Vol. 84, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 14, 1978 Pages 158-165

HEAT INDUCED AGGREGATION OF THE SODIUM DODECYL SULFATE-SOLUBILIZED MAIN INTRINSIC POLYPEPTIDE ISOLATED FROM BOVINE LENS PLASMA MEMBRANE

Michael M. Wong, Nancy P. Robertson and Joseph Horwitz

Jules Stein Eye Institute, UCLA School of Medicine,
Los Angeles CA 90024

Received July 1,1978

SUMMARY: One major component of lens plasma membrane is a glycoprotein that SDS-polyacrylamide gel electrophoresis shows to possess an apparent molecular weight of 26,000. When this protein is solubilized in low ionic strength buffers containing SDS, and heated to 100° for 1 to 3 min prior to electrophoresis, conversion into high molecular weight aggregate results. The heat lability of this protein is greatly enhanced if it is solubilized and heated in buffers containing 0.1 M NaCl. At this ionic strength, incubation for 3 h at 38° results in conversion of 20% of the protein into high melecular weight aggregates. Most other membrane proteins isolated from lens membrane are insensitive to heat treatment. It is concluded that temperature and ionic strength must be recorded and controlled carefully when using SDS-polyacrylamide gel electrophoresis to study this membrane protein.

INTRODUCTION:

The lens fiber cell is one of the best examples for studying the properties of plasma membranes (1). These plasma membranes are also rich in gap junctions and thus serve as a convenient source for examination of the structural and biochemical properties of this cell component (1, 2, 3). In recent years, substantial effort was directed toward elucidating biochemical properties of lens membrane proteins (4, 5, 6, 7). As with other membranous systems, one of the major tools in characterizing lens membrane proteins has been sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). In lens membrane preparations on SDS gels, the protein pattern generally exhibits multiple bands of polypeptides with an apparent molecular weight in the range of 16,000 to 200,000. Even though the SDS-PAGE profile of lens membrane proteins depends to some extent on the method of purification of these membranes (5, 6, 8), it is accepted that the predominant intrinsic membrane protein is a polypeptide

Supported by National Eye Institute, NIH grants # EY 01622, EY 00058, and EY 00331.

0006-291X/78/0841-0158$01.00/0

with an apparent molecular weight of ∿26,000. Bloemendal et al (8) observed that, among the membrane proteins identified, this 26,000 MW polypeptide tends at times to diminish greatly or even to disappear completely from the gel. These investigators attributed the phenomenon to proteolytic action. Other investigators consistantly find the 26,000 MW polypeptide on their gels, but their estimations differ as to the relative abundance of this polypeptide (5, 6). In this communication, we report on what we believe to be the cause of some of the observed discrepancies encountered in dealing with this membrane protein.

MATERIALS AND METHODS:

Bovine lens membranes were prepared by discontinuous sucrose density gradient ultracentrifugation according to the procedure of Bloemendal et al (4). Fractions rich in plasma membranes were collected at the 1.14/1.16 interface of sucrose density. SDS-PAGE was performed on 2mm slabs by modifying the method for tube gels described by Laemmli (9). The stacking gels were 4.5% acrylamide and the running gels were either 7.5, 10, or 13% acrylamide. Membrane fractions were solubilized in either 20 mM Tris-HCl buffer, pH 7.9, containing 1% SDS, or in 20 mM phosphate buffer, pH 6.8, containing 1% SDS. To study the effects of ionic strength, solubilization of membranes was also performed by the addition of 0.1 M NaCl to the samples. Beta-mercaptoethanol (5%) was added to all samples in both buffer systems. Samples were heated either at 38° for 3 h, 57° for 30 min, or 100° for 3 min. Control samples were incubated with beta-mercaptoethanol for 3 h at room temperature. Gels were stained with Coomassie blue as described by Weber, Pringle and Osborn (10). Densitometric tracings of gels were obtained by using a Beckman ACTA MVI spectrophotometer equipped with a Beckman model 2 gel scanner. For carbohydrate detection, gels were fixed and stained according to the periodic acid-Schiff (PAS) basic fuchsin method of Segrest and Jackson (11). The basic fuchsin (MCB Chemicals) was prepared using the protocol of McGuckin and Mckenzie (12). To remove excess dye, the gels were washed in the manner suggested by Zacharius et al (13).

