TWEEN 20-SOLUBLE MEMBRANE PROTEINS OF *ACHOLEPLASMA LAIDLAWII*

Fractionation in the presence of a Tween 20 concentration slightly above its critical micelle concentration and in the absence of detergent by means of agarose-suspension electrophoresis

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1. Introduction

Agarose-suspension electrophoresis (ASE) in the presence of 1% Tween 20 has been used to fractionate the Tween 20-soluble membrane proteins of *A. laidlawii* [1,2], which represent about 60% of the total membrane proteins. Ultimate purification of these proteins requires the removal of the detergent, a step that is particularly difficult when neutral detergents have been used in the fractionation procedure. In the present work it has been investigated the possibility of fractionating these proteins using buffer containing a concentration of detergent slightly above its critical micelle concentration (CMC) or no detergent at all. The Tween 20 concentration was chosen under the assumption that the presence of detergent micelles were necessary to maintain the solubilized membrane proteins in solution and with the idea that, if separation occurred, a slight dilution of the obtained fractions below the CMC value, would allow the removal of the detergent by dialysis. Dialysis could not effectively be used with Tween 20 solutions with concentrations 1% or higher. It is shown that fractionation of the membrane proteins of *A. laidlawii* is possible in both very low detergent- and detergent free-separations* in a fashion similar to that achieved with separations in the presence of 1% Tween 20 [1]. The membrane proteins are stable in solution in detergent-free medium during a period of at least three weeks.

2. Materials and methods

A. laidlawii strain B cells, originally provided by Professor S. Razin, were grown in 2–4 l cultures as described by Razin et al. [3], but at 28°C and collected at the late log phase of growth. Membranes were prepared according to Razin et al. [3] and solubilized with Tween 20 [4]. ASE of the Tween 20-extract and monitoring of the fractions were performed according to Hjertén [5] and Hjertén et al. [6] respectively. It was found that the use of a 0.19% agarose suspension, instead of the 0.17% one used previously, conferred more stability to the starting zone. The zones migrated at a slower rate in 0.19% agarose, but otherwise the results were identical in both cases. Surface tension measurements of Tween 20 solutions were performed with a capillary rise apparatus at 23°C. The concentration of Tween 20 was determined spectrophotometrically [7]. Concentration of ASE fractions after the removal of agarose was performed using Minicon concentrators (Amicon Corporation, Mass., USA).

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*In the text these alternatives will be simply designed as separations in 0.01% Tween 20 and in the absence of detergent, respectively, even when the sample used – a Tween 20-extract of *A. laidlawii* membranes – always contained detergent in a concentration higher than 2%. The detergent remained nevertheless at the start [7].

Polyacrylamide gel electrophoresis (PAGE) in the presence of 0.02 M sodium dodecylsulfate (SDS) and 0.1 M Tris–HCl (pH 8.0), was performed as described elsewhere [8]. A voltage gradient of 10 V/cm during 80–110 min was used. The gels (T = 6%, C = 5%) were stained with Coomassie Brilliant Blue R250.

3. Results

Preliminary measurements showed that 1–5% Tween 20 solutions prepared in 0.1 M Tris–HCl (pH 8.0), dialysed against the same buffer, at 2°C, during 6 days, did not show any significant decrease in concentration. The use of 1 M ammonium sulfate instead of buffer as dialysis solution increased the extent of the dialysis for detergent solutions with concentrations higher than 4%, but did not affect those with concentrations between 1% and 4%.

It was found that CMC values of Tween 20 reported in the literature [9,10] were discrepant by a factor of 6. In view of this fact and the necessity of knowing the CMC of the detergent under the experimental conditions used in the present work, the CMC of Tween 20 was determined in 0.1 M Tris–HCl (pH 8.0), by surface tension measurements. The value obtained was 7 mg detergent per 100 ml buffer, which is very close to that found by Becher [10]. Thus the detergent concentration chosen for ASE runs of Tween 20-extract of *A. laidlawii* membranes was 0.01%.

The results obtained in ASE runs performed both in the presence of 0.01% Tween 20 and in the absence of detergent were almost identical. In fig.1 are shown the results obtained from an ASE run in detergent-free buffer of a Tween 20-extract of *A. laidlawii* membranes and the protein composition of the main fractions as revealed by SDS PAGE. The differences observed between ASE runs performed in the absence of detergent and in the presence of 0.01% Tween 20 were related to the fact that migration of the zones was faster in the former case. Thus, in 0.01% Tween 20 runs, peak D (fig.1A) was resolved into one main and two small peaks. These small peaks contained amounts of protein only detectable by spectrophotometry but not by SDS PAGE, even after concentration of the fractions. But primarily, the results showed that, in

