Biochimica et Biophysica Acta, 508 (1978) 425—430

BBA 77985

SELF-ASSOCIATION OF HUMAN ERYTHROCYTE GLYCOPHORIN A

APPEARANCE OF LOW MOBILITY BANDS ON SODIUM DODECYL SULFATE GELS

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(Received November 23rd, 1977)

Summary

We have examined the self-association of glycophorin A, the major sialoglycoprotein of the human erythrocyte membrane, using sodium dodecyl sulfate (SDS) polyacrylamide gels and circular dichroism. Pure glycophorin A has a tendency to form multiple bands on SDS gels at positions of higher apparent molecular weight than the PAS 1 and PAS 2 bands previously seen. These high molecular weight bands do not have mobilities corresponding to integral polymers of PAS 1 and PAS 2. Circular dichroism spectra of solutions giving rise to these bands or to PAS 1 and PAS 2 bands alone, indicate that these species all have essentially the same peptide conformation.

Introduction

The analysis of human erythrocyte sialoglycoproteins by SDS gel electrophoresis is a subject of considerable interest and complexity [1—10]. This technique has limitations, however, since early estimates of the molecular weight of erythrocyte glycoproteins were highly variable [1,2,4]. The important finding that the PAS 1 and PAS 2 bands are interconvertible [3] and probably represent a dimer-monomer relationship [3,6] reduced the apparent number of individual sialoglycoproteins of the erythrocyte membrane, but drew attention to the potential problem of incomplete dissociation of glycoprotein oligomers on SDS gels. Variability in the SDS gel patterns of erythrocyte glycoproteins depends upon the concentration of glycoprotein during solubilization [3,6,7], the temperature at which they are solubilized

Abbreviation: SDS, sodium dodecyl sulfate.

[3,5—7], SDS concentration [6,10], and ionic strength of the gel system [8]. In addition, we and others [5] have found that isolated sialoglycoproteins have a greater tendency to form multiple high molecular weight bands than sialoglycoproteins in membrane preparations. These high molecular weight bands have been seen on SDS gels of both the major and minor sialoglycoproteins of the human erythrocyte [11]. Because of this complexity, SDS-gel electrophoresis may be unreliable as the sole tool in determining cell surface glycoprotein changes or as a criterion for glycopeptide heterogeneity [9].

The experiments presented here are intended to illustrate the stability and variability of SDS-gel bands arising from a single glycoprotein species and to suggest that these bands represent polymeric forms of a single species, despite their anomalous gel mobility.

Materials and Methods

Specially pure sodium dodecyl sulfate (SDS) was from BDH Chemicals, Ltd. Acrylamide was obtained from Bio-Rad Laboratories, while bis acrylamide and ammonium persulfate were from Ames Laboratories. Schwarz-Mann was the source of the Tris base and urea, both ultrapure. Other materials were from sources previously reported [6].

Glycophorin A was isolated according to the method of Marchesi and Andrews [12], as modified by Furthmayr et al. [11]. Following separation of glycopeptides by gel filtration in Ammonyx-LO, the glycophorin A fraction was exhaustively dialyzed and then lyophilized. This material is referred to as the standard glycophorin A preparation. For some experiments, this glycophorin A was extracted with absolute ethanol once for 60 min at 0°C, and the resulting precipitate was re-extracted for 15 min at 0°C, before lyophilization. This is referred to as ethanol-extracted glycophorin A. For other experiments, glycophorin A was extracted with chloroform/methanol (2 : 1, v/v) at 0°C for 60 min. The precipitate was then re-extracted for 15 min at 0°C with chloroform/methanol/concentrated hydrochloric acid (200 : 100 : 1, v/v), and was finally washed once with absolute ethanol before lyophilization. This material is referred to as $CHCl_3$/MeOH/HCl-extracted glycophorin A.

SDS polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoreis was performed essentially according to the procedure of Fairbanks et al. [1]. Our gels, however, contain only 0.2% SDS. Solubilization of samples is accomplished as noted in the figures and figure legends. Glycoprotein bands are visualized using a periodic acid-Schiff's base staining procedure [6] and quantitated using a Gilford Spectrophotometer with gel scanning attachment. Migration on these gels is expressed relative to the mobility of the tracking dye, pyronin Y.

Circular dichroism

CD spectra were recorded on a Cary Model 61 circular dichrometer. To obtain spectra at a glycoprotein concentration suitable for observing the heat-induced PAS 1 to PAS 2 transition, it was necessary to use a 1 cm path length cell, and measure ellipticity at a full scale range of 0.05°. Spectra were recorded

using a solution of 0.08 mg/ml ethanol-extracted glycophorin A in 10 mM Tris-acetate/2% SDS, pH 7.0, with 4 M urea added or not, as noted in the figure legends. Glycoprotein concentration was determined by quantitative amino acid analysis, using L-norleucine as an internal standard.

