Biochimica et Biophysica Acta, 465 (1977) 275—289

BBA 77631

PURIFICATION AND CHARACTERIZATION OF SPIRALIN, THE MAIN PROTEIN OF THE *SPIROPLASMA CITRI* MEMBRANE

HENRI WRÓBLEWSKI [a,*], KARL-ERIK JOHANSSON [b] and STELLAN HJÉRTEN [b]

[a] *Laboratoire de Biologie Cellulaire, Faculté des Sciences Biologiques, Complexe de Beaulieu, B.P. 25 A, 35031 Rennes Cédex (France) and* [b] *The Membrane Group, Institute of Biochemistry, Biomedical Center, University of Uppsala, Box 576, S-751 23 Uppsala (Sweden)*

(Received October 4th, 1976)

Summary

1. The membrane proteins from *Spiroplasma citri* have been resolved into 16 components by SDS-polyacrylamide gel electrophoresis. By this technique it was also shown that the molecular weights of these proteins ranged from 13 000 to 160 000. One of the proteins, which had an apparent molecular weight of 26 000 was the most abundant and represented more than 22% of total membrane protein. We have designated this protein spiralin. None of the proteins contained carbohydrate.

2. Spiralin has been isolated by a procedure which involves removal of some membrane proteins with the neutral detergent Tween 20, selective solubilization of the Tween residue in DOC and fractionation of the DOC-soluble material by agarose-suspension electrophoresis. The homogeneity of spiralin was demonstrated by analytical polyacrylamide gel electrophoresis under different conditions and by crossed immunoelectrophoresis.

3. Spiralin appeared to bind less DOC than the other membrane proteins of *S. citri*. This observation does not imply, however, that the binding of DOC to spiralin is weak. Spiralin was neither soluble in detergent-free buffers nor in Tween 20, which indicated that it is an intrinsic membrane protein.

4. The amino-acid composition of spiralin was quite different from that of the membrane. Spiralin lacked methionine, histidine and tryptophan, and had a low content of glycine, leucine, tyrosine and phenylalanine, but a high content of threonine, alanine and valine.

* To whom reprint requests should be addressed.

Abbreviations: SDS, sodium dodecyl sulfate; DOC, sodium deoxycholate; TMV, tobacco mosaic virus.

Introduction

Purification of membrane proteins is a fundamental step in the study of biomembrane structure since pure products are required for extensive investigations, including characterization of individual membrane components. Isolated membrane proteins also offer the possibility to build single protein membrane systems, which may be very useful models to correlate membrane structure with function at the molecular level [1].

In the present paper, we describe the purification and partial characterization of a mycoplasma membrane protein, which has been named spiralin because it is the main protein in the cell membrane of *Spiroplasma citri*. This organism, which has the unusual property of having a helical shape without being surrounded by a cell wall [2,3] is, like many other mycoplasmas, very suitable as a model in membrane research. The variability in electrophoretic rates of migration in agarose of DOC-solubilized *S. citri* membrane proteins has been described previously [4]. It should be possible to exploit these differences for the large scale purification of some of these components. This paper describes the purification of spiralin by agarose-suspension electrophoresis [5,6] in veronal buffer in the presence of deoxycholate.

Materials and Methods

Culturing of Spiroplasma citri and preparation of cell membranes

S. citri C 189 (ATCC 27665) was grown as described previously [4]. After osmotic lysis of the cells and digestion of DNA by deoxyribonuclease [3], the cell membranes were washed extensively with diluted β-buffer [7] as described previously [8] and once with distilled water before freeze-drying.

Preparation of antiserum against the cell membrane

Three rabbits were immunized by subcutaneous injections with a suspension of whole membranes mixed with Freund's complete adjuvant as described in a previous paper [4].

Solubilization of the cell membrane with different detergents

The membrane proteins were solubilized with the anionic detergents SDS, Sarkosyl (sodium lauryl-sarcosinate) and DOC as described earlier [9]. Briefly, membrane samples containing 20 mg protein per ml in 0.1 M Tris · HCl buffer (pH 8.0) were solubilized by the addition of an equal volume of either 0.2 M SDS, or 0.2 M Sarkosyl, or 0.5 M DOC in distilled water. The mixtures with Sarkosyl and DOC were kept at 0° C and the mixture with SDS was kept at 5° C for 1 h before centrifugation.

Solubilization of the membrane by this procedure with the neutral detergent Tween 20 was rather inefficient and the following modifications were made. A sample of membrane was mixed with an equal volume of 10% (w : v) Tween 20 in distilled water. The mixture was dialyzed for 48 h at 4° C against 5 mM glycine-NaOH buffer (pH 9.5) containing 1 mM EDTA, 5 mM 2-mercaptoethanol and 0.02% (w : v) sodium azide.

The supernatants obtained by centrifugation of the mixture at 180 000 $\times g$

for 1 h [9] were called SDS, Sarkosyl, DOC and Tween supernatants. The material insoluble in Tween 20, designated Tween residue, was solubilized with 0.25 M DOC in 0.05 M Tris · HCl buffer (pH 8.0) as described above.

Polyacrylamide gel electrophoresis

Experiments performed in tubes. These experiments were run in quartz tubes, which made it possible to detect the protein zones by scanning the gels at 280 nm. The gels were also scanned at 310 nm to identify artifacts [10]. The experiments were performed as described in the legend to Fig. 9 in a previous paper [9]. The gels were subsequently stained for proteins with Amido Black 10 B or with Coomassie Brilliant Blue R-250 and for glycoproteins by the periodic acid-Schiff technique [11].

Slab gel experiments. The experiments illustrated in Figs. 2B, 3, 5A and 5B were performed as described in the legend to Fig. 4, ref. 9 and that in Fig. 2A as described in the legend to Fig. 5, ref. 9. Human albumin, the coat protein of TMV, human carbonic anhydrase B and cytochrome *c* were used as markers for the molecular weight determinations [12,13]. The gels were stained with Amido Black 10 B.

