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SELECTIVE SOLUBILIZATION OF RED BLOOD CELL MEMBRANE PROTEINS WITH GUANIDINE HYDROCHLORIDE

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SUMMARY

The treatment of red blood cell membranes with 6 M guanidine hydrochloride *plus* reduction and alkylation produces a turbid suspension which is resolved by ultracentrifugation into a clear solution and an overlying membranous pellicle. As analyzed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, the guanidine·HCl-soluble fraction is deficient in a major polypeptide component (III), the principal sialoglycoproteins, and some minor bands, all of which are selectively concentrated in the buoyant lipoid layer. These data suggest the particular lipophilicity of these unsolubilized polypeptides and account for the discrepancy between preparations of membrane proteins solubilized in sodium dodecyl sulfate and in guanidine·HCl.

Guanidine·HCl and sodium dodecyl sulfate have recently been employed in the solubilization and analysis of human erythrocyte membrane proteins[1-3]. It seemed desirable to compare the data obtained with these two agents, particularly because some uncertainties have accrued concerning the molecular size and prevalence of the major polypeptides of this membrane. Despite good general agreement, an important discrepancy may be noted: a major polypeptide, which appeared on gel filtration[2] and on polyacrylamide gel electrophoresis[2,3] in sodium dodecyl sulfate as a roughly 100 000-dalton component, was not observed upon gel filtration of guanidine·HCl extracts[1]. It was postulated[3] that the missing component, called Band III, might have remained associated with the lipids following guanidine treatment and was thereby excluded from the subsequent analysis. The present study sought to evaluate this issue by analyzing the polypeptides recovered in the soluble and insoluble fractions derived from guanidine·HCl-treated erythrocyte membranes.

Human erythrocyte ghost membranes were prepared according to the method of FAIRBANKS *et al.*[3]. Guanidine treatment was fashioned after GWYNNE AND TANFORD[1]. Equal weights of packed ghosts (around 4 mg protein/ml) and guanidine·HCl were mixed to approximate a 6 M solution. The cloudy suspension was reduced

Abbreviations: PAS, periodic acid–Schiff; I–VII, numerals designating the major polypeptides demonstrated by electrophoresis; PAS 1–3, the principle sialoglycoproteins demonstrated by electrophoresis[3].

