EFFICIENCY AND SPECIFICITY OF THE SEQUENTIAL EXTRACTION OF MEMBRANE PROTEINS OF *ACHOLEPLASMA LAIDLAWII* WITH THE NEUTRAL DETERGENT TWEEN 20

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Received 3 March 1976

1. Introduction

Solubilization of *Acholeplasma laidlawii* membrane proteins by means of the neutral detergent Tween 20 has lead to the successful isolation and purification of 6 of these proteins [1–3]. The procedure is the only one available at present for the preparation of pure core membrane proteins of *Ach. laidlawii*. It is a long 6–8-step process that for flavoprotein T_{4a}, the most abundant of these proteins, 30% total, yields 0.1 mg protein/liter cell culture. Improvement in the yield of these proteins, for the whole group as well as for particular components, is therefore desirable if further systematic work with them is to be pursued. This demands optimization and scaling up of every one of the preparative steps. A 3–4-fold increase in the yield of cell membranes has already been achieved by selecting determined culturing conditions of the cells [4]. Improvements have also been obtained in the removal of the detergent from the proteins [5,6]. In the present communication a study of some of the parameters involved in the extraction of these proteins from the membrane with Tween 20 is described. It is shown that, by proper selection of the membrane–detergent concentration ratio during solubilization, an increase of the yield of the first extract in 100% can be achieved and a 2–3-fold increase of the total yield can be obtained if successive extractions are performed. Besides it is shown that there is a large degree of selectivity in the composition in Tween 20-soluble membrane proteins of every extract of a multiple-step extraction. This leads to increased efficiency in the isolation of some of these proteins. It is also shown that the membrane protein component D_3 [1], which had not been earlier solubilized from *Ach. laidlawii* membranes, can be released in solution if several extractions of the membranes with the detergent are performed.

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2. Materials and methods

Ach. laidlawii B cells (originally obtained from Professor S. Razin, Haddassah Medical School, Jerusalem) were grown in tryptose medium [7], supplemented with 1% glucose, at 28°C, either static or with agitation. Cell membranes were prepared as in [7]. Membrane concentration was determined by solubilization of aliquots of membrane suspensions in 0.02 M sodium dodecyl sulfate (SDS), 0.02 M Tris–HCl (pH 8.0). The differential extinction coefficient of the membrane ($E^{1\%}_{1\,cm,\,280-310}$) was taken as 8.2 [8]. Extraction of the membranes with Tween 20 (Atlas Chemie, GmbH, Essen) was performed by incubating membrane suspensions with the detergent for 1 h. Several extractions were performed. Various conditions, described in the text, were tried. At the end of the incubation period the membranes were sedimented at 145 000 × *g*, 1 h. The differential absorbance $A_{280}-A_{310}$, measured on aliquots of the supernatant dissolved in 0.02 M SDS, 0.02 M Tris–HCl (pH 8.0), was used to calculate the yield. Analytical polyacrylamide gel electrophoresis (PAGE) of extract aliquots in the presence of SDS was done

as in [9]. A voltage gradient of 10 V/cm, during 80–110 min, was used. The gels (T= 6%, C= 5%) were stained with Coomasie brilliant blue R250.

3. Results

The yield of extraction of *Ach. laidlawii* membranes with Tween 20 at a particular step was estimated by the relationship:

$$[(A_{280}-A_{310}) \text{ of supernatant}/(A_{280}-A_{310}) \text{ of membranes or membranes residues}] \times 100$$

where A_λ represents the absorbance measured at wavelength λ. The supernatant considered in the calculation was that obtained by centrifugation of the incubation mixture of membranes and detergent at the end of the incubation period. The membranes or membrane residue considered in the calculation were those present at the beginning of the same incubation period.

No differences in yield were found whether extraction was performed at 2°C or at 23°C. Nor whether it was performed using static or agitated samples. The variable that more markedly affected the yield was the ratio between membrane concentration and detergent concentration during solubilization. The yields obtained in the first and second extracts are shown in fig.1. It can be seen that the effect of the membrane–detergent concentration ratio is marked during the first extraction but less pronounced during the second one. The yield of the third extraction, performed at a membrane–Tween 20 concentration ratio of 1.44, was found to be 13%. The value represents the average obtained from 8 different membrane preparations. If the yields are referred to the initial amount of membranes present in the sample, that present before any extraction was performed, the results shown in table 1 are obtained. It is seen that large differences in yield can be obtained, depending on the number of extractions performed and the conditions chosen to carry them out. The total efficiency is largely determined by the yield of the first extraction.

The membrane composition of the different extracts was studied by SDS–PAGE. The composition of an extract of a particular sequential order was almost identical among different membrane preparations. The composition was however different between extracts of different sequential order (fig.2). In general terms, a progressive release towards proteins of lower molecular weight was observed by repeated extraction. The first extract contained predominantly proteins D_5 and D_6 and to a minor extent D_{11}. The

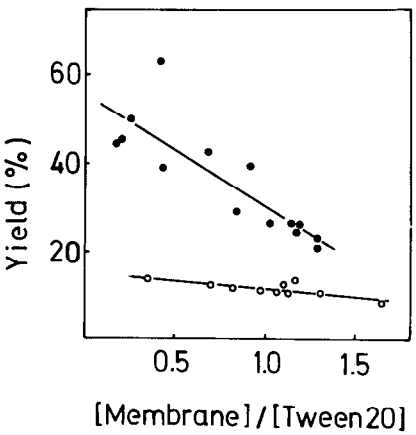

Fig.1. Effect of the membrane–detergent concentration ratio on the yield of extraction of *Ach. laidlawii* membranes with Tween 20. For explanation about the calculation of the yield see the text. Both membrane concentration and detergent concentration are given in g/l. Shown are the regression lines calculated with the experimental points obtained from 14 different membrane preparations. Black circles, first extraction. Open circles, second extraction.