Two-dimensional polyacrylamide-gel electrophoresis. The components in the DOC supernatant were fractionated by two-dimensional electrophoresis as described earlier [9] with the following modifications. The buffer was 0.1 M glycine-NaOH buffer (pH 9.1) in both directions. In the first direction 26 mM DOC was included in the gel and in the catholyte. In the second direction 20 mM SDS was included in the whole system. Other conditions were: voltage, 100 V; current, 70 mA; duration of the run, 2.5 h for both the first and the second direction. Before staining with Amido Black 10 B the DOC was removed from the gel by soaking in 25% isopropanol.

Analysis in pore gradient. The homogeneity of spiralin was checked by polyacrylamide-pore gradient gel electrophoresis. The experiments were run in the GE-4 apparatus from Pharmacia Fine Chemicals, Uppsala, Sweden. The concentration range of the gels, which were prepared in our laboratory, was T = 5—30% and C = 5%. The detergent-free buffer was either 0.1 M glycine-NaOH buffer (pH 9.1) or 80 mM Tris-acetic acid buffer (pH 8.6). The samples were run overnight at a voltage of 200 V and 100 V respectively. Human serum proteins and phycoerythrin, a coloured protein having a molecular weight of 290 000 [14], were used as markers to get a rough estimation of the size of spiralin under these conditions.

Immunoelectrophoresis experiments

Crossed immunoelectrophoresis. The purity of spiralin was determined by crossed immunoelectrophoresis [15,16] in agarose gel containing DOC to prevent aggregation of the amphiphilic membrane proteins [17,18]. Veronal buffer (pH 8.6, $I \doteq 0.03$) was used and 13 mM DOC was included in the gel [4]. In some experiments 13 mM DOC was also added to the catholyte which narrowed the width of the precipitation peaks. The electrophoresis was performed at 10° C in the first direction and at room temperature for the second direction. After completion of the electrophoresis, the plates were incubated at 37° C for 24 h, soaked for 48 h in 0.15 M NaCl, and for 1 h in distilled water. The plates

were then dehydrated and finally stained with Amido Black 10 B.

Fused-rocket immunoelectrophoresis. Fused-rocket immunoelectrophoresis [19] is a very sensitive method for the evaluation of a fractionation procedure, and can indicate which fractions to pool for maximal yield or purity of a certain component. The technique is especially useful if the proteins cannot be easily detected by absorbance measurements. 5 μl aliquots from fractions collected after agarose-suspension electrophoresis were applied, without removal of the agarose suspension, to a double row of wells (diameter: 3 mm) in an agarose gel strip (20 × 100 × 1 mm) in the same order as they were collected. The strip was placed along the long side of a glass plate (85 × 100 mm) and after 2 h incubation at 37°C, to allow diffusion of the proteins, the rest of the plate was covered with a 1 mm thick agarose gel containing antiserum against the *S. citri* membrane proteins. The electrophoresis was then run at room temperature with the sample wells on the cathodic side of the gel. Subsequent procedures were the same as in the crossed immunoelectrophoresis experiments.

Preparative agarose-suspension electrophoresis of the DOC supernatant. For agarose-suspension electrophoresis [5] a 0.17% suspension of agarose [20], is used as the supporting medium. The agarose was obtained from Bio-Rad Laboratories, Calif., U.S.A., the column is commercially available from Mr. Larsson, Sälgvägen 4, S-752 45 Uppsala, Sweden. Usually 0.5 ml of the DOC supernatant of the Tween residue, which contained approximately 3 mg of membrane material, was applied to the column. Before application a pellet of agarose obtained by centrifugation of 0.5 ml of the agarose suspension at 18 000 × g for 3 min was suspended in the sample. Veronal buffer (pH 8.6, I = 0.03) was used and the experiments were performed in the presence of 13 mM DOC in the column and in the catholyte. The conditions were as follows; voltage, 700 V; current, 12 mA; duration of the run, 10 h; temperature of the cooling water, 10°C. Bromophenol blue was applied 2 cm in front of the sample and used as marker. When the zone of bromophenol blue has migrated to the lower part of the column, it started to sharpen and when this phenomenon occurred the electrophoresis was stopped. The proteins were detected by measuring absorbances at 285 nm (instead of 280 nm since the buffer strongly absorbs light at this wavelength) and 310 nm [21]. The proteins were also detected by fused-rocket immunoelectrophoresis [19].

Amino acid analysis

Samples of both unfractionated membrane and spiralin were dialyzed against distilled water and lyopholized. The samples were then hydrolyzed for 24 h and 72 h in 6 M HCl at 110°C in thoroughly evacuated ampoules and the amino acid content was determined with a Durrum D-500 amino acid analyzer. The values for threonine and serine were extrapolated to zero time, assuming zero and first order kinetics, respectively. The amounts of valine, isoleucine and phenylalanine were determined after 72 h hydrolysis if these figures were higher than those obtained after the shorter hydrolysis time [22]. Half-cysteine and methionine were determined as cysteic acid and methionine sulfone, respectively, after performic acid oxidation [23]. The amount of proline was also determined after performic acid oxidation. The tryptophan content was determined with a Biocal BC 200 amino acid analyzer after hydrolysis in 3 M

p-toluene sulfonic acid at 115°C in thoroughly evacuated ampoules for 24 h [24]. The content of the other amino acids was calculated as the average value from the two determinations. Corrections for losses during 24 h hydrolysis were taken as 32% for the amino sugars.

Protein determination

Protein was determined under the assumption that dry membranes contained 46% (w : w) of protein [4].

Results and Discussion

The proteins of S. citri cell membrane

Fig. 1A shows a SDS-polyacrylamide gel electrophoresis of *S. citri* membrane proteins. Up to 16 components could be detected after staining for proteins.