RESULTS:

The electrophoretic pattern of a typical membrane preparation in SDS-acrylamide is shown in Figure 1A. The position of the main intrinsic polypeptide is labeled MIP. A high molecular weight fraction appears at the top of the gel as the termperature of incubation increases. At 38° and 57° this heat-induced aggregate penetrates the stacking gel. On heating the sample to 100°, a portion of the aggregate appears at the top of the 4.5% acrylamide stacking gel (Fig. 1A, column 5). From Fig. 1A and a representative densitometric scan shown in Figure 2, it is evident that the high molecular weight aggregate arises almost exclusively from the main intrinsic polypeptide. Smearing of the high molecular

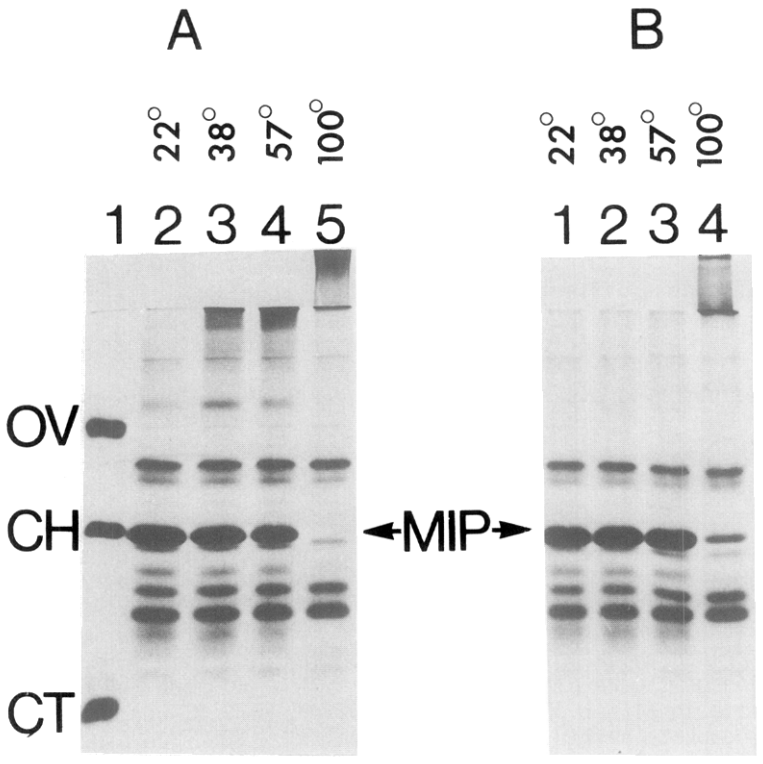

Fig. 1. A. SDS-gel electrophoresis of lens membrane preparation solubilized in high ionic strength buffer. Membranes were dissolved in 20 mM Tris-HCl, pH 7.9, containing 1% SDS, 5% beta-mercaptoethanol, and 0.1 M NaCl. Samples (columns 2 through 5) were incubated at 22° for 3 h, at 38° for 3 h, at 57° for 30 min, and at 100° for 3 min. Each column contains 25 µg of protein. Standared proteins (column 1) were: OV = ovalbumin; CH = chymotrypsinogen; CT = cytochrome c. MIP designates the membrane intrinsic polypeptide.
B. SDS-gel electrophoresis of lens membrane preparation solubilized in a low ionic strength buffer. Membranes were dissolved in 20 mM phosphate buffer, pH 6.8, containing 1% SDS and 5% beta-mercaptoethanol. Samples were heated as described in Fig. 1A. The stacking gel contained 4.5% acrylamide and the running gel contained 13% acrylamide.

