A THIRD FORM FOR THE MAJOR GLYCOPROTEIN OF THE HUMAN ERYTHROCYTE MEMBRANE IN SODIUM DODECYL SULFATE: ELECTROPHORESIS AS BAND PAS-4 AT HIGH IONIC STRENGTH

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Received August 2,1976

SUMMARY: A third form for the major glycoprotein of the human erythrocyte membrane in SDS is described. At ionic strengths greater than 0.25 both PAS-1 and PAS-2 are converted almost quantitatively into a form which migrates in SDS-polyacrylamide gels with a mobility intermediate between them in the position usually associated with PAS-4. This conversion is reversed by reduction of the ionic strength. A novel feature of the conversion to this third form is that it is observed to take place during electrophoresis on a high ionic strength gel, although loaded on the gel in a low ionic strength solubilizing solution. Models for the three forms of this glycoprotein in SDS are proposed.

INTRODUCTION: In 1973 we reported that bands PAS-1 and PAS-2, observed during SDS-polyacrylamide gel electrophoresis of human ghosts, and thought to represent two distinct entities (1,2), were, in fact, interconvertible forms of the same glycoprotein (3). This result has been confirmed by many investigators (4,5,6,7,8) and has been useful in the characterization of the glycoproteins of the human erythrocyte membrane (5,6,9). SDS-polyacrylamide gel electrophoresis has continued to be a widely used and powerful tool in the study of the various glycoproteins from the erythrocyte (8,10,11) and from many other tissues (12,13). We now report a third form for the major glycoprotein from the human erythrocyte membrane as observed in SDS-polyacrylamide gel electrophoresis. This third form, reversibly produced at high ionic strength from either PAS-1 or PAS-2, migrates with a mobility intermediate between PAS-1 and PAS-2, in the position usually assigned to PAS-4 (14,15) and PAS-2' (16).

Abbreviations used are: SDS, sodium dodecyl sulfate; PAS, periodic acid Schiff's stain; PAS-1, PAS-2, PAS-4, periodic acid Schiff-staining bands according to numbering system of Fairbanks et al. (1), and Steck and Dawson (14); EDTA, ethylene diamine tetraacetic acid.

MATERIALS AND METHODS: Acrylamide was purchased from Canalco or Eastman Kodak; N,N'-Methylenebisacrylamide was from Canalco; Pyronin Y was obtained from Matheson; sodium dodecyl sulfate from Sigma Chemical Co. All other reagents were Analytical Grade.

Preparation of Ghost Membranes: Blood was drawn from healthy donors using 0.11 M EDTA as anticoagulant. After removal of the plasma and buffy coat, the packed cells were washed three times in buffer 0.154 M in NaCl and 0.005 M in Tris-HCl adjusted to pH 7.4. Hemolysis was carried out in a low ionic strength buffer 0.010 M in Tris-HCl and 0.0001 M EDTA at pH 7.4. The ghost membranes were sedimented by centrifugation at 20,000 x g for 20 min and washed in hemolysis buffer until nearly white (17). Membranes were stored at - 14°C.

Preparation of Isolated Glycoprotein: The glycoprotein was isolated from the ghost membranes by the method of Hamaguchi and Cleve (18), and stored in water solution at 4°C with a drop each of chloroform and toluene.