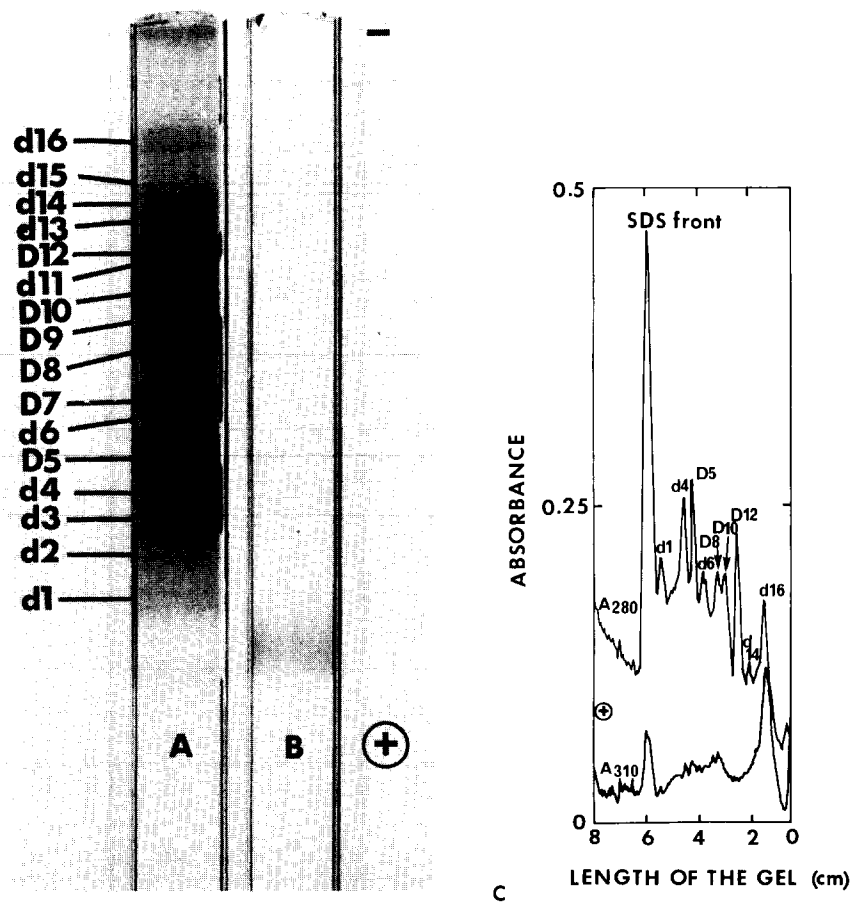

Fig. 1. SDS-polyacrylamide electrophoresis of membrane proteins solubilized with SDS. A. Gel stained with Amido Black 10 B. B. Gel stained with the Schiff reagent. C. Scanning diagram of the electrophoregram at 280 and 310 nm. D5 = spiralin.

Major and minor components are indicated by D and d, respectively, analogous to earlier experiments with *Acholeplasma laidlawii* [9]. A gel, which has been stained for carbohydrates, is shown in Fig. 1B. The carbohydrate positive zone did not coincide with any protein zone but rather with the SDS front, which indicates that there are no glycoproteins in the *S. citri* membrane. The glycolipids contained in the membrane [3] were probably responsible of this staining. Fig. 1C shows an ultraviolet scanning diagram of the gel shown in Fig. 1A before staining. Spiralin (component D 5) is the most heavily stained zone on the gel shown in Fig. 1A. In the scanning diagram, however, spiralin gives a comparatively small peak, which can be explained by its amino acid composition: spiralin lacks tryptophan and must, therefore, have a comparatively low specific absorbance (see *Amino acid composition of the S. citri membrane and of spiralin*, p. 284)

Figs. 2A and 2B show the result of molecular weight estimation of the membrane proteins by electrophoresis in SDS-polyacrylamide gel slabs. This technique permits more accurate comparative electrophoresis of proteins than the gel rod technique used previously [4]. The estimated molecular weights are

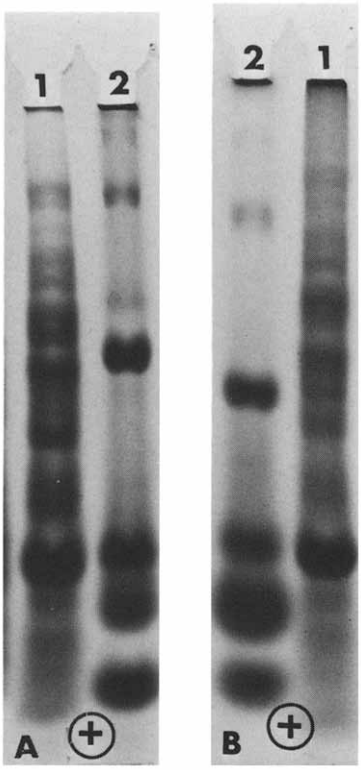

Fig. 2. Molecular weight estimation of membrane proteins by SDS-polyacrylamide gel electrophoresis. Samples: 1, membrane solubilized with SDS; 2, marker proteins: human serum albumin (mol. wt. 68 000), human carbonic anhydrase B (mol. wt. 29 000), the coat protein from TMV (mol. wt. 17 500) and cytochrome *c* (mol. wt. 17 200). Gels: A: electrophoresis in 0.05 M sodium phosphate buffer (pH 7.0) containing 0.02 M SDS and 0.05 M 2-mercaptoethanol; B: electrophoresis in 0.1 M Tris · HCl buffer (pH 8.0) containing 0.02 M SDS.

TABLE I

ESTIMATION OF MOLECULAR WEIGHTS OF *S. CITRI* MEMBRANE PROTEINS

Protein zone	Molecular weight	Protein zone	Molecular weight
d1	13 000	D10	69 000
d2	16 000	d11	81 000
d3	18 000	D12	89 000
d4	22 000	d13	107 000
D5 *	26 000	d14	120 000
d6	35 000	d15	135 000
D7	39 000	d16	160 000
D8	52 000		
D9	63 000		

* D5 = spiralin.

listed in Table I. They range from 13 000 to 160 000, spiralin having a molecular weight of 26 000. The same results were obtained both with (Fig. 2A) and without (Fig. 2B) 2-mercaptoethanol.