Results

Fig. 1A and B shows the SDS gel electrophoresis patterns of a standard glycophorin A preparation solubilized at 2 different temperatures. Fig. 1C and D shows gel patterns of the same standard glycophorin A preparation after absolute ethanol extraction, and Fig. 1E and F shows the same standard glycophorin A preparation after $CHCl_3$/MeOH/HCl extraction. All three of these preparations show a band on SDS gels at a higher apparent molecular weight than PAS 1 unless solubilized at high temperatures. Furthermore, as shown in Fig. 1F, $CHCl_3$/MeOH/HCl-extracted glycophorin A exhibits this band even after heating at 100°C in 2% SDS.

To determine whether the inclusion of 4 M urea in the solubilizing medium would reduce the number of gel bands of glycophorin A, we conducted the experiment shown in Fig. 2.

Fig. 2A shows the change in gel pattern of ethanol-extracted glycophorin A after heating in 10 mM Tris-acetate/2% SDS, pH 7.0. Clearly, heating has

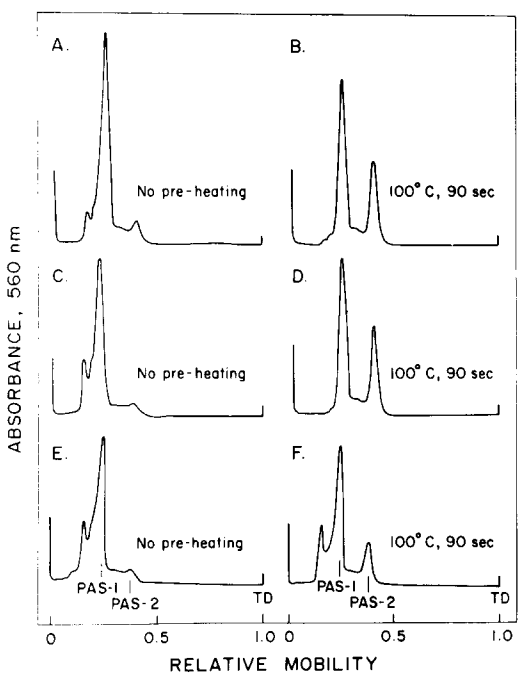
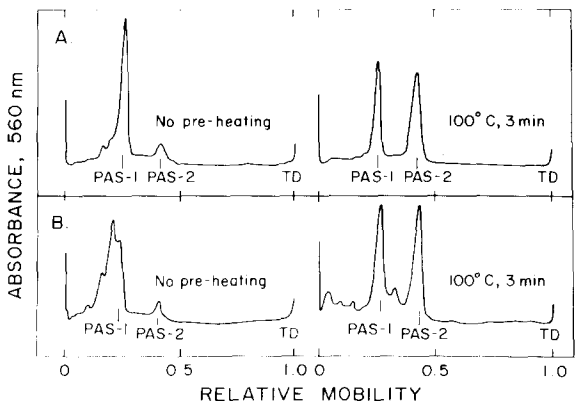


Fig. 1. Gel patterns A and B are of a standard glycophorin A preparation, C and D are of the same standard preparation after ethanol extraction, and E and F are of the same standard preparation after $CHCl_3$/MeOH/HCl extraction. All samples were solubilized in 2% SDS under conditions shown above each pattern and were electrophoresed as given in Materials and Methods. Concentration of glycoprotein in solubilizing medium was 0.5 mg/ml.



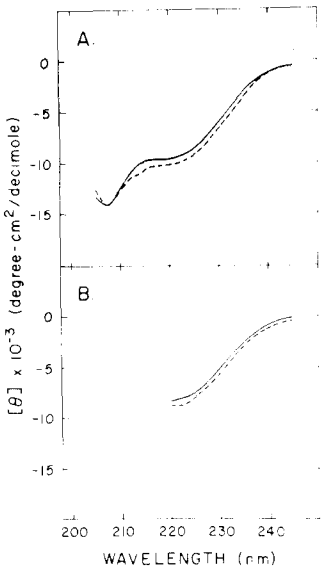


Fig. 2. A shows the gel patterns of an ethanol-extracted glycophorin A preparation, in 10 mM Tris-acetate/2% SDS, pH 7.0, before and after heating at 100°C. B shows the gel patterns of the same ethanol-extracted glycophorin A preparation, in 10 mM Tris-acetate/2% SDS/4 M urea, pH 7.0, also before and after heating at 100°C. Glycoprotein concentration was 0.08 mg/ml in these samples.

Fig. 3. A and B show the CD spectra of the same solutions which gave rise to the gel patterns shown in Fig. 2A and B, respectively. - - - - - -, Sample before heating at 100°C, 3 min; ———, sample after heating at 100°C, 3 min.

caused the conversion of over 40% of the total material from the PAS 1 to the PAS 2 band. Fig. 2B shows the same experiment done in 10 mM Tris-acetate/2% SDS/4 M urea, pH 7.0. In this case, heating causes the conversion of more than 40% of the total material from primarily higher apparent molecular weight bands to PAS 2. It is clear that urea tends to promote the appearance of high apparent molecular weight bands in the sample which was not heated, but has little effect after heating.

