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# THE SEPARATION OF MEMBRANE PROTEINS BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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SUMMARY

A modified TAKAYAMA (K. TAKAYAMA, D. H. MAC LENNANAN, A. TZAGOLOFF AND C. D. STONER, *Arch. Biochem. Biophys.*, 114 (1966) 223.) method for acrylamide gel electrophoresis of membrane proteins is described. The method uses acetic acid, urea, phenol and mercaptoethanol as the solvent for solubilizing the membranes. This method gives more and sharper bands than TAKAYAMA's method and other methods in the literature.

## INTRODUCTION

The relatively large size and the insolubility of membrane proteins in water creates difficulties in the fractionation of membrane proteins by acrylamide gel electrophoresis. The technique of ORNSTEIN[1] has been modified and used by various workers to separate insoluble proteins on acrylamide gels. TAKAYAMA *et al.*[2] used a mixture of phenol, acetic acid, urea and water to solubilize the mitochondrial membrane proteins and used a urea–acetic acid gel to separate them. SCHNEIDERMAN[3] used a mixture of Triton X-100, urea and mercaptoethanol to solubilize the red cell membranes. EVANS[4] modified the method of SUMMERS[5] and used it for the separation of liver cell plasma membrane proteins. The detergent sodium dodecyl sulfate has been used to solubilize the membranes[4]. KIEHN AND HOLLAND[6] also used sodium dodecyl sulfate to solubilize and separate the membrane proteins of cultured human and animal cells. FITZPATRICK *et al.*[7] used 1.2 % lubrol, 0.1 M NaCl in 0.01 M Tris–HCl buffer (pH 7.5) to solubilize the plasma membranes of mammalian kidney. NEVILLE[8,9] employed a mixture of $K_2CO_3$, urea, mercaptoethanol and Triton X-100 to solubilize the proteins of the liver cell plasma membranes and separated them in polyacrylamide gels containing 5 M urea and 0.25% Triton X-100 under a discontinuous acidic pH. NEVILLE's method gives a good separation of the plasma membrane proteins but it is relatively laborious. Recently PANET AND SELINGER[10] modified TAKAYAMA's method for the gel electrophoresis of rat liver microsomal membranes. They used a nonionic detergent and mercaptoethanol in addition to phenol and acetic acid.

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Attempts were made to find a simpler method which would better separate the membrane proteins and which could be used for a variety of membranes. A modification of the method of TAKAYAMA *et al.*[2] was developed and was found to give good results.

## METHODS AND MATERIALS

Acrylamide and methylene bisacrylamide were purchased from Eastman Kodak and were recrystallized before use. Analytical grade urea and phenol were purchased from Baker. Glacial acetic acid was obtained from Mallinckrodt. Aniline blue black was purchased from Canalco and the coomassie brilliant blue from Edward Gurr Ltd., London.

The plasma membranes were prepared by the method described previously[11]. The microsomes were prepared by the method of DALLNER *et al.*[12]. The mycoplasma membranes were kindly supplied by Dr. J. Das of the Department of Microbiology, University of Rochester. The rat brain particulate fraction was prepared by centrifuging a 1% homogenate of rat brain in 0.001 M bicarbonate (pH 7.4) at 600 × *g* for 30 min and washing it twice by resuspension and centrifugation at 600 × *g*. Proteins were assayed by the LOWRY method[13].

The membranes were dissolved in the following solvent: 20% acetic acid, 24% urea, 40% phenol and 5% mercaptoethanol. This differed from TAKAYAMA's method in that it contained mercaptoethanol.