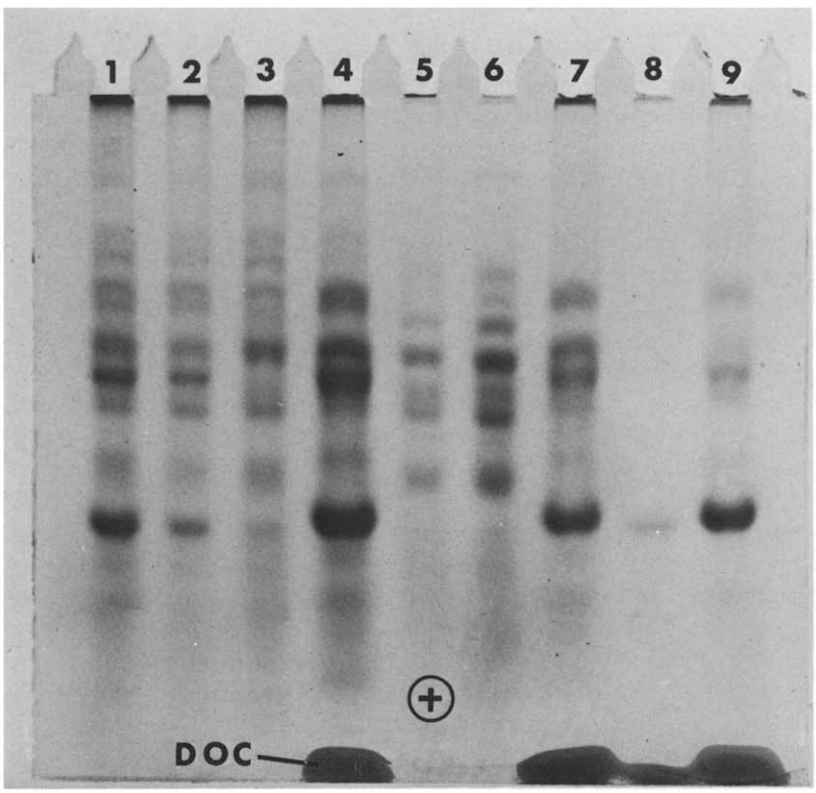

Fig. 3. SDS-polyacrylamide gel electrophoresis of proteins solubilized by different detergents. Samples: 1, 1, 25 µl of SDS supernatant; 2, 10 µl of SDS supernatant; 3, 25 µl of Sarkosyl supernatant; 4, 50 µl of DOC supernatant; 5, 50 µl of Tween supernatant; 6, 100 µl of the Tween supernatant; 7, 30 µl of DOC supernatant; 8, 10 µl of DOC supernatant of the Sarkosyl residue; 9, 50 µl of DOC supernatant of the Tween residue.

Solubilization of the proteins by different detergents

The aim of these experiments was to select a detergent for the extraction of spiralin from the membrane as efficiently and as selectively as possible. The membrane was solubilized by the ionic detergents SDS, Sarkosyl and DOC and by the neutral detergent Tween 20. The electrophoregram of the proteins solubilized by SDS was used as a reference since this detergent quantitatively solubilized all the membrane proteins.

The electrophoregram of Fig. 3 shows that spiralin was solubilized most efficiently and selectively by DOC. The protein was extracted quantitatively whereas some other proteins remained in the residue, especially those having the higher molecular weight. By comparison with the other proteins of the membrane, spiralin was poorly soluble in Sarkosyl. It is to be noted that Tween 20 solubilized the same proteins as DOC, with the exception of spiralin which seemed to be insoluble in this detergent. The results indicated that, in contrast to other detergents, DOC had a rather high affinity for spiralin, and this was exploited for a preparative purpose in the following way. The membrane was first extracted with Tween 20 to remove the proteins soluble in this detergent. The residue was then extracted by DOC to give a spiralin rich supernatant (compare the compositions of samples 1, 7 and 9 in Fig. 3).

Purification of spiralin by agarose-suspension electrophoresis

Fig. 4 shows the electrophoregram and the fused-rocket immunoelectrophoresis of an agarose-suspension electrophoresis of the DOC extract of the Tween residue. These results indicate that the proteins were separated into three zones. The fractions corresponding to the different zones were pooled into three fractions as indicated on the electrophoregram. After concentration they were analyzed by SDS-polyacrylamide gel electrophoresis (Figs. 5A and 5B). Fraction I contained most of the proteins present in the DOC supernatant of the Tween residue, but comparatively little of spiralin. Fraction II contained only spiralin. Fraction III contained mainly component d 11. Fig. 5B summarises the extraction procedure for spiralin.

Most of proteins bind approximately the same amount of SDS per weight unit, and consequently become equally charged per unit area [25]. It is usually not possible to separate proteins efficiently by agarose suspension electrophoresis in the presence of SDS, since electrophoresis in this non-sieving medium separates molecules chiefly according to their surface charge density [5]. Amphiphilic proteins can bind large amounts of the anionic detergent DOC, often without denaturation [26,27]. It can thus be expected that intrinsic membrane proteins will behave in agarose suspension electrophoresis in the presence of DOC in a similar manner to SDS-solubilized proteins. Most of the DOC soluble proteins from *S. citri* membrane behaved as expected in agarose suspension electrophoresis, but spiralin and component d11 moved more slowly. Our interpretation of this result is that spiralin binds less DOC per unit area than the majority of other DOC soluble proteins from *S. citri* membrane. This does not necessarily mean that the binding of DOC by spiralin is weak.

