HUMAN ERYTHROCYTE MEMBRANE GLYCOPROTEIN: A RE-EVALUATION OF THE MOLECULAR WEIGHT AS DETERMINED BY SDS POLYACRYLAMIDE GEL ELECTROPHORESIS

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SUMMARY. Molecular weights for the human erythrocyte membrane glycoprotein and other glycoproteins calculated relative to protein standards on SDS acrylamide gel electrophoresis depend upon the percent crosslinking of gels. This anomaly is due to a decreased binding of SDS to the oligosaccharide side chains relative to the polypeptide backbone; removal of sialic acid fails to correct the anomaly. Previous molecular weights proposed for the human erythrocyte membrane glycoprotein utilizing SDS gel electrophoresis are probably incorrect and a new value of 55,000 is proposed based on corrected SDS-gel data.

Reported molecular weights for the monomeric unit of the major glycoprotein of the human red cell membrane range from 31,000[1] to 160,000[2]. The lower value was obtained by ultracentrifugal studies of glycoprotein preparations that were purified from membranes treated with hot phenol. More recent studies with the SDS-acrylamide gel electrophoresis technique[3] have indicated molecular weights of 108,000[4] and 160,000[2]. We have found that when the purified membrane glycoprotein is studied by acrylamide gel electrophoresis in SDS, its apparent molecular weight varies with the degree of acrylamide crosslinking. This paper describes experiments which show that polypeptide chains which contain a substantial amount of covalently-bound carbohydrate migrate at rates which are not proportional to their molecular mass. This anomalous behavior is especially marked in the cases of glycoproteins and tryptic glycopeptides derived from cell membranes.

MATERIALS AND METHODS. The following proteins were used to construct standard plots of log molecular weight versus mobility in the different acrylamide gel concentrations studies: Bovine serum albumin (Pentex), M.W. 68,000; aldolase

(Pharmacia), M.W. 40,000; α-chymotrypsinogen A (Pharmacia), M.W. 25,700; myoglobin (Pentex), M.W. 17,200.

The human erythrocyte membrane glycoprotein was isolated and purified by the procedure of Marchesi and Andrews[5]. Tryptic glycopeptides were prepared from the erythrocyte glycoprotein by incubation of the glycoprotein with 2% L-1-tosylamido-2-phenylethylchloromethyl-1-ketone-treated trypsin (Worthington) in 0.10 M Tris-HCl, pH 8.2, at 37°C for 24 hrs. A glycopeptide was purified from the digestion mixture by fractionation on columns of Dowex 50-X2, Sephadex G-150, and DEAE-cellulose (In preparation). The porcine ribonuclease was a gift of Drs. C.H.W. Hirs and I. Kabasawa of Indiana University. Desialized glycoprotein and glycopeptide were prepared by treating the samples with Type VI Clostridium perfringens neuraminidase (Sigma) in 0.05 M Tris-maleate buffer, pH 5.3. After 1 hr. at 37°C, 100% of the sialic acid had been released. The desialized samples were run directly on SDS gel electrophoresis.

Four concentrations of acrylamide gels (5, 7.5, 10 and 12.5%) were used for calculation of apparent molecular weights for the three native and the two desialized glycoproteins. A constant proportion of acrylamide to N-N^{1}-methylene-bisacrylamide was maintained in all gels (25:1). The electrophoresis was carried out by a modification of the procedure of Shapiro et al.[3]. The proteins were dissolved in 1% SDS containing 10^{-2} M dithiothreitol and incubated for 1 hr. at 37°C. Before electrophoresis an equal volume of 0.002 M sodium phosphate buffer, pH 7.1 in 8 M urea was added for a final SDS concentration of 0.5%. After electrophoresis the gels were fixed overnight and stained with either Coomassie brilliant blue or periodic acid Schiff. Standards and glycoproteins were run separately and together with identical results.

RESULTS AND DISCUSSION. The mobilities of the four protein standards when plotted against the log of their molecular weights are linear for each of the four acrylamide gel concentrations studied (Fig. 1). When the apparent molecular weights of the different glycoproteins (native human RBC glycoprotein, tryptic glycopeptide and porcine ribonuclease) are extrapolated from the

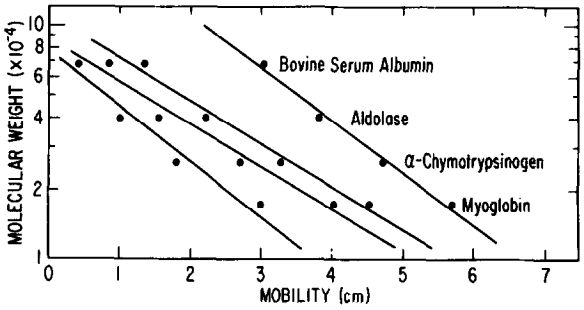

Fig. 1. Log of molecular weight versus mobility by SDS gel electrophoresis of four standard proteins run in increasing concentrations of acrylamide. The plots from right to left are the 5, 7.5, 10, and 12.5 percent gels.

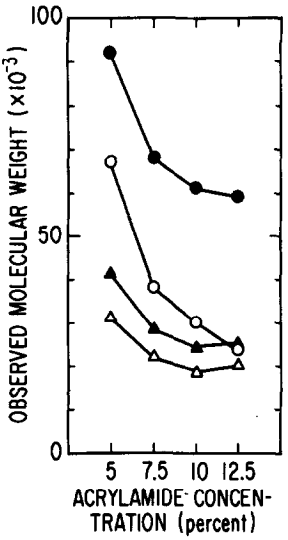

Fig. 2. Observed molecular weights of three glycoproteins calculated by electrophoretic mobility relative to the standard curves in Fig. 1 versus acrylamide gel concentration. (●———●) Human erythrocyte membrane glycoprotein; (o———o) human erythrocyte membrane tryptic glycopeptide; (▲———▲) porcine ribonuclease, higher molecular weight species; (△———△) porcine ribonuclease, lower molecular weight species.

appropriate standard curves and plotted against gel concentration (Fig. 2), a markedly nonlinear relationship is found in each case.