Table 1
Yields of sequential extractions of *Ach. laidlawii* membrane with Tween 20[a]

Extraction order	Initial membrane conc. 5 g/l	Initial membrane conc. 30 g/l
First	52	26
Second[b]	7	10
Third[c]	5	8
Total	64	44

[a] Yields are expressed with respect to the amount of membrane present in the sample before any extraction was performed

[b] Performed at a membrane–detergent ratio of 0.20

[c] Performed at a membrane–detergent ratio of 1.44

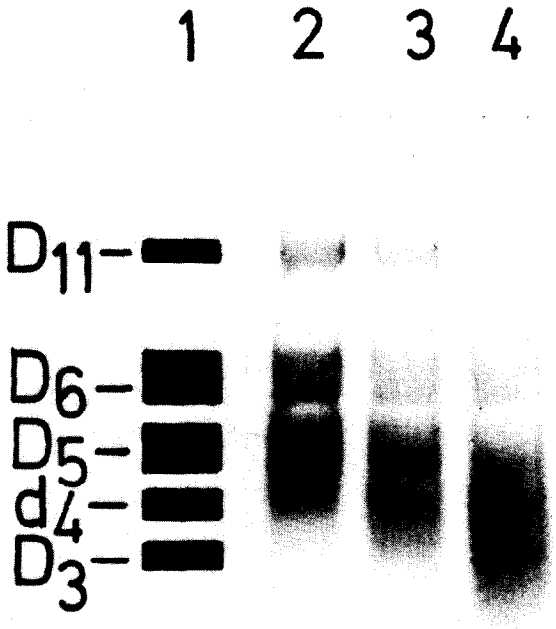

Fig.2. SDS–PAGE of Tween 20-extracts of *A. laidlawii* membranes. Extracts obtained from 8 different membrane preparations were analysed and showed identical results. 1: Scheme (as in [1]) of the bands present in the extracts. 2: First extract. 3: Second extract. 4: Third extract.

third extract contained predominantly d_4 and D_3. The second extract had an intermediate composition between the first and the third (for the nomenclature of the electrophoretic bands see [1]).

4. Discussion

The results of the present work show that the most significant parameter when attempting to vary the efficiency of the extraction of membrane proteins of *Ach. laidlawii* with Tween 20 is the membrane–detergent concentration ratio of the solubilization mixture. If a high total yield of the extraction is desired it is preferable to perform solubilization at low membrane concentrations (~5 g/l). Differences in the yield of the first extraction of 100% can be obtained in this way. Further improvements in yield can be obtained by additional extractions. The significance of these results becomes particularly evident when comparison is made between the yield obtained with a one-step extraction performed at high membrane concentration (30 g/l, table 1), as earlier used, with the yield obtained with several extractions at low membrane concentration. It is seen that an improvement of 2–3-times in total yield is obtained. Notice must also be paid to the yields of different sequential extractions. These differences determine that how the first extraction is performed decides the magnitude of the total yield. As pointed out below, however, high total yield may not be the only desirable feature of an extraction.

Other factors like temperature or different forms of agitation have little or no significant effect on the yield. However, the fact that extraction can be performed at 2°C with the same efficiency than at 23°C is advantageous from the viewpoint of avoiding the effects of laboratory temperature upon the membrane components during solubilization.

The different selectivity shown by extracts of different sequential order offers the possibility of improving the efficiency in the isolation of particular components of the Tween 20-soluble membrane proteins of *Ach. laidlawii*. The first extract contains three components, but mainly the two quantitatively important proteins D_5 and D_6. These correspond to proteins T_{4a} (flavoprotein) and T_{4b} which together represent about 50% of the Tween 20-soluble membrane proteins. The third extract contains proteins d_4 and D_3. Protein D_3 had not been solubilized with the one-step extraction used earlier [1]. It is thus a new member of the Tween 20-soluble membrane proteins of *Ach. laidlawii*, whose further purification can now be attempted using the third Tween 20-extract as the starting source. Since the composition of the second extract is intermediate between that of the first and the third, its use can lead to additional improvement in yield in the preparation of either T_{4a} or d_4.

Thus the modifications in both yield and selectivity that can be achieved during extraction of membrane proteins of *Ach. laidlawii* with the neutral detergent Tween 20, described in the present work, can be useful aids in the preparation of these proteins. This result, as mentioned earlier, is particularly significant in view of the fact that extraction with Tween 20 is at present the only available procedure that leads to the preparation of pure membrane proteins from *Ach. laidlawii*.

Acknowledgements

The author wants to thank Professor Stellan Hjertén for the facilities provided to perform this work. The support of the following institutions is gratefully acknowledged: Swedish International Development Authority; Swedish Institute; Swedish Natural Science Research Council; Von Kantzow Foundation.

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