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Amphiphilic Nature of Spiralin, the Major Protein of the *Spiroplasma citri* Cell Membrane

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Spiralin could not be solubilized in the absence of detergents, and it was shown by charge-shift crossed immunoelectrophoresis that this protein was capable of binding detergents under nondenaturing conditions. These properties indicate the amphiphilic nature of spiralin, which therefore should be regarded as an intrinsic membrane protein. The efficiency of mild (ionic and neutral) detergents to solubilize spiralin was as follows: deoxycholate > lauroyl sarcosinate, cholate, taurocholate, taurodeoxycholate > Triton X-100 > Brij 58 > Tween 20, indicating that mild ionic detergents were more effective than neutral ones. Solubilization of spiralin was quantitative with sodium deoxycholate. It was also shown that although a membrane protein is not extractable by a given detergent from the membrane, this does not necessarily mean that the protein is not soluble in this detergent.

Spiralin is the most abundant protein in the membrane of *Spiroplasma citri* (17), a mycoplasma which differs from other mycoplasmas by its helical shape (4). The cellular function of this protein is unknown, although some of its properties have been described. In brief, spiralin is a protein of 26 kilodaltons (estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and which lacks methionine, tyrosine, and tryptophan (17). It is possible to quantitatively extract this protein from the *S. citri* membrane with the bile salt sodium deoxycholate (DOC) or with sodium dodecyl sulfate (17). Sodium lauroyl sarcosinate (Sarkosyl) and Tween 20, on the other hand, are relatively ineffective for releasing spiralin from the membrane (15, 17). Some of these observations suggest that spiralin is an intrinsic membrane protein. The purpose of the present work was to validate this assumption by showing that this protein is insoluble in detergent-free buffers and is able to bind detergents in nondenaturing conditions. It is indeed of utmost importance to know whether or not a membrane protein is amphiphilic to correctly interpret structural data concerning this protein and to develop a rational strategy to ascertain its function. The solubility of spiralin in several mild detergents was also investigated.

S. citri strain C 189 (ATCC 27665) (11) was grown as previously described (14) except that the concentration of foal serum in the culture medium was reduced to 5% (vol/vol). The cells were harvested by centrifugation (15,000 × *g*, 15 min, 4°C), dispersed into 0.1 M Tris-hydrochloride (pH 8.0), and disrupted by sonication (20 kilocycles, 2 × 1 min, 0°C). The membranes were pelleted by centrifugation (40,000 × *g*, 1 h, 4°C) and extensively washed with 0.1 M Tris-hydrochloride buffer (pH 8.0). Spiralin was purified from the membranes by agarose suspension electrophoresis (7, 8) as described elsewhere (17). To prepare a spiralin-lecithin proteoliposome, 50 mg of DL-α-lecithin was added to 10 ml of Veronal buffer (pH 8.6; ionic strength, 0.03) containing 15 mg of spiralin, 26 mM DOC, and 1 mM 2-mercaptoethanol. The lipid was solubilized by sonication (20 kilocycles, 3 × 1 min, 0°C), and 5 ml of distilled water was then added. The solution was then dialyzed for 72 h against 6 × 1 liter of Veronal buffer (pH 8.6, I = 0.03, 0.02% NaN_3) to remove the detergent. The last buffer change contained 10 mM $MgCl_2$. The spiralin-lecithin proteoliposome was pelleted by centrifugation (40,000 × *g*, 1 h, 4°C), washed twice with 10 mM ammonium bicarbonate buffer (pH 8.0), and lyophilized after acidification of the buffer with acetic acid. Phycoerythrin from *Ceramium rubrum* was kindly supplied by Irja Johansson (Institute of Biochemistry, Uppsala, Sweden). Crossed immunoelectrophoresis (CIE) (3, 9) was performed in 1-mm-thick agarose gels cast on glass plates (10 by 8.5 cm). The agarose concentration was 1% (wt/vol) in Veronal buffer (pH 8.6, I = 0.03) containing detergent(s) (2, 10). Triton X-100, DOC, and cetyltrimethylammonium bromide (CTAB) were used at the concentrations and in the combinations described elsewhere (7). Electroimmunoassay

(14) was performed in agarose gels of the same size as those for CIE; the Veronal buffer contained 14 mM DOC in the gel and in the catholyte. Antisera containing antibodies directed against spiralin or phycoerythrin were prepared by following a procedure described previously (14). Other procedures concerning immunoprecipitation in gel experiments were described in previous publications (16, 17).

