* G MEMBRANES AND OF TWO POLYPEPTIDE GROUPS FROM THEIR *n*-BUTANOL "SOLUBLE" FRACTION

The data are calculated from the extrapolated amino acid contents of the preparations after different times of hydrolysis (see Materials and Methods). The percentages of protein recovery as amino acid residues were 32 for Group 1 and 102 for Group 3.

Amino acid residue	Membranes (moles/100 moles)	Polypeptide Group 1 (moles/100 moles)	Polypeptide Group 3 (moles/100 moles)
Tryptophan	0.69	0.78	0.85
Lysine	3.76	4.55	4.91
Histidine	1.44	1.65	1.58
Arginine	5.75	5.84	6.41
Aspartic acid	9.47	9.32	9.61
Threonine	5.79	5.80	5.91
Serine	4.47	4.45	5.19
Glutamic acid	10.33	12.60	11.22
Proline	2.26	2.89	2.69
Glycine	4.59	12.53	11.27
Alanine	13.56	12.74	12.51
Cysteine*	—	—	—
Valine	7.86	7.56	7.52
Methionine	2.31	1.96	1.58
Isoleucine	4.02	3.35	3.88
Leucine	9.72	9.01	9.31
Tyrosine	1.95	1.96	2.39
Phenylalanine	4.27	3.10	3.08
$\frac{\text{Basic (Lys+Arg+His)}}{\text{Acidic (Asp+Glu)}} =$	0.55	0.55	0.62
Hatch index [10] =	1.29	1.54	1.70
Percent polar residues according to Vanderkooi and Capaldi [11] =	38.43	45.52	45.52
$\frac{\text{Asp+Glu+Lys+Arg+Ser+Thr(polar)}}{\text{Val+Leu+Ileu+Met+Met+Phe (apolar)}} =$	1.40	1.70	1.70

* Not measured. Cystine was not detected. According to the sensitivity limits of our experimental procedure, its contents must be <0.2 mole per 100 moles.

about the non-identity between the two groups. As shown in Table II, three N-terminal residues were found in each group, differing one from another except for alanine. However, this common residue is predominant in Group 3 while it is only a minor component of Group 1. The limited heterogeneity of these groups is not surprising taking into account that the fractionation procedure was based on molecular weight. As shown in Table II, it is also worth noting the good agreement between the estimated molecular weights by this procedure and those obtained by dodecylsulphate-gel electrophoresis [1]. This is particularly striking owing to the possible glycoprotein nature of some components of the groups [1].

TABLE II

N-TERMINAL AMINO ACIDS OF THE POLYPEPTIDE GROUPS ISOLATED BY SODIUM DODECYLSULPHATE PREPARATIVE ELECTROPHORESIS OF A "SOLUBLE" FRACTION FROM *STREPTOMYCES ALBUS* MEMBRANES

The protein amounts (Lowry et al. method [2]) were 2 mg for Group 1 and 1 mg for Group 3. Analysis was carried out as described under Materials and Methods. Based on the 32 % Lowry protein recovered as amino acid residues in Group 1 (see Table I), its actual protein content was taken as 0.64 mg. Its content of amino terminal residues per mg protein was corrected according to this and its molecular weight is therefore tentative.

Polypeptide group	Amino acid residue	Ratio	nmoles N-terminal residues/mg protein	Estimated molecular weight
1	Glutamic acid–lysine–alanine	1.7 : 1.3 : 1	66.7*	15 000
3	Alanine–methionine–leucine or isoleucine	2 : 1.7 : 1	18.86±1.5	53 000±5 000

* Corrected value (see above).

Ultracentrifugation analysis

Analytical ultracentrifugation confirmed the individuality of the two polypeptide groups as well as their existence in a "soluble" form. Results are shown in Fig. 3, the $S^0_{20,w}$ values being 1.54 S for Group 1 and 2.46 S for Group 3.