SDS-Polyacrylamide Gel Electrophoresis: The polyacrylamide gels, 5.6% in acrylamide and 0.21% in bisacrylamide, were prepared according to the method of Fairbanks et al. (1), except where noted. For the experiments on the effects of NaCl concentration, the gels were 1% in SDS, 0.039 M in Tris, 0.012 M in sodium acetate, 0.00176 M in EDTA, adjusted to pH 7.4 with acetic acid, and containing varying additions of NaCl. The electrophoresis buffer for these experiments was identical in ionic components to the gels as noted above except that NaCl was added such as to bring the ionic strength to 0.10. For the experiments on the effect of sodium phosphate concentration, the gels were prepared 1% in SDS with only sodium phosphate as the buffer establishing the pH at 7.4, and 0.0008 M or less in EDTA. The electrophoresis buffer for these experiments was 0.024 M in sodium phosphate, 0.0008 M in EDTA, and 1% in SDS for a total ionic strength of 0.10. Samples were solubilized in 3% SDS with the SDS/glycoprotein-protein ratio held at 200/1, w/w, and kept at either room temperature or heated to 100°C for 15 min. Other than the Pyronin Y tracking dye and sucrose, there were no other additions to the solubilization solutions. Glycoprotein was determined as protein by the method of Lowry using bovine serum albumin as the standard. 15 µg of protein was layered per gel and electrophoresis was carried out at a potential difference of 75 volts between electrodes. Electrophoresis was continued until the Pyronin Y dye front had migrated 9.1 cm. The gels were then stained for carbohydrate by the periodic acid Schiff procedure (1), and the gels scanned at 560 nm on a Beckman DU 2400 spectrophotometer fitted with a Gilson Linear Transport attachment No. 222.

RESULTS AND DISCUSSION: Fig. 1A shows the effect of the concentration of sodium chloride in the gels on the patterns obtained from the electrophoresis of the major human erythrocyte glycoprotein after it has been solubilized at room temperature in 1% SDS. At the lower ionic strengths, as shown in gels (a), (b), and (c) (ionic strengths 0.10 to 0.15), there is the customary predominance of the Pas-1 band (1,3). However, by the time the ionic strength of 0.20 is reached, about half of the Schiff's reagent color is found in the PAS-4 position. At ionic strengths of 0.25 and above, nearly all of the Schiff's reagent color appears in the PAS-4 region (14). Curve A in Fig. 2, which is a densitometric scan of gel (a) in Fig. 1A, shows more precisely the