Using protein molecular weight markers, we found that the higher apparent molecular weight bands, shown in Fig. 2B, do not migrate as integral polymers of PAS 1 and PAS 2. Despite their anomalous mobility, though, the simplest explanation for the high apparent molecular weight bands of glycophorin A is thaty they are oligomers of a single glycoprotein species. An alternative explanation is that these bands are very different conformational forms of glycophorin A, binding less SDS, and thus having decreased mobility on gels. To test these hypotheses, we examined, by circular dichroism, the conformation of glycophorin A species in the solutions used for Fig. 2.

Fig. 3 shows the CD spectra of these solutions. We have previously examined the CD spectra of glycophorin A in SDS at concentrations of glycoprotein where no PAS 1 to PAS 2 transition occurs after heating, and we found essentially no change in CD spectra due to heating alone. In Fig. 3A, the CD spectra of glycophorin A in SDS, before and after heating, indicates that very little, if any, change has occurred in glycopeptide conformation despite the

considerable conversion of PAS 1 to PAS 2 seen in Fig. 2A. Similarly, Fig. 3B shows that glycophorin A, in SDS and 4 M urea, shows essentially no change in conformation after heating, despite the very considerable conversion of high apparent molecular weight species to PAS 2 seen in Fig. 2B. The small differences in the spectra of either Fig. 3A or 3B are close to the experimental error of the instrument under these conditions.

We conclude, then, that the glycophorin A species giving rise to the PAS 1 and PAS 2 bands on SDS gel have the same conformation, and that the apparently higher molecular weight bands of glycophorin A share this conformation and are probably oligomers of PAS 1 and PAS 2. Analysis of the PAS 1 and PAS 2 bands, using Ferguson plots, corroborates these conclusions, indicating that these bands do indeed have different molecular weights [10] and this, rather than conformational difference, accounts for their differences in mobility.

Discussion

Investigators in this field have realized for some time that the SDS gel bands PAS 1 and PAS 2 are in equilibrium with each other and are forms of a single species of erythrocyte glycoprotein [3—7,10]. We have shown here that this glycoprotein, glycophorin A, can also exhibit apparently higher molecular weight bands, and that these forms are quite stable, particularly after organic solvent extraction of the glycoprotein. These apparently higher molecular weight bands do not migrate on SDS gels as integral polymers of the PAS 1 and PAS 2 bands, and yet, after heating, they can be quantitatively converted to the PAS 1 and PAS 2 forms. To investigate the nature of these multiple species, we examined the CD spectra of glycophorin A in solution under conditions which would dramatically change its SDS gel electrophoresis pattern. The absence of significant changes in the CD spectra of the glycoprotein solutions indicates that all these SDS gel bands are derived from glycophorin A species having very similar conformations. We believe, then, that these multiple bands are oligomeric forms of a single glycophorin A species, which migrate anomalously on SDS gels.

We would like to point out that these studies were conducted with a single glycoprotein component of the human erythrocyte membrane. Other glycoproteins of this membrane can also show multiple high molecular weight bands on SDS gels [11]. In view of this tendency for erythrocyte glycoproteins to form stable oligomers, we urge that SDS gel electrophoresis not be used as a sole criterion for determining glycopeptide heterogeneity or in quantitating glycoprotein changes in other membrane systems.

Acknowledgement

We would like to thank Dr. Joseph Coleman for the use of the Cary Model 61 circular dichrometer.

References

1 Fairbanks, G., Steck, T. and Wallach, D. (1971) Biochemistry 10, 2606—2617
2 Tanner, M. and Boxer, D. (1972) Biochem. J. 129, 333—347

3 Marton, L. and Garvin, J. (1973) Biochem. Biophys. Res. Commun. 52, 1457–1462
4 Azuma, J., Janado, M. and Onodera, K. (1973) J. Biochem. (Tokyo) 73, 1127–1130
5 Tuech, J. and Morrison, M. (1974) Biochem. Biophys. Res. Commun. 59, 352–360
6 Furthmayr, H. and Marchesi, V. (1976) Biochemistry 15, 1137–1144
7 Silverberg, M., Furthmayr, H. and Marchesi, V. (1976) Biochemistry 15, 1448–1454
8 Potempa, L. and Garvin, J. (1976) Biochem. Biophys. Res. Commun. 72, 1049–1055
9 Mueller, T., Dow, A. and Morrison, M. (1976) Biochem. Biophys. Res. Commun. 72, 94–99
10 Silverberg, M., Chow, C. and Marchesi, V.T. (1977) Biochim. Biophys. Acta 494, 441–446
11 Furthmayr, H., Tomita, M. and Marchesi, V.T. (1975) Biochem. Biophys. Res. Commun. 65, 113–121
12 Marchesi, V. and Andrews, E. (1971) Science 174, 1247–1298