SOLUBILIZATION OF MYCOPLASMA MEMBRANES BY THE NONIONIC DETERGENT TRITON X-100

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Received 22 April 1969

1. Introduction

The organization of the protein and lipid in the plasma membrane of mycoplasmas has been the subject of several recent reports [1–4]. From the appearance of a single symmetrical schlieren peak in membrane material solubilized by sodium dodecyl sulphate (SDS) it was inferred that the membrane is built of centrifugally homogeneous lipoprotein subunits [1]. This was disproven when the protein was found to be separable from the lipid in the SDS-solubilized membrane preparation by prolonged centrifugation in sucrose density gradients [2, 3] or electrophoresis in polyacrylamide gels [4]. Though the use of SDS has failed to demonstrate lipoprotein subunits in mycoplasma membranes, this is not sufficient to discard the subunit theory, because by virtue of its marked ability to separate lipid from protein the strong anionic detergent might also break up any existing membrane subunits. It therefore seemed worthwhile to analyze the products of solubilization of the mycoplasma membranes by the milder nonionic detergent Triton X-100. The results reported here show that Triton X-100 does not disaggregate the *Mycoplasma laidlawii* membrane into lipoprotein subunits, but rather into separate lipid-detergent and protein-detergent micelles. Furthermore, membrane lipids are far more effectively solubilized by the detergent that the membrane proteins, resulting in the formation of an insoluble protein-rich residue. The solubilization of membrane proteins by the detergent is selective: some protein species are found only in the soluble fraction, and others only in the insoluble residue. This suggests the use of Triton X-100 for the fractionation of mycoplasma membrane proteins.

2. Materials and methods

Mycoplasma laidlawii (oral strain) was grown in a modified Edward medium [5]. To label the membrane lipids, 1 μC of [1-^{14}C]-oleic acid (The Radiochemical Centre, Amersham, England) was added to each litre of the growth medium. Since oleic acid is incorporated into the phospholipids and glycolipids which constitute over 90% of the total membrane lipids of *M. laidlawii* [6] determination of radioactivity is a convenient measure of the proportion of lipid in various membrane fractions. The organisms were harvested after 18-20 hr incubation at 37°C, washed twice in 0.25 M NaCl and their membranes were isolated by osmotic lysis [3]. The membranes were washed three times and resuspended in β-buffer diluted 1:20 in deionized water [3]. The amount of membrane protein in the suspensions was determined according to Lowry et al. [7].

Solubilization of the membranes in Triton X-100 (redistilled, Packard Instrument Co.) was carried out at room temperature for 10 min. The treated membrane suspension was then centrifuged at 105000 *g* for 2 hr at 4°C. The supernatant fluid, referred to as the soluble fraction, was carefully separated. Analysis of the protein species of the soluble and insoluble membrane fractions was done in acidic polyacrylamide gels containing 7.5% acrylamide, 35% acetic acid and 5 M urea [3]. Polyacrylamide gel electrophoresis was also used

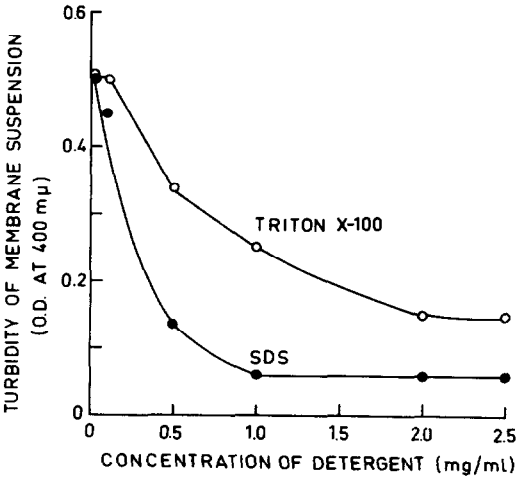

Fig. 1. Solubilization of *M. laidlawii* membranes by Triton X-100 and by sodium dedecyl sulphate (SDS). Various amounts of either detergent were added to the membrane suspension (containing 0.3 mg protein/ml), and turbidity readings were made after 10 min incubation at room temperature.

for testing the association of protein and lipid in the soluble fraction. The alkaline gel system and the procedure employed were as described previously [4]. Radioactivity in membrane fractions was determined in a Packard Tri-Carb liquid scintillation spectrometer [4].

3. Results

Triton X-100 solubilized *M. laidlawii* membranes less effectively than SDS, as evidenced by the considerable residual turbidity noted even at the highest Triton X-100 concentration tested (fig. 1).

A far higher percentage of membrane lipid than of protein was released by Triton X-100 into the soluble fraction, particularly at the lower detergent/membrane protein ratios (fig. 2). That the membrane components in the soluble fraction are indeed soluble and do not consist of minute particles was shown by centrifuging the fraction at 105 000 g for an additional 6 hr, when less than 10% of the protein and 4% of the lipid sedimented. Prolonged sucrose density centrifugation of the soluble fraction separated most of the