It was not possible to release spiralin from the *S. citri* membrane with detergent-free buffers, even in the presence of 2-mercaptoethanol and EDTA, in contrast to several other proteins. This criterion is often regarded as sufficient to decide whether a membrane protein is intrinsic or extrinsic. The use of this criterion alone, however, may sometimes be misleading (12). For example, an extrinsic membrane protein cannot be released from a membrane with detergent-free buffers if the membrane is in the form of a closed vesicle and the protein is localized on the inner surface of the membrane. It is therefore necessary to invoke stricter criteria, such as the capacity to bind a nondenaturing detergent. This property is specific to amphiphilic proteins (e.g., intrinsic membrane proteins) (5, 13) and simple techniques such as charge-shift CIE (6) are now available to demonstrate this property. It is indeed possible to induce a shift in the electrophoretic mobility of a protein–Triton X-100 complex by the addition of a small amount of an ionic detergent which will modify the net charge of the complex (6). Charge-shift CIE is a two-dimensional electrophoresis in which the first directional electrophoresis is a charge-shift electrophoresis and the second an immunoelectrophoresis (1). Figure 1 shows that spiralin did not move electrophoretically at pH 8.6 in the presence of the neutral detergent Triton X-100. Anodic or cathodic mobilities were induced by DOC (anionic detergent) and by CTAB (cationic detergent), respectively, when these detergents were added to Triton X-100. The electrophoretic migration of the water-soluble protein phycoerythrin was not affected by the two ionic detergents. It should nevertheless be noted that the precipitation lines corresponding to both spiralin and phycoerythrin exhibited a shoulder in the presence of CTAB. This phenomenon eludes explanation at present. If necessary, it is possible to use detergents in only the first dimension of charge-shift CIE (1); however, it is better to use them in both dimensions, since well-developed precipitation lines are not obtained in the absence of an ionic detergent in the cases of proteins which, like spiralin, do not move electrophoretically at a pH in the range usually used for CIE.

To estimate the solubility of spiralin in different mild (anionic or neutral) detergents, the proteoliposome spiralin-lecithin was treated with four bile salts (DOC, cholate, taurocholate, and taurodeoxycholate), Sarkosyl, and three nonionic detergents (Triton X-100, Brij 58, and Tween 20). The solubilized protein was then titrated with a monospecific serum containing antibodies directed against spiralin by electroimmunoassay in the presence of DOC. The proteoliposome was chosen as the starting material because it was not possible to solubilize pure spiralin (i.e., free of lipid, or detergent, or both) with mild detergents. It was first necessary to denature the protein (e.g., with guanidinium-hydrochloride) in the presence of the mild detergent. Spiralin could be solubilized in those conditions and remained in solution in the detergent after removal of guanidinium-hydrochloride. In contrast, it was possible to directly solubilize

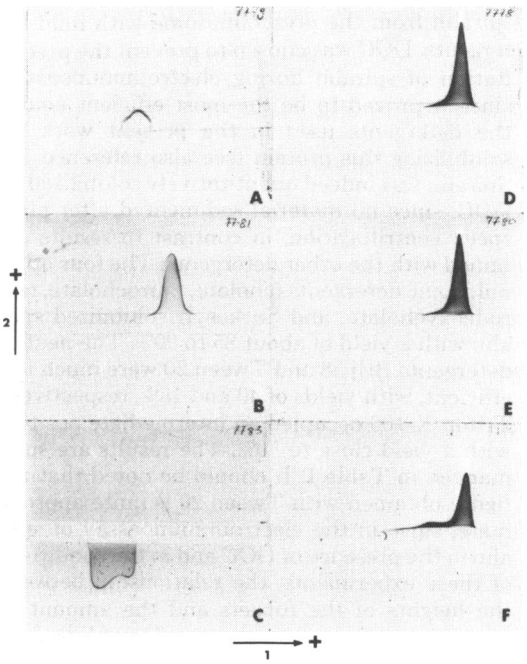

FIG. 1. *Charge-shift CIE of spiralin (A, B, and C) and of phycoerythrin (D, E, and F). Each well was loaded with 10 μg of protein. The amount of gel was 100 μl·cm^{-2}. The antiserum concentration was 3.5 μl·cm^{-2} in both experiments. Detergents were as follows: 2% Triton X-100 (A and D), a mixture of 2% Triton X-100 and 1% DOC (B and E), and a mixture of 2% Triton X-100 and 0.2% CTAB (C and F). First direction: current, 15 mA/plate; duration of the run, 70 min; temperature, 5°C. Second direction: current, 4 mA/plate; duration of the run, 18 h; room temperature. Anode at right and top (see the arrows). The immunoprecipitates were stained with amido black 10 B.*

spiralin from the proteoliposome with mild detergents. DOC was chosen to prevent the precipitation of spiralin during electroimmunoassay, since it proved to be the most efficient among the detergents used in the present work for solubilizing this protein (see also reference 17). Spiralin was indeed quantitatively solubilized by DOC, since no material sedimented after high-speed centrifugation, in contrast to results obtained with the other detergents. The four other mild ionic detergents (cholate, taurocholate, taurodeoxycholate, and Sarkosyl) solubilized spiralin with a yield of about 85 to 90%. The neutral detergents Brij 58 and Tween 20 were much less efficient, with yields of 40 and 15%, respectively. Triton X-100 occupied an intermediate position with a yield close to 70%. The results are summarized in Table 1. It should be noted that the figure obtained with Tween 20 is quite approximate, since in the electroimmunoassay of spiralin in the presence of DOC and in the conditions of these experiments, the relationship between the heights of the rockets and the amount of titrated protein was unfortunately not linear for spiralin concentrations <0.4 mg/ml (i.e., <20% solubilized material; see Fig. 2).