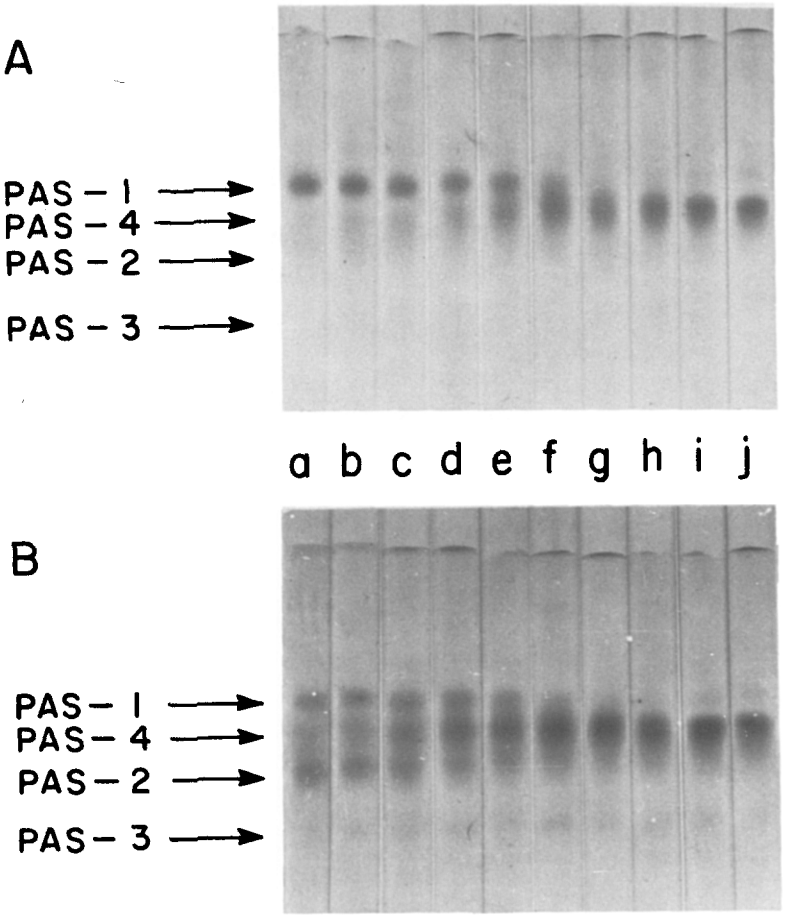

Fig. 1. Effect of the concentration of sodium chloride in the gels on the patterns obtained from the electrophoresis of the major human erythrocyte membrane glycoprotein in polyacrylamide gels containing 1% SDS. (A) Solubilization at room temperature; (B) Solubilization at 100°C for 15 min. The composition of the gels in terms of the concentration of NaCl, and total ionic strength respectively, were as follows: (a) 0.005 M, 0.10; (b) 0.029 M, 0.13; (c) 0.054 M, 0.15; (d) 0.079 M, 0.18; (e) 0.105 M, 0.20; (f) 0.129 M, 0.23; (g) 0.155 M, 0.25; (h) 0.179 M, 0.28; (i) 0.204 M, 0.30; (j) 0.329 M, 0.43. The gels were stained by the Schiff's periodic acid procedure (1). For details see text.

mobilities and densities of the PAS-1 and PAS-4 bands at an ionic strength of 0.10. Comparison of the peak of the PAS-4 band at an ionic strength of 0.28 (Curve B in Fig. 2) with the shoulder at ionic strength 0.10 (Curve A in Fig. 2), shows that there is an almost exact correspondence in their positions. This relative position of band PAS-4 agrees with that found by other investigators (14).

Fig. 1B shows the patterns obtained from a parallel series of gels with the same graduated ionic strengths produced by added NaCl but in which the solubilization was carried out at 100°C. Gel (a) (ionic strength 0.10) shows the expected pattern with the greatly increased density at PAS-2 due to heating in SDS (3). However, as the ionic strength rises there is a rapid increase in the Schiff's color density at the PAS-4 position and a decreased density at both the PAS-1 and PAS-2 positions - gels (b) through (f), (ionic strengths 0.13 through 0.23). As in Fig. 1A, at ionic strengths of 0.25 and above, nearly all of the Schiff's reagent color is found in the PAS-4 position. It appears that both PAS-1 and PAS-2 are converted by high ionic strength in the gels into a species which runs at the PAS-4 position. Curve C in Fig. 2 is the scan of a

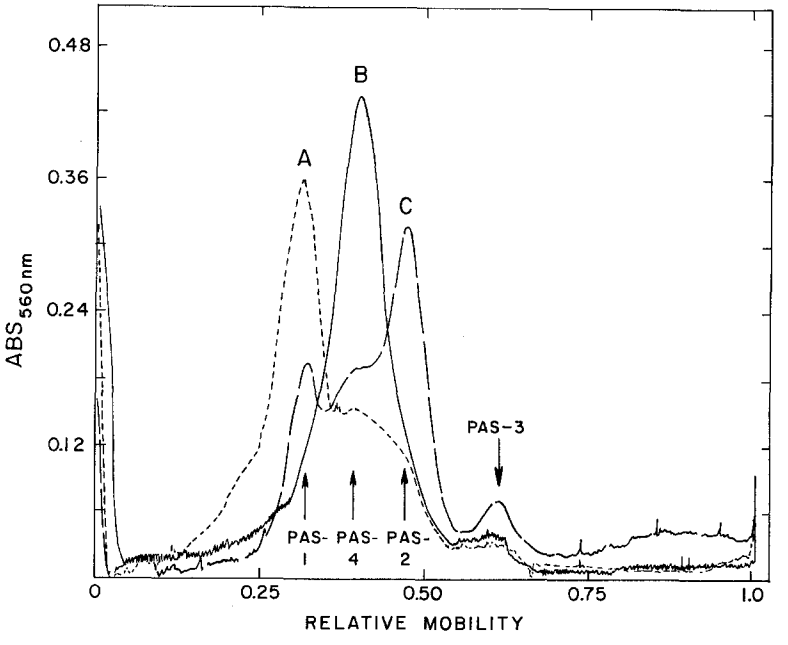

Fig. 2. Electrophoretic profiles of the major human erythrocyte glycoprotein after electrophoresis on 5.6% polyacrylamide gels in 1% SDS. (A) 15 µg of glycoprotein solubilized at room temperature in 3% SDS layered on a gel adjusted to ionic strength 0.10 with NaCl; (B) 15 µg of glycoprotein solubilized at 100°C in 3% SDS layered on a gel adjusted to ionic strength 0.28 with NaCl; (C) 15 µg of glycoprotein solubilized at 100°C in 3% SDS and layered on a gel adjusted to an ionic strength of 0.10 with NaCl. Each gel was stained by the Schiff's periodic acid procedure (1).