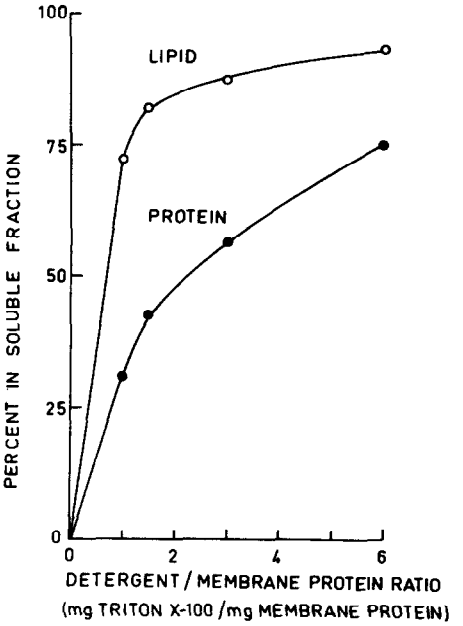

Fig. 2. Solubilization of *M. laidlawii* membrane protein and lipid as a function of the detergent/membrane protein ratio. Various amounts of membranes, containing ^{14}C-labelled lipids, were suspended in 2 ml volumes of a 1.3% (w/v) Triton X-100 solution, incubated at room temperature for 10 min, and centrifuged at 105 000 g for 2 hr at 4°C. Protein and radioactivity were determined in the supernatant fluids representing the soluble fractions, and in the sediments.

membrane protein from the membrane lipid (fig. 3). Polyacrylamide gel electrophoresis of the soluble fraction in the alkaline system also showed a highly variable ratio of labelled lipid to protein in the different gel sections, supporting the thesis that the lipid and protein in the soluble fraction are separate.

Fig. 4 shows that the solubilization of membrane proteins by Triton X-100 is to some extent selective. Thus, some protein bands in the electrophoretic pattern of the soluble fraction could not be detected in the pattern of the insoluble fraction, and vice versa.

4. Discussion

Our findings indicate that Triton X-100 does not disaggregate *M. laidlawii* membranes into lipoprotein subunits, even at a low detergent/membrane

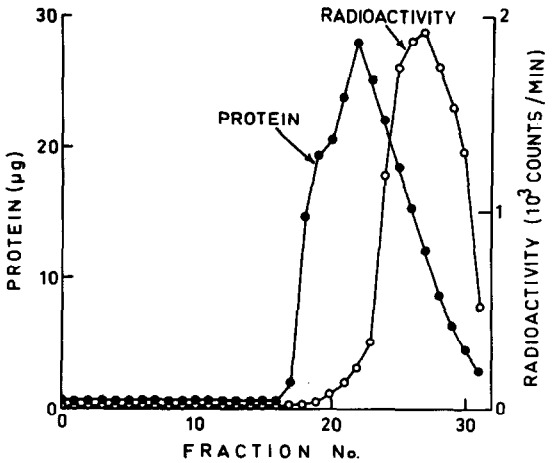

Fig. 3. Separation by sucrose gradient centrifugation of lipid from protein in the soluble membrane fraction. Samples (0.15 ml) of the soluble fraction containing 250–300 μg protein were layered over 3.2 ml linear sucrose gradients (5 to 28% sucrose) containing 0.5% (w/v) Triton X-100. The gradients were centrifuged at 42000 rpm in a swing-out rotor of an M.S.E. Super-Speed 65 ultracentrifuge for 20 hr at 5°C. Fractions (0.05 ml) were collected and were assayed for protein and radioactivity. The top of the gradient is on the right.

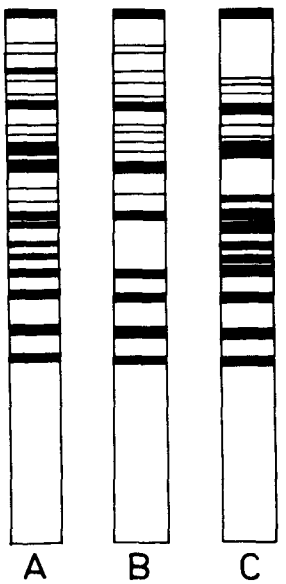

Fig. 4. Electrophoretic analysis of *M. laidlawii* membrane proteins in polyacrylamide gels containing 35% acetic acid and 5 M urea. A. Whole lysate of membranes solubilized by Triton X-100 (2 mg detergent/mg membrane protein). B. The soluble fraction of the membrane lysate obtained after centrifugation of the lysate at 105 000 g for 2 hr. C. The insoluble fraction of the membrane lysate obtained after centrifugation of the lysate.

protein ratio. The detergent solubilizes most of the membrane lipid, but only part of the protein, leaving an insoluble protein-rich residue. This corroborates the recent report of Salton et al. [8] on the removal of over 90% of the lipids of the *Micrococcus lysodeikticus* plasma membrane by deoxycholate, leaving an insoluble membraneous residue consisting almost entirely of protein. On the other hand Shibuya et al. [9], using the non-ionic detergent Nonidet P-40, claim to have isolated lipoprotein subunits from spinach chloroplast lamellae at a certain detergent/chlorophyll ratio. It should however be stressed that whereas the subunit architecture of chloroplast membranes is fairly well established, the evidence for a subunit pattern in plasma membranes is rather meagre [10]. From the detergent solubilization studies made so far it is impossible to deduce the molecular organization of the protein and lipid in the plasma membrane, but the findings of Salton et al. [8], as well as our own, both indicate the existence of protein to protein bonds which are more resistant to breakage by detergent action than the lipid to lipid or lipid to protein bonds.

The selective solubilization of some of the membrane proteins by Triton X-100 may be of use in the fractionation of membrane proteins. Being a mild detergent, Triton X-100 has also an advantage over SDS in having slighter denaturing effects. Thus, solubilization of *M. laidlawii* membranes by Triton X-100 did not destroy their ATPase activity whereas SDS did (Razin and Barash, unpublished observations). However, our efforts to purify the solubilized ATPase by the conventional protein fractionation methods failed so far, apparently because of the difficulties encountered in the removal of Triton X-100 from the solubilized membrane preparation.

Acknowledgement

This study was supported by Grant FG-Is-174 from the U.S. Department of Agriculture under Public Law 480.

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