Effect of dodecylsulphate on gel electrophoresis patterns

In a previous work, we showed the modification of dodecylsulphate electrophoretic patterns of the "soluble" fraction by changing the dissociating conditions [1]. Two alternatives were suggested: (i) conversion to very low molecular weight peptides; (ii) changes in their hydrodynamic properties [12]. On the basis of the results presented above, the first alternative appears very unlikely. In an attempt to

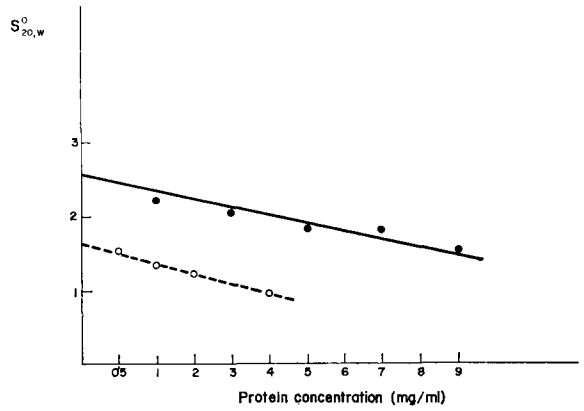

Fig. 3. Concentration dependence of the sedimentation coefficients of the polypeptide groups 1 (○ - - - ○) and 3 (● — ●). Protein concentration was estimated by the method of Lowry et al. [2]. Measurements were performed as described under Materials and Methods.

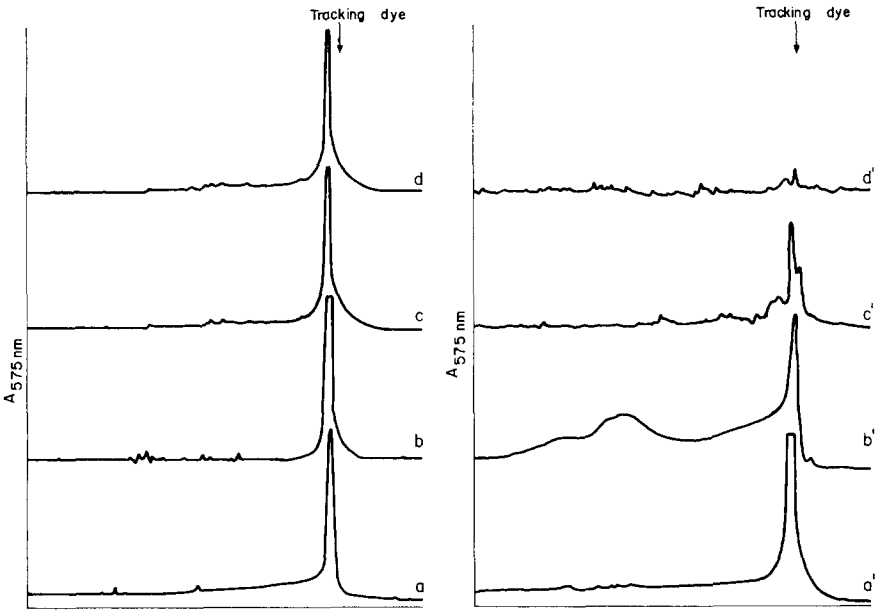

Fig. 4. Densitometric tracings of the electrophoretic patterns of polypeptide groups 1 and 3 under different conditions: Group 1 (a) and Group 3 (a′) exhaustively dialysed (see the text) were electrophoresed without sodium dodecylsulphate in gels and buffers. Group 1 (b) and Group 3 (b′) were dialysed against 50 mM Tris–HCl (pH 7.5)–0.1 % dodecylsulphate and electrophoresed in the same conditions as above. Group 1 (c) and Group 3 (c′) were treated with 1 % sodium dodecylsulphate for 5 min at 25 °C and run with 0.1 % dodecylsulphate in gels and buffer. Group 1 (d) and Group 3 (d′) heated with 1 % sodium dodecylsulphate (85 °C, 5 min) and run as for c and c′. Electrophoretic migration was from left to right. All samples contained 50 μg protein estimated by the method of Lowry et al. [2].

check the second possibility, we examined the electrophoretic behaviour of the isolated polypeptides under different conditions. The densitometric tracings of the gels obtained in these conditions are shown in Fig. 4. Analysis by gel electrophoresis of the fractions isolated as described above in the absence of dodecylsulphate, reveals the presence of a fast-moving band for both groups (see a and a′ in Fig. 4) and a spreading aggregate more evident for Group 3 (see a′). These results indicate that the polypeptides exist partially as aggregates, but are able to enter the gels. The detection of a band of relative mobility 1.0 for Group 3 in these conditions is surprising. Electrophoresis in the presence of dodecylsulphate results in a better definition of both groups. The addition of mercaptoethanol does not modify the gel patterns. Group 1 shows the presence of a fast-moving component independent of the conditions of treatment with detergent (b, c, and d in Fig. 4). On the other hand, Group 3 presents a more complex response. The addition of 0.1 % dodecylsulphate results in the individualization of two bands roughly corresponding to the original Groups 2 and 3 (see b′). As previously observed [1], the aggregate and the above-mentioned bands disappear by increasing the dissociating conditions, but this is not paralleled with an increment in the fast-moving component, (c′ and d′ in Fig. 4). At the same time, a marked decrease in total stain is observed. Gel electrophoresis in the presence of Triton X-100 shows in Fig. 5 the different behaviour of the two groups while revealing a similar