The modifications of the electrophoretic mobility of spiralin by the addition of small quantities of ionic detergents to Triton X-100 micelles showed that this protein could bind detergents under nondenaturing conditions. This accounts for the amphiphilic nature of spiralin, which therefore should be regarded as an intrinsic membrane protein. This conclusion is not in contradiction with the fact that the hydrophobicity of this protein, calculated from its amino acid composition (17), is in the range of most water-soluble proteins. The solubility of a protein is a function of the spacial distribution of its polar and apolar amino acid residues. It is the presence of at least one exposed region highly enriched with apolar amino acids which is responsible for the amphiphilic properties of a protein, independent of the hydrophobicity conferred by its overall amino acid composition (12). The present work shows also that spiralin was not equally soluble in all nondenaturing detergents, in spite of its amphiphilic properties. The capacity of a given detergent to solubilize spiralin seemed to depend primarily on the nature of the hydrophilic moiety of the detergent, since ionic detergents proved to be significantly more efficient than neutral ones. Moreover, the example of Sarkosyl shows that the ability or inability of a detergent to extract a membrane protein cannot be used to predict whether that protein will be soluble in the detergent. Indeed, even though isolated spiralin was highly soluble in Sarkosyl, the protein was extracted from the *S. citri* membrane by this detergent with a very low yield (15, 17). This fact is probably a conse-

TABLE 1. *Solubilization of spiralin by mild (ionic and neutral) detergents*[a]

Detergent	M_r[b]	Detergent concn[c]		Spiralin[d]	
		g/100 ml	mM	mg/ml	%
DOC	414.6	4.1	100	2.0	100
Cholate	430.6	4.3	100	1.7	85
Taurodeoxycholate	521.6	5.2	100	1.8	90
Taurocholate	537.7	5.4	100	1.8	90
Sarkosyl	293.4	2.9	100	1.8	90
Triton X-100	646.6	3.0	46	1.4	70
Brij 58	1.222[e]	3.0	27[f]	0.8	40
Tween 20	1.230[e]	3.0	24[f]	0.3	15
No detergent				T	0

[a] Suspensions of proteoliposome containing 4 mg of spiralin per ml of Vernal buffer (pH 8.6, I = 0.03) were mixed with the same volume of detergents in distilled water. The suspensions were left for 1 h at 4°C and then centrifuged (180,000 × *g*, 1 h, 4°C). Spiralin was quantified by electroimmunoassay in the supernatants as described in the text.

[b] M_r, Molecular weight (relative).

[c] Increasing these concentrations above the reported values did not improve the yields of solubilized spiralin.

[d] The amounts of solubilized spiralin (average values calculated from two independent determinations). A yield of 100% means that the protein was quantitatively solubilized.

[e] The average molecular weight.

[f] Based on the average molecular weight.

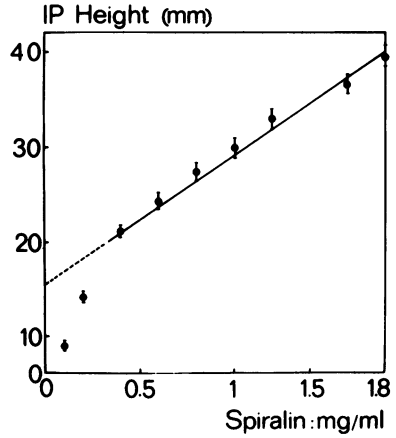

FIG. 2. *Standard curve for spiralin in 13 mM DOC determined by electroimmunoassay with a monospecific antiserum (antispiralin) at a concentration of 5 $\mu l \cdot cm^{-2}$ gel. Each well was loaded with 5 μl of sample. Current, 4 mA/plate; duration of the run, 18 h; room temperature. The immunoprecipitates were stained with amido black 10 B. The curve is based on four independent experiments. The correlation between the height of the immunoprecipitate in millimeters (y axis) and the concentration of spiralin in the dilutions in milligrams·milliliter^{-1} (x axis) was linear for concentrations ≥ 0.4 mg·ml^{-1}.*

quence of the complexity of the interactions between proteins and lipids in biological membranes. The extraction efficiency of a protein from a membrane by a detergent depends on the relative affinities of the protein for the detergent and its membrane environment. It will be possible to extract the protein with a good yield only if its affinity for the detergent is significantly greater than its affinity for the lipids, or other proteins in the membrane, or both.

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