The composition of the solvent mixture for running the gel was as follows: Solution A, 6% acrylamide, 0.23% methylenebisacrylamide, 28% urea and 37.3% acetic acid; solution B, 1.6% ammonium persulfate and 60% urea. Solutions A, B and tetraethylethylene diamine were mixed in the ratio of 3:1:0.02. The gels were allowed to polymerize at room temperature for about 60 min. The samples were layered on the top of the gels, then 10% acetic acid was used to fill the rest of the tube. The upper and the lower electrode chambers contained 10% acetic acid. Electrophoresis was conducted at the room temperature in a Savant unit, model DEC-12. A current of 1.5 mA per tube was passed for about 60 min and then 4 mA per tube for another 4 to 5 h. After the run the gels were fixed for about 60 min in 12.5% trichloroacetic acid and then stained overnight in a mixture of 0.25% aniline blue black and 0.125% coomassie brilliant blue in 7.5% acetic acid. Destaining was carried out in a Canalco destainer.

The present procedure differs from TAKAYAMA's method in the following respects: Mercaptoethanol at relatively high concentration has been used in the 'sample solvent'. Mercaptoethanol more effectively solubilizes the membranes so that more bands are obtained. Mercaptoethanol also prevents discoloration of the 'sample solvent' during storage. In this method 6% acrylamide gels have been used instead of 7.5% in TAKAYAMA's method. The urea concentrations in the gel have been increased from 4 to 6 M. In TAKAYAMA's method polymerization of the gels are carried out by heating the tubes in an oven for 15 min, whereas in the present method gels are polymerized at room temperature. In our experience slow polymerization of the gels gives a better resolution of proteins with sharper bands than does rapid polymerization of the gels. Finally, the gels were fixed in 12.5% trichloroacetic acid before staining. Fixation of the gels prior to staining helps to show up some minor bands which remain invisible when the fixation step is omitted. During the long time

of the staining procedure some proteins may be eluted out from the gels if they are not fixed prior to staining. In TAKAYAMA's method no fixation step is used before staining.

## RESULTS AND DISCUSSION

Fig. 1 shows the effect of the 'sample solvent' on the separation of the rat liver plasma membrane proteins by acrylamide gel. 75% acetic acid alone (Gel A) partially solubilized the membrane and gave a fair separation of the proteins. Addition of urea (Gel B) gave a similar pattern with slight differences. Urea plus acetic acid still yielded slightly turbid membrane dispersions. Addition of phenol to the 'sample solvent' (TAKAYAMA's solvent Gel C) increases the solubilizing power of the solvent as is evident by the greater transparency of the membrane dispersion. In TAKAYAMA's solvent the membranes yield an almost completely transparent dispersion. However, TAKAYAMA's method (Gel C) gives a poorer resolution of the protein bands compared to those obtained in gels A and B where no phenol was added. The reason for the poorer resolution of the membrane proteins in phenol-containing solvent (TAKAYAMA's) is not understood. However, the incorporation of mercaptoethanol in the sample solvent considerably improved the separation of the membrane proteins (Gel D). The sharpness of the different protein bands with minimal smearing indicates that among the solvents tested, the mercaptoethanol-containing one may prevent the reaggregation of proteins and thereby decreases smearing and yields sharper bands