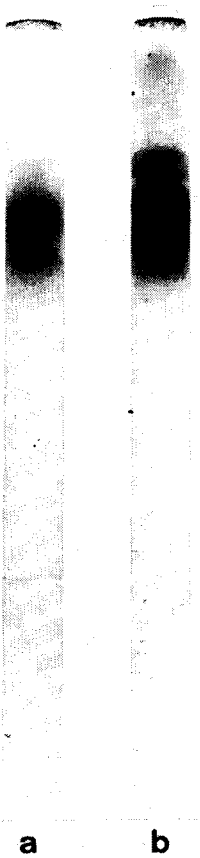

Fig. 5. Gel electrophoresis of polypeptide groups 1 (a) and 3 (b) in presence of Triton X-100. For experimental details see the text. Both samples contained 50 μg protein by Lowry et al. [2].

intensity of coomassie stain. From all these results it seems reasonable to conclude that changes in the hydrodynamic properties of Group 3 may modify its detection properties (i.e. lack of protein fixation or staining) and/or its electrophoretic mobility due to an abnormally high negative charge. Both effects can be due to a high capacity for binding dodecylsulphate and/or to bind it with high affinity.

Sodium dodecylsulphate binding

The "soluble" fraction bound a maximum of 0.40 mg dodecylsulphate/mg protein (protein estimated by the method of Lowry et al. or 0.55 mg of detergent/mg protein after correction for the overestimation of the protein concentration of Group 1 (see comments in Tables I and II). When compared to the binding of this detergent by soluble proteins [9, 13], the amount bound by this fraction was low. Therefore, although the binding experiments were carried out without pretreatment with guanidinium · HCl and reduction of possible S–S bridges, the anomalous behaviour of isolated Groups 1 and 3 to dodecylsulphate does not seem to be due to a high detergent-binding capacity. To test the possibility of a high affinity for binding, we exhaustively dialysed the fraction containing the bound dodecyl-[^{35}S]sulphate against 50

mM Tris (pH 7.5) and water. After several changes and 168 h of dialysis, the fraction still retained 0.037–0.046 mg of detergent bound/mg protein (concentration of protein estimated by the method of Lowry et al. [2]) or 0.051–0.063 mg/mg protein after correction of protein concentration (see above).

Circular dichroism

We considered it of interest to examine the circular dichroism of the two polypeptide groups in terms of the residually bound (high affinity) detergent. Fig. 6 shows the circular dichroism spectra of Group 1 in near and far ultraviolet light. The spectrum in near ultraviolet light indicated again the presence of nucleotide material. Interestingly, the spectrum in far ultraviolet light, with negative extrema at 222 and

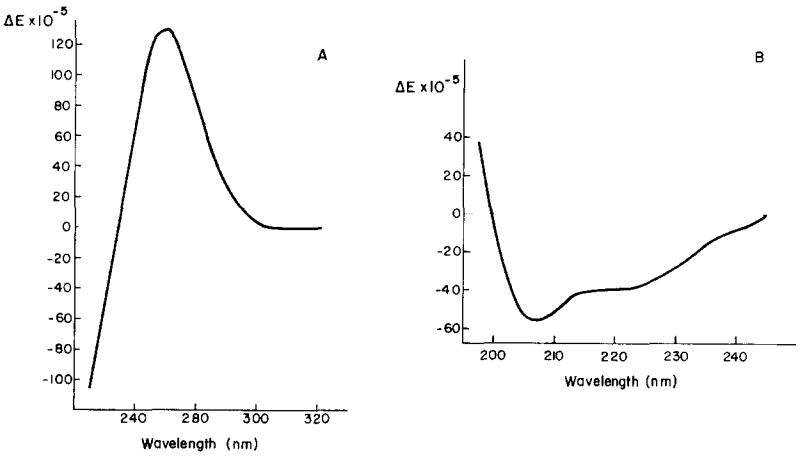

Fig. 6. Circular dichroism in the near (A) and far (B) ultraviolet light of polypeptide group 1. This fraction was dialysed and its spectra recorded as described under Materials and Methods using a 1-mm light path. Protein concentration by Lowry et al. [2] was 0.887 mg/ml.