gel of ionic strength 0.10, the glycoprotein for which was solubilized at 100°C for 15 min. It shows the expected predominance and relative position of the PAS-2 band. In addition, the PAS-4 shoulder is readily seen. Curve B of Fig. 2 shows the profile obtained from a sample solubilized at 100°C in 3% SDS without added salt, run in a gel of ionic strength 0.28. It is clear that the PAS-4 band constitutes almost the entire scan, there being in addition only the small peak at a relative mobility of about 0.60 corresponding to PAS-3. Although indistinct in Fig. 1A and 1B, PAS-3 was easily seen in the original gels at all ionic strengths used and with its expected mobility of about 0.60 (14).

The experiments shown in Fig. 1 above were repeated using sodium phosphate instead of sodium chloride to adjust the ionic strength. The results were essentially the same, that is, both PAS-1 and PAS-2 were converted almost quantitatively to material with the mobility of PAS-4 at ionic strengths of 0.26 or greater. At lower ionic strengths the distribution of the species was almost identical to that produced by sodium chloride.

The data reported above strongly support the proposal of yet a third form for the major glycoprotein of the human erythrocyte plasma membrane in SDS. This third form appears to be readily derived from either of the two previously described forms - PAS-1 and PAS-2 - which are themselves interconvertible (3-8). This new form can be derived from either PAS-1 or PAS -2 and constitutes the sole form of the major glycoprotein when the ionic strength exceeds 0.26. Its mobility in 1% SDS polyacrylamide gels (5.6%) corresponds to that previously described for PAS-4 (14,15), and overlaps the position taken by PAS-2' (16). The formation of this third form appears to be reversible since if the glycoprotein is solubilized in a 3% SDS solution of high ionic strength, and then dialyzed against 1% SDS, it reveals mainly bands PAS-1 and PAS-2 when electrophoresed on low ionic strength gels (ionic strength = 0.10).

The intermediate mobility found for the PAS-4 band between that of PAS-1 and PAS -2 poses a problem in interpretation, since we have already proposed that PAS-1 is a dimer and PAS-2 is a monomer of the 31,000 dalton species (3).

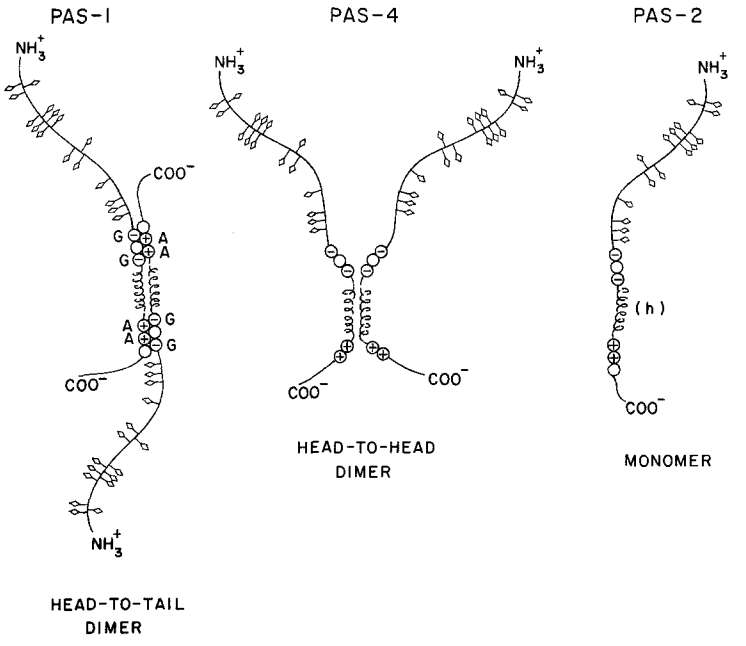

Fig. 3. Schematic models for the three forms of the major human erythrocyte glycoprotein in SDS. ◊, carbohydrate side chains carrying sialic acid residues; G, glu residue; A, arg residue; (h) hydrophobic segment composed of residues 73-96 (19). The bound SDS is omitted from the drawings.