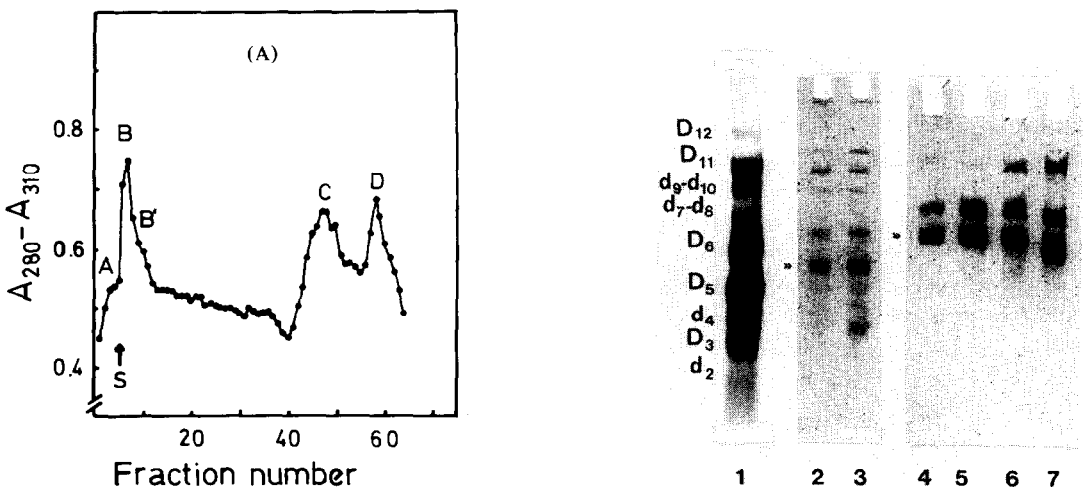

Fig.1.(A). ASE in the absence of detergent of a Tween 20-extract of *A. laidlawii* membranes. Agarose concentration: 0.19%. Sample volume, 300 μl. Buffer, 0.1 M Tris–HCl (pH 8.0). Voltage, 800 V. Current, 24 mA. Duration of the run 15 h. Use was done of the full length of the electrophoresis column. S, start. Anode located to the right. (B). SDS PAGE of fractions obtained from the ASE run shown above. Since the duration of the run was not the same for all the samples, the position of band D5 (T4a–flavoprotein) has been indicated by arrows as a reference. The samples were concentrated 5 times prior to the run. 1: Tween 20-extract of *A. laidlawii* membranes. 2: Pooled fractions No. 6–8 (peak B). 3: Pooled fractions No. 9–12 (peak B'). 4: Fraction No. 44 (peak C). 5: Pooled fractions No. 47–48 (peak C). 6: Fraction No. 50 (peak C). 7: Pooled fractions No. 57–59 (peak D). For the nomenclature of the bands see ref. [4].

ASE performed in the absence of detergent or in the presence of 0.01% Tween 20, fractionation occurred in a similar fashion as in the presence of 1% Tween 20 [1]. The following differences were however noticed. In the absence of detergent or in the presence of 0.01% Tween 20: (i) the time required for separation was reduced in 50%, (ii) the lipids remained near the start partially mixed with the detergent [7], (iii) small amounts of protein (fig.1B) remained near the start mixed with the detergent and the lipids,** (iv) the resolution achieved for peak C (containing T4a-flavoprotein and T4b, that represent about 50% of the Tween 20-soluble membrane proteins), using the maximum possible length of the column, was not quite as complete as in 1% Tween 20 runs; in the descending branch of this peak were present small amounts of protein D11, which is either absent or present in smaller amounts in 1% Tween 20 runs. The ultraviolet–visible absorption spectra of fractions collected from peaks A, B, C, and D, revealed in all of them the presence of an absorption band with a maximum close to 280 nm, very likely due to protein. The spectra of fractions from peaks A and B revealed the presence of Tween 20 [7] and those of fractions C and D the presence of flavoprotein [1]. The yield of ultraviolet-absorbing material ($A_{280}-A_{310}$) of peak C was 22–26%, which should be compared with the value 16–19% of the homologous peak of ASE-runs performed in the presence of 1% Tween 20. The proteins of fractions obtained from detergent-free runs were stable in solution at 2°C for a period of at least 3 weeks, but their concentration lead to the formation of precipitate.

4. Discussion

From the results of the present work it becomes clear that fractionation of the Tween 20-soluble membrane proteins of *A. laidlawii* can be performed by means of ASE in the presence of 0.01% Tween 20 (a concentration 100 times lower than that previously used [1]) and in the absence of detergent. The fractionation patterns obtained in both cases are almost identical. Due to a slower migration of the zones in separations performed in 0.01% Tween 20, the resolution achieved is slightly higher than in detergent-free separations. The resolution of the latter can be however increased using longer columns. The fractionation pattern obtained with both types of separations is quite similar to that obtained from separations performed in the presence of 1% Tween 20. Separation of the protein from the lipids is sharper in runs performed without detergent or with low detergent concentration. Recoveries of the fractions containing the quantitatively significant protein T4a and T4b are comparable in all cases. The results also show that, after release from the membrane of *A. laidlawii* of the proteins that are more soluble in Tween 20, there is no need for the presence of the detergent to keep them in solution, at least for a period of 3 weeks. The mixture of proteins that remains in small amounts together with the detergent and the lipids near the start is enriched in those proteins which are predominantly left in the residue upon solubilization of the membrane with Tween 20, i.e., the most insoluble membrane proteins.

**Measurements performed on fractions obtained from ASE runs in the presence of 1% Tween 20 revealed the absence of proteins in the peak near the origin and negligible amounts of proteins with a mobility like D11 and D6 in the peaks containing the lipids.

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