Crossed immunoelectrophoresis of spiralin

Homogeneity of spiralin was checked by crossed immunoelectrophoresis

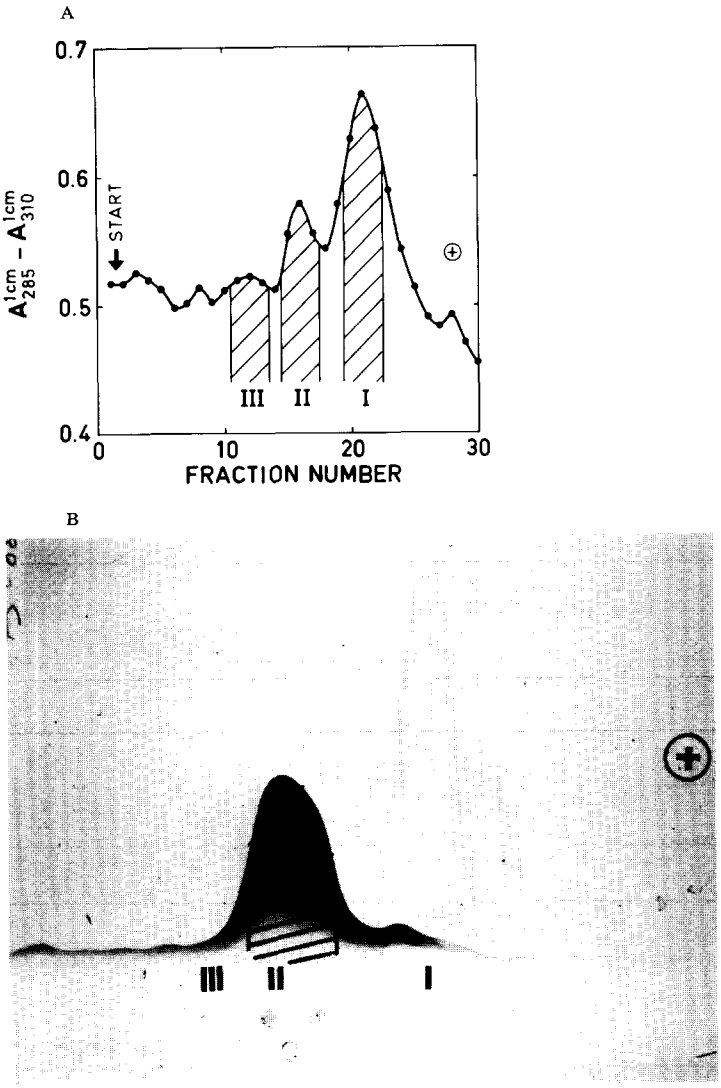

Fig. 4. Fractionation of the DOC supernatant of the Tween residue by agarose-suspension electrophoresis. A. Absorbance curve of the fractions. The fractions corresponding to the hatched areas in the electrophoregram were concentrated and checked for homogeneity. B. Fused-rocket immunoelectrophoresis of the same fractionation.

with a serum containing antibodies directed to the whole membrane of *S. citri*. Spiralin (Fig. 6A) was compared with the unfractionated DOC supernatant of the Tween residue. The spiralin sample contained a large excess of the protein to show that it was not contamined by minor components. In immunoelectrophoresis, the most dominant precipitation line was the one corresponding to spiralin. This precipitation line was extremely heavily stained and the area covered by it was always slightly coloured. Since spiralin is the major component in the *S. citri* membrane, production of antibodies in the rabbit against other proteins than spiralin may be diminished. Spiralin could also be more immunogenic than other proteins in the membrane.

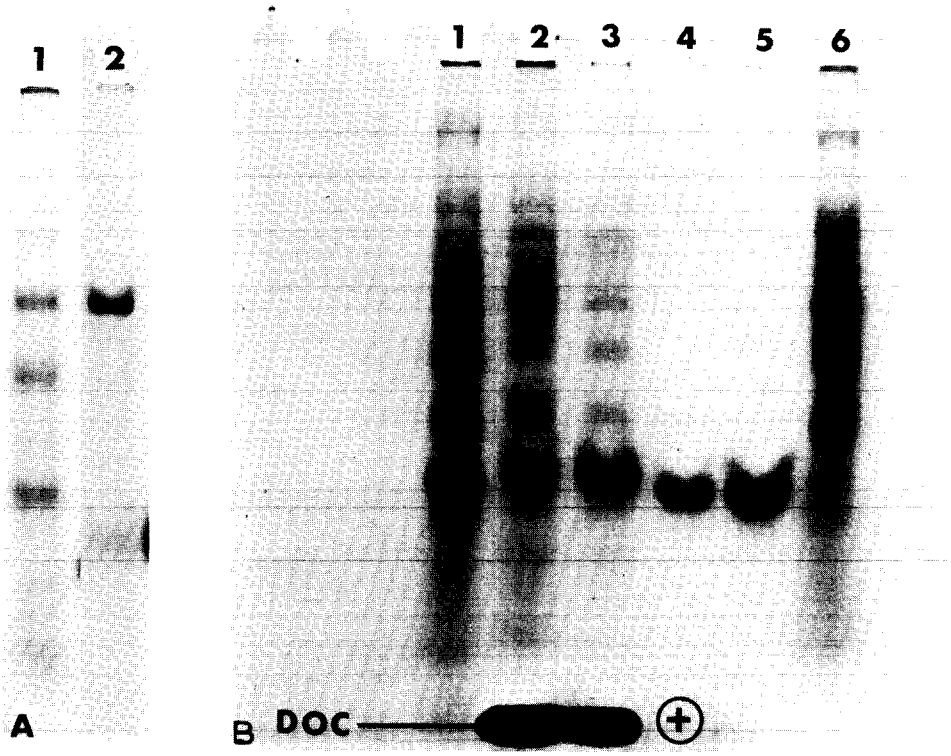

Fig. 5. SDS-polyacrylamide electrophoresis of fractions I, II and III of the agarose suspension electrophoresis. Samples: A: 1, 50 µl of fraction I; 2, 50 µl of fraction III; B: 1, 30 µl of SDS supernatant; 2, 50 µl of DOC supernatant; 3, 50 µl of the DOC supernatant of the Tween residue; 4, 50 µl of fraction II (purified spiralin); 5, 100 µl of fraction II; 6, 100 µl of Tween supernatant.

Electrophoretic behaviour of spiralin in different systems

Spiralin was analyzed by SDS-polyacrylamide electrophoresis in gels of different concentrations. The protein was homogeneous in all the experiments (Figs 7A—C). It was also possible to perform electrophoresis of purified spiralin on an analytical scale in the absence of detergent and without precipitation (Fig. 7D). However, when spiralin was analyzed by polyacrylamide gradient gel electrophoresis without detergent (Figs. 8A and 8B), the zone was diffuse and its position corresponded to a molecular weight much larger than 300 000. This observation has also been noted for purified membrane proteins of *A. laidlawii* (Johansson, K.E. and Hjertén, S., unpublished), and indicates that the proteins precipitated in the gels at a critical protein concentration. Fig. 9 shows a two-dimensional polyacrylamide-gel electrophoresis of the DOC supernatant. All proteins, except spiralin, fall on an approximately straight line, indicating that spiralin may differ from the other proteins of *S. citri* membrane in DOC binding.