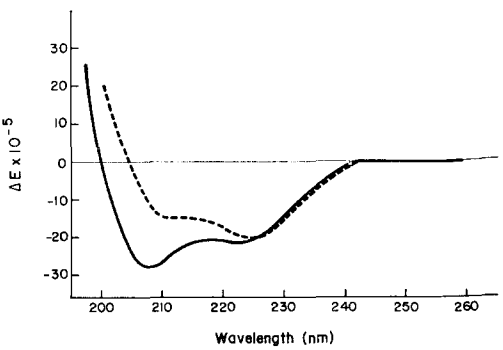

Fig. 7. Circular dichroism spectra in the far ultraviolet light of Group 3. The spectrum of this fraction was recorded with a 1-mm light path after dialysis as described in Materials and Methods (—). Protein concentration was 0.2 mg (Lowry et al. [2])/ml. Addition of phosphate buffer (pH 7.4) and sodium dodecylsulphate gave an identical spectrum (see the text). The spectrum of the fraction at pH 0.78 is also shown (- - -).

208 nm, was very similar to those obtained by other authors on enzyme proteins in the presence of dodecylsulphate [14, 15] except for a lower value of the dichroism. Group 3 did not give any circular dichroism in near ultraviolet light, in agreement with previous observations (i.e. absence of nucleotide material) and its circular dichroism in far ultraviolet light was similar to that of Fraction 1 (Fig. 7). The addition of sodium dodecylsulphate to this fraction did not modify its CD spectrum. This suggests that its initial features may well be due to firmly bound dodecylsulphate. Furthermore, drastic changes in pH are needed for a transition in the spectrum of the fraction (Fig. 7). Even at a pH value as low as 0.78 the transition was not yet complete.

DISCUSSION

The isolation of the two major polypeptide groups present in a "soluble" fraction of *n*-butanol-extracted *S. albus* membranes has provided information on approx. 20 % of the total membrane protein. We have shown that the fast-moving component is, at least in part, of protein nature. This is interesting in connection with the constant and predominant presence of similar components in sodium dodecylsulphate electrophoretic patterns of bacterial membranes (refs 16 and 17 and Muñoz, E., unpublished). These studies, while showing the great similarity in the amino acid composition of both groups of proteins, have presented evidence of their non-identity. Thus, the use of amino acid composition as a criterion of identity between isolated membrane proteins [18] appears very questionable. As judged by the values of several indexes based on their amino acid composition, the two polypeptide groups can be identified as "extrinsic", peripheral or "detachable" proteins [11] as suggested previously [1]. In this regard, the analogy of their amino acid composition to that of other "extrinsic" membrane proteins, such as bacterial and mitochondrial ATPases, is striking [19].

The limited complexity of the two polypeptide groups studied in this work, which accounted for about the 20 % of total membrane protein, suggests that certain repeating structures may exist in *S. albus* membranes. In this context, the study of the polypeptides of a similar size that pass into the insoluble phase after butanol extraction of the membranes [1] would be of interest.

Of particular importance in this work is the finding of residually bound dodecyl sulphate in the isolated fractions. This fact does not appear to be related to an extremely high level of detergent binding. On the contrary this amount is low as one would expect, since it has been generally admitted that glycoproteins [20, 21] and highly-charged proteins [13] bind less sodium dodecylsulphate than non-carbohydrate-containing proteins and relatively uncharged proteins. It is nonetheless surprising that the molecular weights of these polypeptides calculated from N-terminal amino acid determination are consistent with those estimated by dodecylsulphate-gel electrophoresis [1]. This is in contrast with previous findings in erythrocyte membrane glycoproteins [20]. The most plausible explanation for this may be the "high affinity" binding of dodecylsulphate by the *n*-butanol-"soluble" proteins of *S. albus* membrane. It is now well established that the action of sodium dodecylsulphate on proteins from non-membrane origin causes a change in their circular dichroism to give spectra similar to that of the α-helix [14, 15]. Subsequent removal of the detergent led to a

further change in the spectra of these proteins suggesting the appearance of appreciable amount of β structure [14]. It is interesting that the low amount of tightly bound detergent apparently imparts to these membrane proteins the same conformation that loosely bound detergent does to soluble enzymes [14, 15]. We do not know how general this property may be. If restricted to this case, *S. albus* membrane proteins offer a good model for studying the interaction between dodecylsulphate and protein. On the other hand, if it was of general application, it would raise important questions about the significance of immunological and reconstitution studies carried out with dodecylsulphate-dissociated membranes [22]. It is well to remember that the tight binding of dodecylsulphate by chymotrypsin has been recently reported [15].

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