Figure 3 illustrates schematically our model to explain the mobility differences among the various forms. The intermediate mobility of the PAS-4 band dimer is rationalized on the grounds of its intermediate cross-section assuming that the net charge per unit mass is approximately the same for each of the three species. Thus we would predict that the monomer would provide the smallest effective Stokes radius and therefore would have the highest mobility, the head-to-head dimer would have a similar radius regarding length but would be twice as wide giving it a somewhat lower mobility, and finally the head-to-tail dimer would have the largest effective radius being much longer. This proposal is largely consistent with the postulate of Reynolds and Tanford (15), that the length of the rod formed by a polypeptide in SDS is the decisive factor in mobility of the complexes in SDS gel electrophoresis.

Additional grounds for the proposed arrangement of the two dimer models

can be derived from considerations of charge distribution. In the head-to-tail dimer the 20-23 negative charges due to the sialic acid residues on the glycosylated portion of each monomer (19) molecule are widely separated, also the oppositely charged amino acid residues next to the hydrophobic segment of each monomer are brought into potentially close apposition. (arg close to glu). Both of these distributions of charge are consistent with a low electrostatic free energy. On the other hand, at high ionic strength, these charge effects are less important, and it is possible to imagine that as the amino acids of the hydrophobic segment come into register, the forces developed may become decisive, resulting in the production of the head-to-head dimer.

In any case, no matter what the subunit and/or conformational states prove to be, the potential for the conversion of the glycophorin into a species with the mobility of PAS-4 will have to be considered in evaluation of SDS-polyacrylamide gel patterns obtained from human erythrocyte membranes.

ACKNOWLEDGMENTS: This investigation was supported in part by the Edris Fund.

REFERENCES

1. Fairbanks G., Steck, T. and Wallach D. (1971) Biochemistry 10 2606-2617
2. Morrison, M., Mueller T. and Huber C. (1974) J. Biol. Chem. 249 2658-2660
3. Marton L. and Garvin, J. (1973) Biochem. Biophys. Res. Commun. 52 1457-1462.
4. Tuech, J. and Morrison, M. (1974) Biochem. Biophys. Res. Commun. 59 352-360.
5. Furthmayr H. and Marchesi, V. (1976) Biochemistry 15 1137-1144.
6. Silverberg, M., Furthmayr H. and Marchesi, V. (1976) Biochemistry 15 1448-1454
7. Azuma J., Janado M. and Onodera K. (1973) J. Biochem. (Tokyo) 73 1127-1130.
8. Steck, T. (1974) J. Cell Biol. 62 1-19.
9. Garvin, J.E, Potempa, L. A. and Marton L. S. G. (1975). J. Cell Biol. 67 130a.
10. Richards, F. M., and Wang, K. (1974) J. Biol. Chem. 249 8005-8018.
11. Nicholson, G. (1976) Biochem. Biophys. Acta 457 57-108.
12. Brivio-Haugland, R. P., Louis, S. L., Musch, K., Waldeck, N. and Withams, M.A. (1976) Biochem. Biophys. Acta 433 150-163.
13. Nicolson, G., (1976) Biochem. Biophys. Acta 458 1-72.
14. Steck, T. and Dawson, G. (1974) J. Biol. Chem. 249 2135-2142
15. Tanner, M. and Boxer, D. (1972) Biochem. J. 129 333-347
16. Mueller T. and Morrison M. (1974) J. Biol. Chem. 249 7568-7573.
17. Dodge, J., Mitchell, C. and Hanahan, D. (1963) Arch. of Biochem. Biophys. 100 119-130
18. Hamaguchi, A. and Cleve, H. (1972) Biochem, Biophys. Res. Commun. 47 459-464.
19. Tomita M. and Marchesi, V., (1975) Proc. Nat. Acad. Sci. (U.S.A.) 72 2964-2968