Amino acid composition of the S. citri membrane and of spiralin

Table II shows the amino acid and amino sugar composition of spiralin and

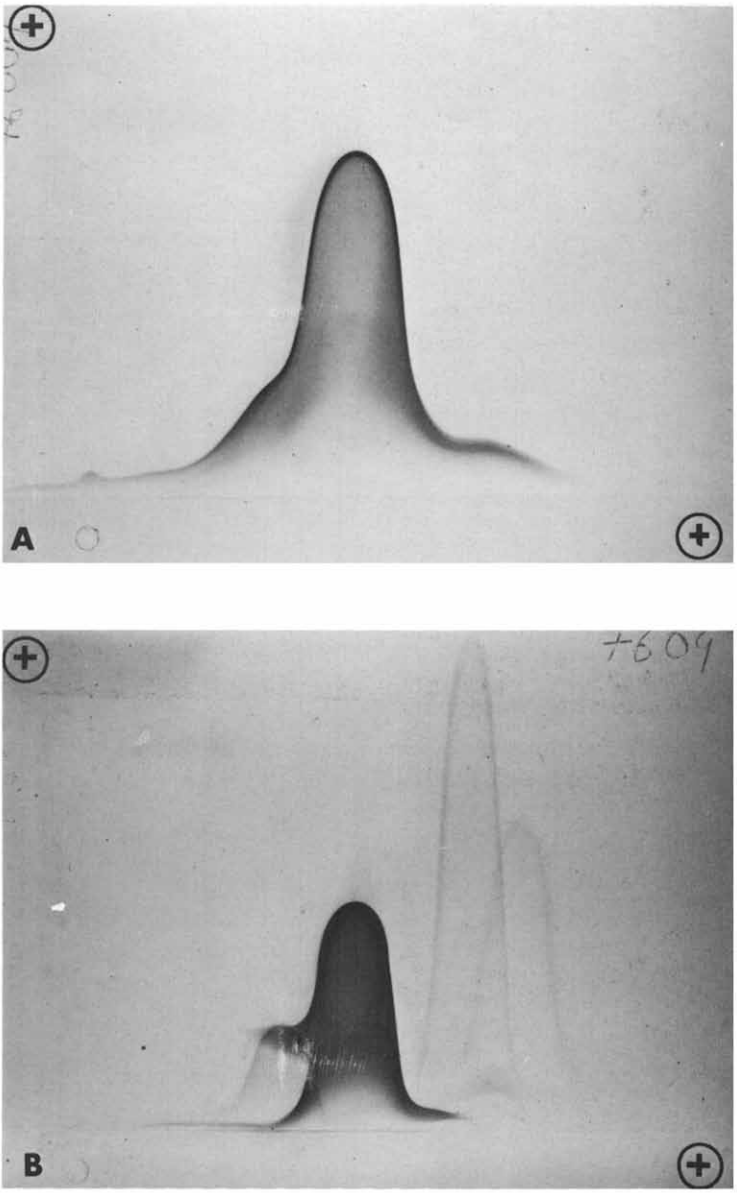

Fig. 6. Crossed immunoelectrophoresis of purified spiralin. Plates: A, Fraction II (purified spiralin); B, DOC supernatant of the Tween residue. The experimental conditions have been published earlier [4].

of the unfractionated membrane. The membrane contained amino sugars, but not spiralin, which confirms that this component is not a glycoprotein. This means that the electrophoretic behaviour of spiralin depended only on its amino acid composition and distribution.

The amino acid composition of spiralin was quite different from that of the membrane. Spiralin did not contain methionine, histidine, and tryptophan, and had a low content of glycine, leucine, tyrosine and phenylalanine, but a high

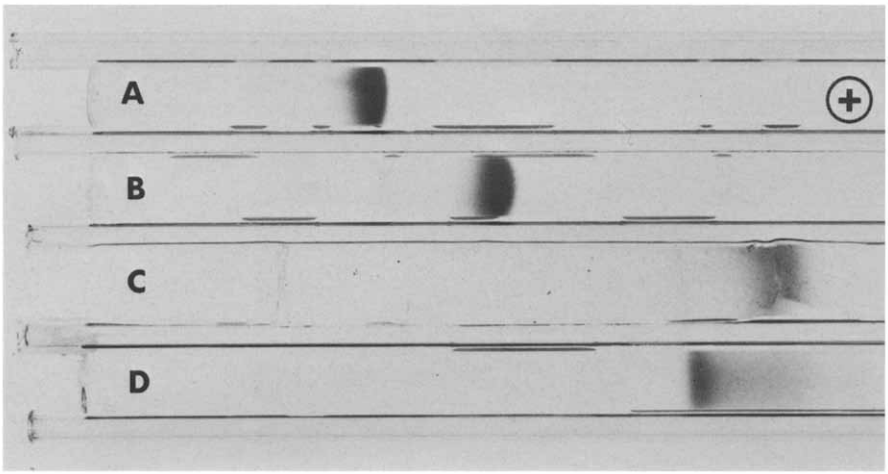

Fig. 7. Polyacrylamide gel electrophoresis of spiralin in different gel systems. Buffers: A, B and C, 0.1 M Tris · HCl buffer (pH 8.0) containing 20 mM SDS; D, 0.1 M glycine-NaOH buffer (pH 9.1) without detergent. Compositions of the gels: A: T = 8% C = 3%; B: T = 6%, C = 3%; C: T = 4%, C = 2%; D: T = 8%, C = 3%; [30].

content of threonine, alanine and valine. The unknown amino acid might have been ornithine or methylated lysine, but it was probably an artifact since it was not present in the membrane sample.