weight fraction, as shown in Figures 1A and 2, indicates that these aggregates are not composed of discrete multimers of the 26,000 MW polypeptide. To further check this point, samples were electrophoresed on 7.5% running gels. Again, the SDS-acrylamide pattern was similar to that observed in Fig. 1A, indicating a continuum of aggregation. The gel in Fig. 1 was purposely overloaded to visualize minor bands and to study temperature effects on other membrane proteins. Figure 1 and the densitometric scan in Fig. 2 show that

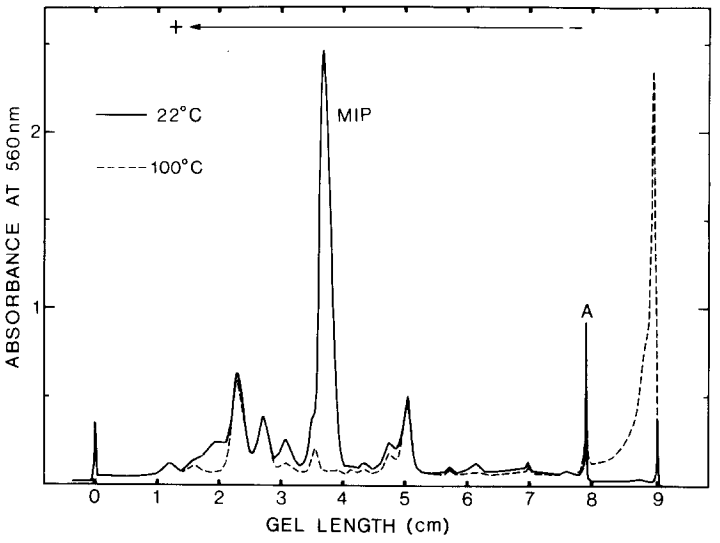

Fig. 2. Densitometric scan of membrane proteins which were incubated at 22° and 100° in high ionic strength buffer. The gels scanned were columns 2 and 5 of Fig. 1A. MIP designates the membrane main intrinsic polypeptide. The line labeled A is a scanning artifact arising from the interface between the stacking gel and the running gel.

some other minor polypeptide bands diminish upon heating, but the effect of temperature on the main intrinsic polypeptide is overwhelming. Densitometric scans of the sample incubated at 38° (not shown) indicate that approximately 20% of the main intrinsic polypeptide was converted to high molecular weight aggregates while no other changes were observed for the remaining polypeptide bands.

Figure 1B shows the SDS-PAGE pattern of another membrane preparation which was dissolved in phosphate buffer containing 1% SDS without 0.1 M NaCl. The sample was heated in the same manner as in Fig. 1A and was then electrophoresed. No heat-induced aggregations are observed at 38° or 57° (see Fig. 1B). Densitometric scans of these samples also failed to detect any heat-induced effects. However, incubation of the sample at 100° for 3 min converted a large fraction of the main intrinsic polypeptide into high molecular weight aggregates (see Fig. 1B, column 4). From a densitometric scan of this sample (not shown), it was estimated that about 80% of the main intrinsic polypeptide