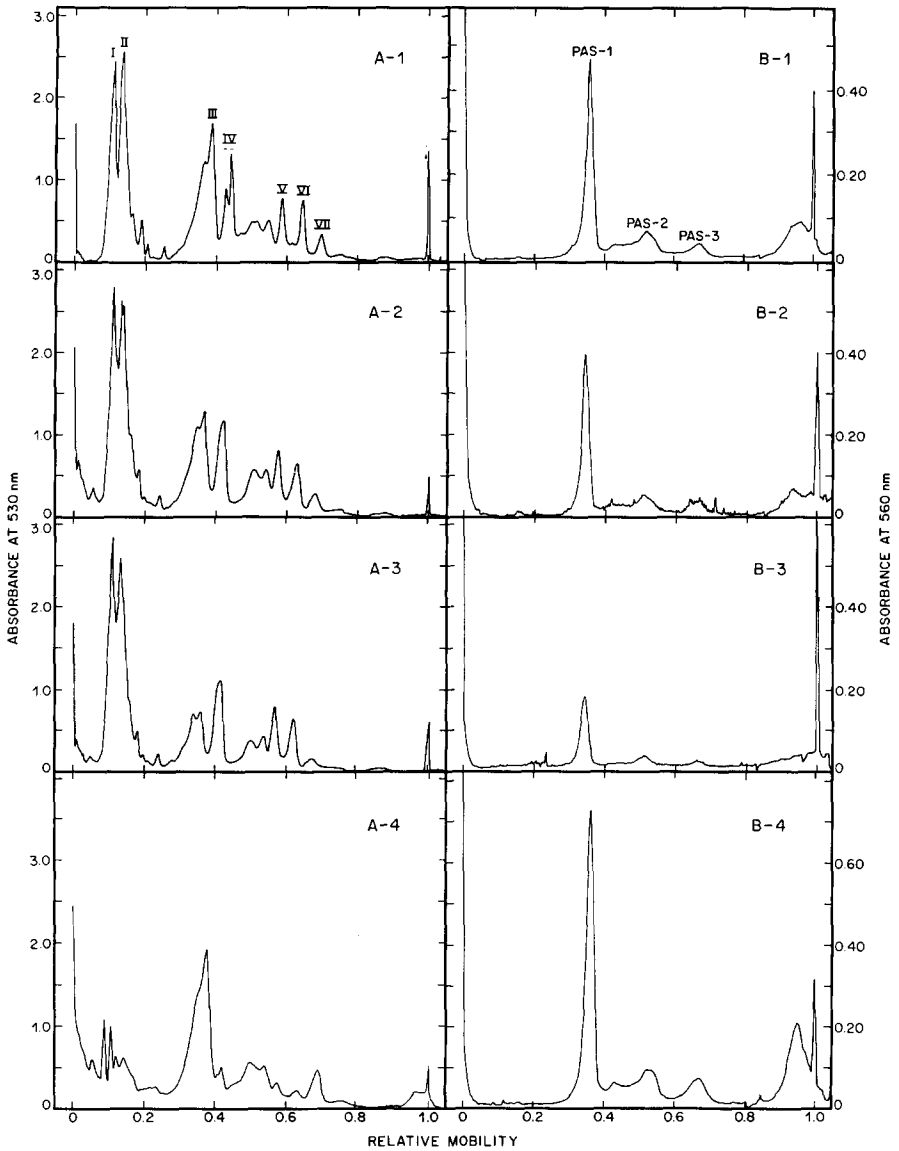

Fig. 1. Electrophoretograms of membrane proteins and sialoglycoproteins extracted with guanidine·HCl. Dialyzed samples were prepared and run on polyacrylamide gels according to the method of FAIRBANKS *et al.*[3], except that the gels were made 5.0% in acrylamide monomer and 0.2% in sodium dodecyl sulfate. As a result, Band IV split into a doublet (which is resolved here only on Scans A-1 and A-4). Approx. 20–40 μg protein were loaded on each gel. Polypeptide mobilities are plotted relative to that of the ink stab which records the position of the tracking dye. Column A (left): protein stain. Gels were stained with Coomassie brillant blue and scanned at 530 nm. The major polypeptides are designated I-VII (refs. 3, 5). Column B (right): carbohydrate stain. Duplicate gels were treated with PAS and scanned at 560 nm. PAS 1-3 are the major sialoglycoproteins; the broad peak migrating just behind the tracking dye is associated with the membrane lipids[2,3]. Row 1 represents untreated ghosts; Row 2 depicts the guanidine·HCl-treated unfractionated mixture; Row 3 represents the clear solution; and Row 4 the membranous residue obtained by centrifuging guanidine·HCl-treated ghosts.

with 0.01 M dithiothreitol at 37° for 15 min and then alkylated with 0.1 M iodoacetamide at 37° for 15 min. (Protection of the sulfhydryl groups is necessary to avoid irreversible aggregation of certain polypeptides.) Centrifugation at 15° for $9 \cdot 10^6 \times g \cdot$ min resolved the suspension into a clear solution and an opaque pellicle on its surface. The latter has been found to contain the membrane lipids[1,4]. It appeared by phase contrast microscopy to be composed of membrane vesicles of varying size. The clear solution and membranous pellicle were collected separately, diluted with 0.01 M β-mercaptoethanol in 0.005 M sodium phosphate (pH 8.0), and dialyzed overnight against two changes of the same solution.

The dialyzed samples were analyzed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate by a modification of the methods of FAIRBANKS *et al.*[3]. Fig. 1 summarizes the results. It is seen that, except for a small amount of aggregated material near the top of the gel, the normal electrophoretic pattern of the ghost proteins was conserved following treatment with guanidine·HCl (Gel A-1 *versus* A-2 and B-1 *versus* B-2). However, upon centrifugation, the polypeptides were unequally partitioned between the clear solution and membrane residue (Gel A-3 *versus* A-4 and B-3 *versus* B-4). The "soluble" fraction was depleted of Components III and VII and some minor bands, such as those migrating between the doublet IV and Band V. In addition, the sialoglycoproteins (PAS 1–3),which are not well stained by Coomassie blue but are visualized by the peroidic acid–Schiff (PAS) reaction[3], were also diminished in the soluble fraction. In a reciprocal fashion, the insoluble residue was selectively enriched in all these polypeptides as well as the PAS-positive lipids. The relatively small amounts of the guanidine·HCl soluble polypeptides recovered with the lipoid pellicle could result from cross-contamination or could reflect their continued association with the membrane residue.

The selective enrichment of certain polypeptides in the residue could indicate their hydrophobicity. In particular, Component III and the sialoglycoproteins appear to span the thickness of the isolated red blood cell membrane[5–7], suggesting that an intimate, hydrophobic association with lipids may exist *in situ*.

These data indicate that solubilization with guanidine·HCl, as with many other agents[8], is selective in that certain polypeptides are differentially released while others remain associated with an insoluble membrane matrix. Similar findings on the sialoglycoproteins of ox erythrocyte membranes have recently been published[4]. The view[9,10] that total membrane protein solubilization is achieved by guanidine·HCl treatment is not supported by these data (nor was it suggested in the original study[1]). On the other hand, we have found that substituting guanidine thiocyanate for the hydrochloride results in a completely clear solution; no pellicle forms following ultracentrifugation. For this reason, it may be of advantage in the study of membrane proteins dissolved in concentrated guanidine solutions.

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