Hydrophobicities of spiralin and of whole membrane proteins have been cal-

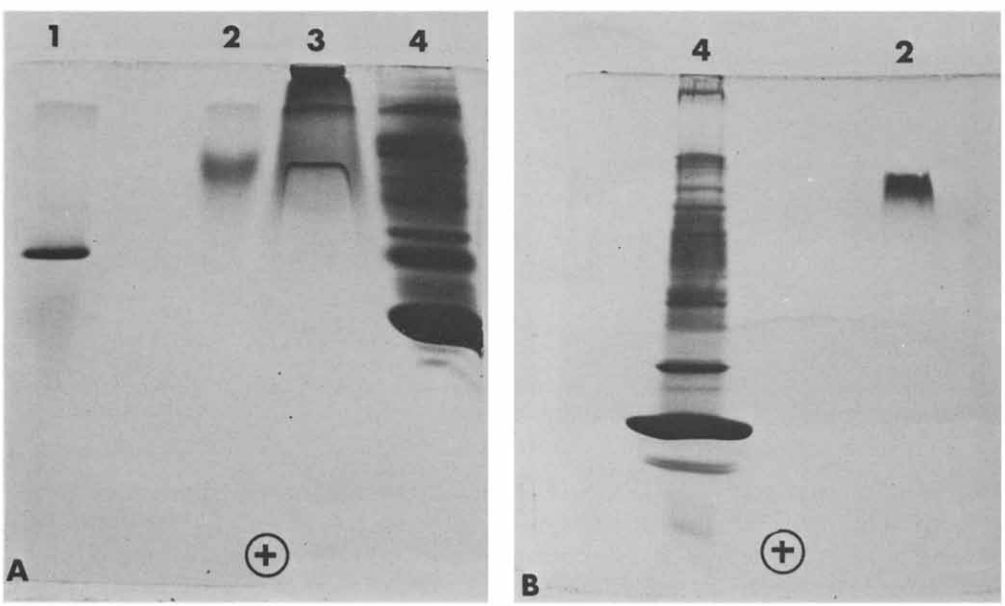

Fig. 8. Electrophoresis of spiralin in polyacrylamide-pore gradient gels. Buffers: A, 0.1 M glycine-NaOH buffer (pH 9.1); B, 80 mM Tris-acetic acid buffer (pH 8.6). Samples: 1, phycoerythrin; 2, spiralin; 3, DOC supernatant; 4, human serum.

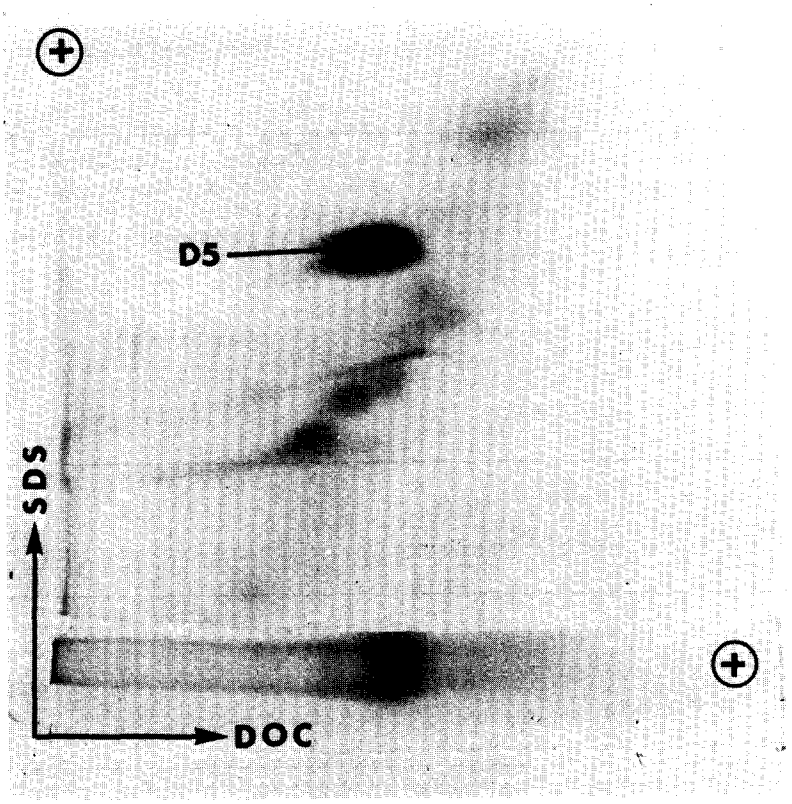

Fig. 9. Two-dimensional polyacrylamide-gel electrophoresis of the DOC supernatant. A photograph of a stained section of the run in DOC has been inserted on the bottom of the SDS gel. D5 = spiralin.

culated from their amino acid composition by multiplying the hydrophobicity of each amino acid [28] by its concentration expressed as mole per cent and summing the products. The hydrophobicities for the *S. citri* membrane and spiralin were found to be 102 and 93 kcal/mol, respectively. These values are not extreme and should be compared with the values for purified membrane proteins from *A. laidlawii* which are in the range of 95—100 kcal/mol (Johansson, K.E. and Hjertén, S., unpublished). The hydrophobicity values of water soluble proteins are often in this range. This shows that spiralin is not an extremely hydrophobic protein. However it is not possible to extract it from *S. citri* cell membrane with aqueous solutions, even with the procedure of Marchesi et al. [29] or with Tween and only poorly with Sarkosyl. Furthermore it precipitated when DOC was removed from the solution by dialysis (see next section) and so spiralin must be regarded as an intrinsic membrane protein. These solubility properties suggest that spiralin may be bound to the membrane by a sequential or a conformational hydrophobic anchor which is buried in the apolar region of the phospholipid bilayer.

The amount of spiralin in the S. citri membrane

Spiralin was extracted from 160 mg (dry weight) of membranes containing

TABLE II

THE AMINO ACID COMPOSITION OF THE *S. CITRI* MEMBRANE AND ITS MAIN COMPONENT SPIRALIN

The amount of amino sugar is expressed as mol per 100 mol of amino acid. The deviation in per cent of the amino acid composition of spiralin compared to the membrane is also given in the table. By deviation we mean the difference (in per cent) in amino acid composition between spiralin and the unfractionated membrane. The signs + and — indicate that spiralin contains more and less, respectively, of the corresponding amino acid. The values for histidine and arginine have been placed between parentheses, since the low values for these amino acids in spiralin are not accurate. The amount of each amino acid residue in spiralin has been calculated assuming a molecular weight of 26 000 for this protein.