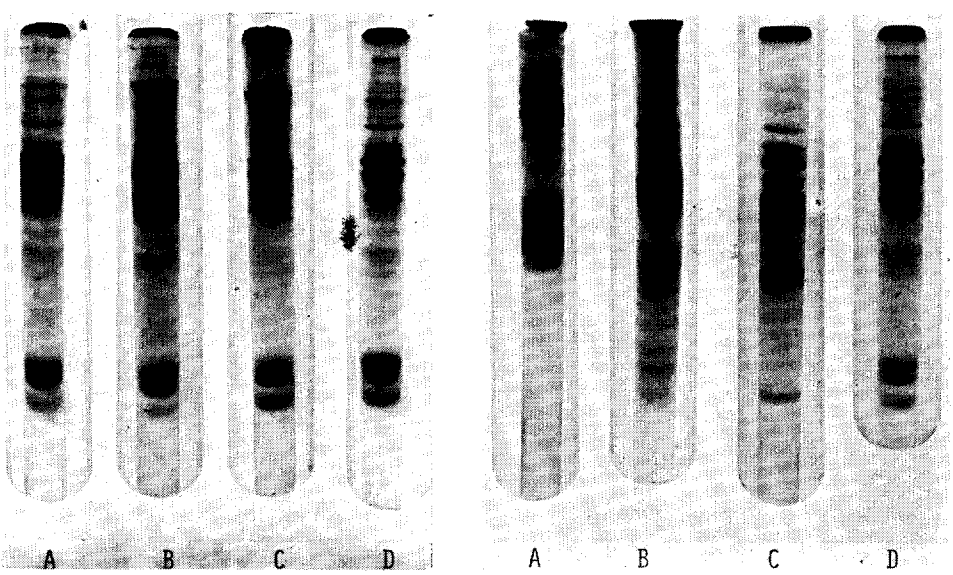


Fig. 1. Acrylamide gel electrophoretic patterns of rat liver cell plasma membrane. The electrophoresis was carried out as explained in the text. A, membranes treated with 75% acetic acid; B, membranes treated with 75% acetic acid *plus* 6 M urea; C, membranes treated with TAKAYAMA's solvent; D, membranes treated with the modified solvent.

Fig. 2. Acrylamide gel electrophoretic patterns of different membranes: Electrophoresis was carried out as explained in the text. A, Total rat brain particulates; B, microsomal membranes of rat liver; C, mycoplasm membranes; D, rat liver plasma membranes.

SH agents have been used by SCHNEIDERMAN[3], NEVILLE[8,9] and PANET AND SELINGER[10].

Fig. 2 shows that the present method can be used successfully for a variety of different membrane systems (rat brain membranes, A; microsomal membranes, B; mycoplasm membranes, C; liver plasma membranes, D). In the case of the liver cell plasma membranes (Gel D) at least 30 bands were observed. A densitometer scan of the gel is shown in Fig. 3. The gel patterns of the plasma membranes are similar to those reported by NEVILLE[8] for the rat liver plasma membrane, but more bands could be resolved by the present method than by NEVILLE's technique. NEVILLE states that 15 bands in his system were clearly resolved and another 10 were too faint to be seen in the photographic reproduction and densitometer trace. The present method yields 18 major bands and 12 minor bands all of which are visible in the photographic reproduction. The present method also gives more protein bands for the microsomal membrane than does the method of PANET AND SELINGER[10].

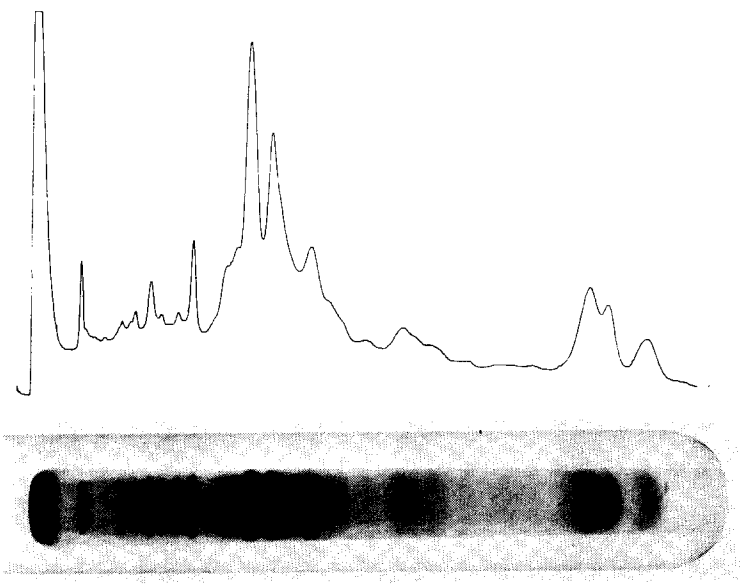

Fig. 3. Acrylamide gel electrophoretic pattern and densitometric tracing of rat liver plasma membranes. Electrophoresis was carried out as explained in the text. The tracing was done on a Gilford scanner.

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