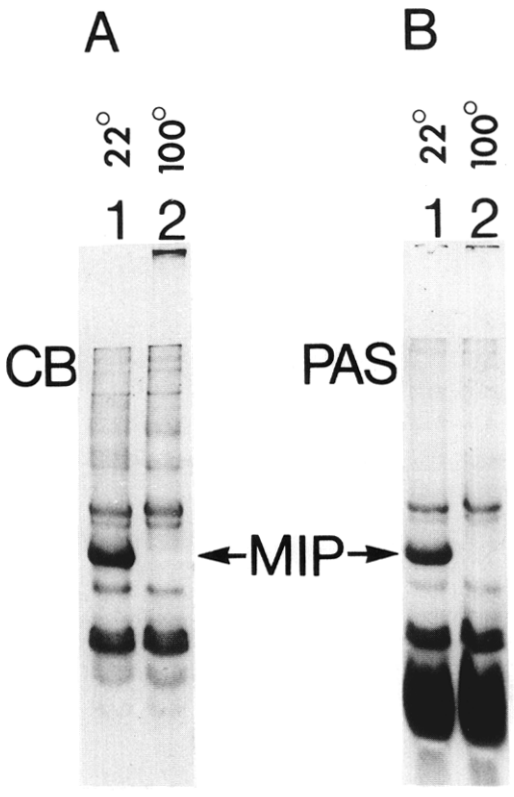

Fig. 3. SDS-gel electrophoresis of lens membrane preparation stained by the periodic acid-Schiff procedure. For comparison, half of the gel slab (Fig. 3A) was stained with Coomassie brilliant blue. MIP designates the membrane main intrinsic polypeptide. The running gel contained 10% acrylamide. Membrane samples were prepared as described in Fig. 1A.

was converted into high molecular weight aggregates. The differences in SDS-PAGE pattern between Figures 1A and 1B are due to the ionic strength of the heat-treated samples and not because of the different buffers used. Identical patterns (seen in Fig. 1A) were observed for membranes solubilized in Tris or phosphate at high ionic strength (0.1 M NaCl), and identical patterns (seen in Fig. 1B) were observed for both buffer systems at low ionic strength.

Figure 3 shows a membrane preparation stained by the periodic acid-Schiff procedure. Note that the only glycoprotein affected by heating is the main intrinsic polypeptide. Heavy PAS staining at the bottom of the gel arises from a membrane glycolipid. As shown in Fig. 3, this glycolipid is not affected by

heating. The protein pattern of the isolated fiber membranes (prepared as described in METHODS) may be contaminated with some soluble crystallins (1, 7). The soluble crystallins migrate in the molecular weight region of 18,000 to 32,000. To ascertain that the heat-induced aggregation of the main intrinsic polypeptide is not dependent on the presence of other proteins or glycolipids, we purified the main intrinsic polypeptide still further. Membrane fractions were dissolved in 20 mM Tris-HCl buffer, pH 7.9, containing 1% SDS, and chromatographed on a Sepharose CL-6B column (1.5 cm x 120 cm) which was eluted with the same buffer. Polyacrylamide gel electrophoresis of main intrinsic polypeptide isolated in this manner showed it to be essentially free from all the other polypeptides and glycolipids. Heating the purified samples of the main intrinsic polypeptide again resulted in induced aggregation identical to that observed in the total membrane protein fractions. Thus the heat lability of the main intrinsic polypeptide is independent of other proteins or glycopeptides present in the preparation.

DISCUSSION:

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a widely used and powerful tool in the study of membrane proteins. It is well known that many proteins, especially glycoproteins, behave anomalously with respect to their apparent molecular weight when subjected to SDS-PAGE (14). Studies of the properties of a sialoglycoprotein from human erythrocytes showed that heating the protein with SDS at 100° caused depolymerization of this major membrane glycoprotein (15). Later it was demonstrated that the aggregation/deaggregation equilibrium depends on the buffer used. Inter-conversion of a high molecular weight fraction into a low molecular weight fraction at higher temperatures does not take place in phosphate-buffered samples, but does occur in Tris buffer systems (16). More recent work has shown that the variability in SDS-PAGE patterns of erythrocyte glycoproteins depends also upon the concentration of the glycoprotein during solubilization, on the SDS concentration, and the ionic strength of the system (17).

As with the erythrocyte glyprotein, we found that the SDS-PAGE patterns of the main intrinsic polypeptide isolated from lens fiber cells depended on the conditions under which the sample was prepared. Heating depolymerized the erythrocyte protein, but the effect of temperature on the main intrinsic polypeptide of the lens membrane was to aggregate the protein. Our results show that the ionic strength of the sample is of utmost importance if the samples (which were solubilized in SDS) are to be heated prior to electrophoresis. Heating samples to 100° can cause a loss of up to 80% of the protein even in low ionic strength buffer. On the other hand, at 0.1 M ionic strength, heating the sample to a mild temperature of 37° can result in significant aggregation.