Amino acid	*S. citri* membrane (mol %)	Spiraline		
		mol %	Deviation (%)	Residues molecule
Asp	11.53	13.53	+19	33
Thr	6.94	9.41	+36	23
Ser	5.08	4.58	—10	11
Glu	10.23	10.44	+2	26
Pro	3.62	4.21	+16	10
Gly	7.24	5.07	—30	13
Ala	8.32	12.50	+50	31
1/2 Cys	0.67	0.64	—5	2
Val	7.76	11.91	+50	29
Met	1.65	0	—	0
Iso	8.10	6.86	—15	17
Leu	7.43	4.92	—34	12
Tyr	3.04	2.26	—26	6
Phe	3.48	1.55	—55	4
His	1.20	0.11	(—91)	0
Unknown	0	0.25	—	0
Lys	10.11	11.50	+14	28
Trp	0.74	0	—	0
Arg	2.84	0.25	(—91)	1
GlCn	0.21	0	—	
GalN	traces	0	—	

74 mg of protein. The pure spiralin from agarose suspension electrophoresis was dialyzed at 5° C for 72 h against 8 l of veronal buffer (pH 8.6, I = 0.03) containing 0.02% sodium azide and for 48 H against 6 l of 10 mM ammonium bicarbonate buffer (pH 8.0) to remove the DOC. Spiralin precipitated during the dialysis and 12 mg of the protein were recovered by freeze-drying after acidification of the buffer with acetic acid. The yield calculated by dividing the hatched area in Fig. 4 B by the total area of the peak, was of 71.4%. If it is assumed that the extraction of spiralin with DOC was quantitative, and losses during dialysis were negligible, then the starting material contained at least 10.5% of the membrane dry weight, i.e. at least 22.1% of total membrane protein. Though the function of spiralin is yet unknown, such a predominant protein probably plays an important role in the physiology of the *S. citri* cell. It is tempting to speculate that spiralin has a structural function related to the helical morphology of the *S. citri* cell.

Acknowledgements

The many valuable discussions with Dr. David Eaker are gratefully acknowledged. We want to thank Irja Blomqvist and Anne-Marie Touzalin for help with some of the experiments. We also thank Dr. David Grant for constructive criticism. Human carbonic anhydrase and TMV were generous gifts from Professor Bror Strandberg and Dr. Per Oxelfeldt, respectively. The work has been supported financially by the Centre National de la Recherche Scientifique (LA No. 256, Contrat C.N.R.S.-Université), by the Swedish Natural Science Research Council, the Wallenberg Foundation, the Foundation of Ollie and Elof Ericsson, and the von Kantzow Foundation.

References

1 Vanderkooi, G. (1974) Biochim. Biophys. Acta 344, 307—345
2 Cole, R.M., Tully, J.G., Popkin, T.J. and Bove, J.M. (1973) J. Bacteriol. 115, 367—386
3 Razin, S., Hazin, M., Ne'eman, Z. and Rottem, S. (1973) J. Barteriol. 116, 1421—1435
4 Wroblewski, H. (1975) Biochimie 57, 1095—1098
5 Hjertén, S. (1963) J. Chromatogr. 12, 510—526
6 Johansson, K.E. (1974) In Protides of the Biological Fluids (Peeters, H., ed.), Vol. 21, pp. 151—156, Pergamon Press, Oxford
7 Razin, S., Morowitz, H.J. and Terry, T.M. (1965) Proc. Natl. Acad. Sci., U.S. 54, 219—225
8 Johansson, K.E., Blomqvist, I. and Hjertén, S. (1975) J. Biol. Chem. 250, 2463—2469
9 Hjertén, S. and Johansson, K.E. (1972) Biochim. Biophys. Acta 288, 312—325
10 Fries, E. and Hjertén, S. (1975) Anal. Biochem. 64, 466—476
11 Zacharius, R.M., Zell, T.E., Morrison, J.H. and Woodlock, J.J. (1969) Anal. Biochem. 30, 148—152
12 Shapiro, A.L., Vinuela, E. and Maizel, J.V. (1967) Biochem. Biophys. Res. Commun. 28, 815—820
13 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406—4412
14 O'Carra, P. (1965) Biochem. J. 94, 171—174
15 Laurell, C.B. (1965) Anal. Biochem. 10, 358—361
16 Clarke, H.G.M. and Freeman, T.A. (1967) In Protides of the Biological Fluids (Peeters, H., ed.), Vol. 14, pp. 503—509, Elsevier, Amsterdam
17 Johansson, K.E. and Hjertén, S. (1974) J. Mol. Biol. 86, 341—348
18 Bjerrum, O.J. and Lundahl, P. (1974) Biochim. Biophys. Acta 342, 69—80
19 Svendsen, P.J. (1973) Scand. J. Immunol. 2, suppl. 1, 69—70
20 Hjertén, S. (1971) J. Chromatogr. 61, 73—80
21 Hjertén, S., Höglund, S. and Ruttkay-Nedecky, G. (1970) Acta Virol. 14, 89—101
22 Eveleigh, J.W. and Winter, G.D. (1970) in Protein Sequence Determination (Needleman, S.B., ed.), pp. 91—123, Springer-Verlag, New York
23 Moore, S. (1963) J. Biol. Chem. 238, 235—237
24 Lin, T.Y. and Chang, Y.H. (1971) J. Biol. Chem. 246, 2842—2848
25 Reynolds, J.A. and Tanford, C. (1970) J. Biol. Chem. 245, 5161—5165
26 Helenius, A. and Simons, K. (1972) J. Biol. Chem. 247, 3656—3661
27 Makino, S., Reynolds, J.A. and Tanford, C. (1973) J. Biol. Chem. 248, 4926—4932
28 Nozaki, Y. and Tanford, C. (1971) J. Biol. Chem. 246, 2211—2217
29 Marchesi, S.L., Steers, E., Marchesi, V.T. and Tillack, T.W. (1970) Biochemistry 9, 50—57
30 Hjertén, S. (1962) Arch. Biochem. Biophys. suppl. 1, 147—151