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THE INADEQUACY OF 6 M GUANIDINE HYDROCHLORIDE AS A DISPERSIVE AGENT FOR MEMBRANE PROTEINS

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SUMMARY

It is shown that when erythrocyte membranes are dispersed in 6 M guanidine hydrochloride some of the protein, a fraction rich in sialic acid, remains associated with the lipid. The guanidine is also shown to be incapable of dissociating protein aggregates formed during other methods of membrane protein isolation.

The complexity of the interactions of membrane proteins as demonstrated by the multitude of solubilisation and fractionation schemes now available, has lead to a search for conditions which destroy intermolecular interactions[1-3]. The most recent method suggested[3] utilises 6 M guanidine hydrochloride, a solution known to disrupt all but covalent bonds in almost all soluble proteins examined[4]. In our experience some caution must be exercised before it is assumed that this disruptive effect is generally applicable to membrane proteins.

GWYNNE AND TANFORD[3] exposed human erythrocyte ghosts to 6 M guanidine hydrochloride and after centrifugation at 100000 × *g* for 2 h recovered in the clear solution in the lower part of the centrifuge tube 60% of the membrane's protein relatively free of lipid. The protein solution contained a significant amount of a protein with a molecular weight of 192000 together with smaller molecules ranging down to 14000. The molecular weight of this large entity was initially determined by gel exclusion chromatography and confirmed by sedimentation equilibrium centrifugation. Its apparent hydrodynamic radius and ORD spectrum were consistent with its being in a single unassociated disordered polypeptide chain. GWYNNE AND TANFORD[3] are fully aware of the possible limitations of guanidine hydrochloride as a general solvent for membrane proteins but lest their caution pass unheeded we wish to report certain interactions of membrane proteins that are not dissociated by 6 M guanidine hydrochloride.

We were able to repeat, using ox erythrocyte membranes, the essentials of the investigation on human ghosts. The ghost suspension was made 6 M with respect to guanidine hydrochloride by addition of solid, and after 2 h at 37° in the presence of 20 mM dithiothreitol the mixture was centrifuged for 2 h at 45000 rev./min in a Spinco 50 rotor. The two layers were separated but the upper opaque fatty layer could not be collected without some contamination from the lower clear layer. The following observations (Table I) were made on the centrifuged mixture: (i) The distribution

TABLE I

THE RELATIVE CONCENTRATIONS OF PROTEIN, SIALIC ACID AND PHOSPHATE BETWEEN THE TOP AND BOTTOM LAYERS PRODUCED BY CENTRIFUGATION OF GHOST PREPARATIONS IN GUANIDINE HYDROCHLORIDE

Centrifugation was performed using a Spinco 50 rotor, 45000 rev./min for 2 h.

	Protein	*Sialic acid*	*Phosphate*
A. Centrifugation of ghost suspension in 6 M guanidine hydrochloride + 20 mM dithiothreitol:			
Total suspension	1.00	1.00	1.00
Top layer	1.26	1.81	2.30
Bottom layer	0.80	0.43	0.45
B. Centrifugation of bottom layer obtained from A:			
Total solution	1.00	1.00	N.D.*
Top layer	1.25	2.40	N.D.
Bottom solution	0.83	0.08	N.D.

* N.D., not determined.

of lipid. After the relatively short centrifugation most of the lipid migrated to the top of the tube into the thick viscous layer. The large negative sedimentation coefficient indicated by this result implies that the lipid was present as aggregates of considerable size. (ii) The concentrations of protein (measured by a modified Lowry method[5]) showed a marked enrichment of the upper layer. Therefore some protein moved upwards although its own density is certainly greater than that of the guanidine solution (1.15). (iii) The distribution of sialic acid. The sialic acid, which in these membranes is attached to protein, also tended to pass into the upper layer where its concentration relative to protein was greater than in the lower phase and in the uncentrifuged mixture. These effects were repeated when the lower phase was recentrifuged. The three observations show that the guanidine has, perhaps surprisingly, not destroyed interactions between lipid molecules and between lipid molecules and certain proteins, particularly sialoproteins.

As the forces responsible for the interactions between proteins (*i.e.* combinations of electrostatic, van der Waals and hydrophobic forces in varying proportions[6]) are of the same nature as those between lipids, and between lipids and proteins, the effect of the reagent was tested on proteins which were isolated from the membrane by other methods. The proteins of ox erythrocyte ghosts were prepared as a virtually lipid-free aqueous solution by treatment of the ghost with *n*-butanol[7]. When the freshly prepared ghost was treated and the resulting protein solution, which contained about 90 % of the membrane proteins, was centrifuged in phosphate buffer the largest component had a sedimentation coefficient ($s_{20,w}$) around 20S, and addition of 6 M guanidine hydrochloride and 20 mM dithiothreitol reduced the $s_{20,w}$ of the largest particle to 12S. However, if the membrane protein was solubilised in two stages, first extracting the ghost with either dilute acetic acid or 0.5 mM ethylenediaminetetracetic acid (EDTA) which released about 30 % of the protein, and subsequently solubilising the remaining protein by butanol[8], the butanol-treated extract now contained a particle sedimenting at 40–50 S whose corrected sedimentation coefficient was not affected by guanidine and sulphydryl reagent. (In the solution prepared by direct treatment of the ghosts

with guanidine and thiol reagent all the material was of 2.65 S and less) Thus guanidine is unable to disaggregate a complex which formed under quite mild conditions of protein extraction.

The elucidation of the interactions between membrane proteins is probably a prerequisite to the analysis of the protein composition of membranes. Ideally, such an analysis should be conducted under conditions which do not irreversibly destroy the native conformations of the proteins, but in view of the extreme complexity of the interactions much useful information can be obtained by more vigorous methods. However, the efficacy of these procedures must be carefully assessed in each case and not assumed from the effects they produce on soluble globular proteins. The disruptive effects of 6 M guanidine hydrochloride on almost all globular proteins examined are well known[1] but our findings indicate that this generalisation may not be extended to membrane proteins.

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