Broekhuyse and Kuhlmann (6) noted recently that, to avoid formation of an insoluble residue caused by heating membrane preparations for 3 min at 100°, it was necessary to change the heating procedure to 3 h at 37°.

Vermorken et al (18) in studying changes in membrane protein patterns in relation to lens cell differentiation, found that the most striking difference between the protein patterns of plasma membrane derived from epithelium and fibers concerns the 26,000 MW polypeptide. Their SDS-PAGE gels show that this polypeptide is virtually absent in the pattern of epithelial plasma membranes, while there is no difference in the distribution of other polypeptides. They used the SDS-PAGE method of Laemmli (9), which calls for boiling the samples for 1.5 min. In view of our findings, it is clear that the above results must be carefully reevaluated. Especially at temperatures approaching 100°, the difference of a few seconds in the time the samples are heated can result in drastic differences in the amount of protein being converted to high molecular weight aggregates. Thus, temperature and ionic strength must be recorded and controlled carefully for any meaningful, comparative study.

In their recent work on human erythrocyte Glycophorin-A, Schulte and Marchesi (19) suggested that SDS-PAGE should not be used as the sole criterion for determining glycopeptide heterogeneity or in quantitating glycoprotein changes in other membrane systems. Indeed, our work with the main intrinsic polypeptide

of the lens fiber plasma membrane supports their suggestion and provides another example of the unique problems encountered with some membrane glycoproteins when analyzed by SDS-gel electrophoresis.

REFERENCES:

1. Bloemendal, H. (1977) Science 197, 127-138
2. Peracchia, C. (1978) Nature 271, 669-671
3. Goodenough, D. (1978) Biophysical Journal Abstracts 21, 147a
4. Bloemendal, H. Zweers, A., Vermorken, F., Dunia, I. and Benedetti, E.L. (1972) Cell Differentiation 1, 91-106
5. Alcala, J., Lieska, N. and Maisel, H. (1975) Exp. Eye. Res. 21, 581-595
6. Broekhuyse, R.M. and Kuhlman, E.D. (1978) Exp. Eye Res. 26, 305-320
7. Benedetti, E.L., Dunia, I., Bentzel, C.J., Vermorken, A.J.M., Kibbelaar, M. and Bloemendal, H. (1976) Biochem. Biophys. Acta 457 353-384
8. Bloemendal, H., Vermorken, A.J.M., Kibbelaar, M., Dunia, I., and Benedetti, E.L. (1977) Exp. Eye Res. 24, 413-415
9. Laemmli, U.K. (1970) Nature 227, 680-685
10. Weber, K., Pringle, J.R., and Osborn, M. (1972) In: Methods in Enzymology Vol. XXVI part C pp 3-43, Hirs, C.H.W. and Timasheff, S.N., Eds., Academic Press N.Y.
11. Segrest, J.P. and Jackson, R.L. (1972) in: Methods in Enzymology Vol. XXVIII part B pp 54-63, Ginsburg, V. Ed., Academic Press New York
12. McGuchin, W.F. and McKenzie, B.F. (1958) Clin. Chem. 4, 476-483
13. Zacharius, R.M. Zell, T.E., Morrison, J.H., and Woodlock, J.J. (1969) Anal. Biochem. 30, 148-152
14. Tanford, C. and Reynolds J.A. (1976) Biochem. Biophys. Acta 457, 133-170
15. Marton, L.S.G., and Garvin, J.E. (1973) Biochem. Biophys. Res. Comm. 52, 1457-1462
16. Tuech, J.K. and Morrison, M. (1974) Biochem. Biophys. Res. Comm. 59, 352-360
17. Potempa, L.A., and Garvin, J.E.(1976) Biochem. Biophys. Res. Comm. 72,1049-1055
18. Vermorken, A.J.M., Hilderink, J.M.H.C., Dunia, I. Benedetti,E.L. and Bloemendal, H. (1977) FEBS Letters, 83, 301-306
19. Schulte, E.H. and Marchesi, V.T. (1978) Biochem. Biophys. Acta 508, 425-430