Removal of the sialic acid residues from the human erythrocyte glycoprotein and tryptic glycopeptide by neuraminidase further accentuates the anomalous migratory patterns of these molecules (Fig. 3). Allowing for the loss of molecular weight represented by sialic acid from the human erythrocyte membrane

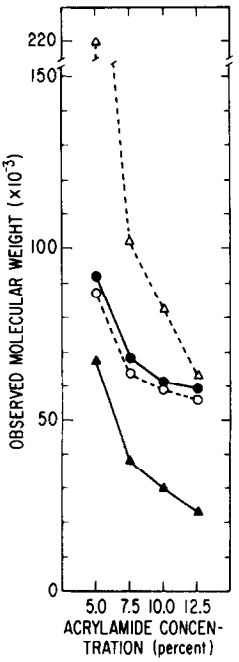

Fig. 3. Observed molecular weights of native and desialized glycoproteins on SDS gel electrophoresis versus acrylamide gel concentration. (●———●) Native human erythrocyte membrane glycoprotein; (o------o) desialized human erythrocyte membrane glycoprotein; (▲———▲) "native" human erythrocyte membrane tryptic glycopeptide; (Δ------Δ) desialized human erythrocyte membrane glycopeptide.

glycoprotein and glycopeptide (25 and 50% respectively), the desialized glycoprotein has a 20% increase in molecular weight and the desialized tryptic glycopeptide more than a 100% increase for each gel concentration examined.

The low mobility of the desialized glycopeptide even on 5% gels suggests a low binding of SDS to this molecule. The neuraminidase results indicate that the negatively charged sialic acid residues partially compensate for this decreased binding of SDS. It is unlikely that the low mobility of the desialized glycopeptide represents aggregation since this molecule forms sharp bands well away from the top of standard acrylamide gels.

The apparent low binding of SDS to the desialized glycopeptide suggests a possible explanation of the anomalous electrophoretic behavior of the glycoproteins relative to standard polypeptides. If less SDS were bound to glycopeptides on a weight basis then the complex would have a lower charge/mass

ratio than standard polypeptides. The effect of this on the mobility of the SDS-glycoprotein complex would be most pronounced at low gel concentrations where the sieving effect is least. This explanation is supported by the results shown in Table 1; the human erythrocyte membrane glycoprotein and tryptic glycopeptide bind only 50 and 4% respectively of the SDS bound by protein standards per gram of protein. A low binding of SDS to glycoproteins is consistent with the shape of the curves in Fig. 2 where the apparent molecular weight values approach assymptotically the real molecular weights with increasing gel crosslinking. It is also likely this corrected apparent molecular weight is still slightly higher than the real one. This is shown to be the case most clearly for porcine ribonuclease since this glycoprotein (20-35% carbohydrate)[6] is the best characterized of the three included in this study. Reinhold et al.[6] have shown that porcine ribonuclease has a dimorphic molecular population with molecular weights of 21,000 and 17,000. The corrected molecular weights from Fig. 2 for this glycoprotein are 24,000 and 20,000.

These results show that molecular weights for major glycoproteins cannot

Table 1. Binding of S^{35}-sodium dodecyl sulfate to proteins. Each protein (4 mg) in 4 ml of 0.1M potassium phosphate buffer, pH 7.2, containing 10^{-3}M dithiothreitol was dialyzed against 400 ml of the same buffer which contained 2 X 10^{-3}M S^{35}-sodium dodecyl sulfate (Amersham, 343,545 cpm/mg). After 72 hours the samples were removed and radioactivity determined with a Beckman Scintillation Spectrometer.

Protein	Total cpm bound x 10^{-3}	g SDS / g protein
Bovine serum albumin	1,009	0.73
Ovalbumin	1,213	0.88
Orosomucoid	560	0.41
Erythrocyte glycoprotein	531	0.38
Erytrocyte glycopeptide	32	0.023

be estimated accurately on the basis of gel electrophoresis in SDS utilizing a single concentration of acrylamide. Gel concentrations over 10% appear to give the most accurate results, although even with these gels the results might be high by a considerable factor (e.g. the desialized glycopeptide on 12.5% gels has an apparent molecular weight of 58,000).

As a consequence of the anomalous behavior of glycoproteins on SDS gels the most recently reported molecular weights for the human erythrocyte membrane glycoprotein[2,4] are apparently incorrect. Blumenfeld et al.[2] and Lenard[4] have reported molecular weights for this glycoprotein of 160,000 and 108,000 respectively based upon the mobility of polypeptides in 5% gels. In our 5% gels we find an apparent molecular weight of 92,000 (Fig. 2). If we correct the mobility of the membrane glycoprotein by a factor equal to the correction required to normalize the mobility of porcine ribonuclease, the lower value of 59,000 for the erythrocyte glycoprotein suggests a real molecular weight of approximately 50-55,000. Since the erythrocyte glycoprotein is 60% carbohydrate, the proposed molecular weight is consistent with a polypeptide chain of roughly 200 residues, a value in excellent agreement with chemical data based on cyanogen bromide peptides (In preparation).

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