Biochimica et Biophysica Acta, 426 (1976) 271–287

BBA 77215

EFFECTS OF pH DURING RECOMBINATION OF HUMAN ERYTHROCYTE MEMBRANE APOPROTEIN AND LIPID

ERNST WEHRLI*, SUSANNE MOSER and PETER ZAHLER

Theodor Kocher Institute and Department of Anatomy, University of Bern, Central Laboratories of the Swiss Blood Transfusion Service SRK, Freiestrasse 1, Bern (Switzerland)

(Received July 23rd, 1975)
(Revised manuscript received September 29th, 1975)

SUMMARY

The recombinates from human red cell membrane proteins and lipids resulting from dialysis of the components in 2-chloroethanol against aqueous buffers from pH 2–12 have been studied by density gradient centrifugation, polyacrylamide gel electrophoresis and freeze-fracture electron microscopy.

Between pH 4 and 10 most of the proteins were found in the recombinates whereas below pH 4 and above pH 10 only part of them were recovered in the lipoprotein band after density gradient centrifugation.

At low pH, increasing incorporation of the "major glycoprotein" into the recombinates was detected by gel electrophoresis and in parallel increasing amounts of particles were found in the freeze-fractured membrane faces.

The necessity of working at low pH values from pH 2–4, however, and a critical evaluation of all the data presently available leads to the conclusion that the 2-chloroethanol technique is not adequate for recombination studies tending to membrane reconstitution.

INTRODUCTION

In previous work Zahler and Weibel [1] studied the chemical and physical nature of erythrocyte membrane apoprotein and lipid solubilized and separated in 2-chloroethanol and recombined by dialysis against neutral aqueous buffer. They showed that the recombined and original erythrocyte membranes were structurally and chemically similar. However difficulties arose in the interpretation of the total absence of functional activities in their recombinates. Since their structural study has been restricted to information obtained from thin sectioning, a re-evaluation of these data by the freeze-fracturing technique was undertaken in the first part of this study. This technique reveals the hydrophobic central plane of membranes [2, 3]

* Present address: Cytologisches Laboratorium, Institut für allgemeine Botanik, Universität Zürich, Zollikerstr. 107, CH-8008 Zürich, Switzerland.

whereby lipid-protein interactions involving the hydrophobic core of a membrane appear as distinct particles on the fracture faces [4, 5]. Since such interactions are essential for all biological membranes, freeze-fracturing can be used as a method to compare recombined and native erythrocyte membranes.

Subsequent work in other laboratories [6–9] has shown that the properties of the erythrocyte apoprotein-lipid recombinates are dependent upon the way in which the recombinates are formed. For example, extraction of the proteins with *n*-butanol [6] results in a system in which recombination occurs predominantly on the basis of electrostatic interactions, while recombinates of lipids and proteins isolated from erythrocyte membranes with acetic acid [7, 8] are formed mostly by hydrophobic interactions. Recombination of components solubilized by 2-chloroethanol was thought to be nearly independent of electrostatic considerations, but as the previous authors studied recombination at pH 7.6, no definite conclusions about the system in general could be obtained. For this reason, we continued in the second part of this study the work of Zahler and Weibel [1] by forming erythrocyte apoprotein-lipid recombinates over the pH range from 2–12. The resultant recombinates have been characterized by density gradient centrifugation, polyacrylamide gel electrophoresis and freeze-fracture electron microscopy.

MATERIALS AND METHODS

Isolation of red cell membranes

Fresh preparations of human red blood cells (A^+, Rh^+) were provided by the Swiss Blood Transfusion Service SRK, Bern. Membranes were isolated by the procedure of Dodge et al. [10] modified as in ref. 1. Hemolysis was performed in 8 volumes of 15 mosM phosphate buffer at pH 8 and 4 °C. Time for hemolysis and subsequent washing was minimized and care was taken to obtain preparations completely free of white cells. The washing procedure was stopped when the sedimented ghosts were slightly pinkish (hemoglobin content of 1–4 % as measured densitometrically in polyacrylamide gel electrophoresis). Hemoglobin was used as an internal marker to follow the recombination behaviour of a nonmembrane protein. The membranes were frozen and stored at −25 °C at a protein concentration of 10 mg/ml.

Isolation of membrane proteins

Lipid-free membrane proteins were isolated by solubilization and gel filtration in 2-chloroethanol water (90 % v/v, pH 2) as previously described [1]. The proteins were stored in 2-chloroethanol at −25 °C and used within 6 days.

Recombination

Red cell membranes were solubilized in 2-chloroethanol (1 vol. membranes + 9 vols. 100 % 2-chloroethanol, pH 2). The resulting clear solution was concentrated by rotary vacuum evaporation to 2 mg protein per ml. For recombination, the organic solvent was removed by dialysis against the buffers described below without prior separation of proteins and lipids (recombination I). Recombinates II were obtained after separation of the apoproteins and lipids [1] and mixing the components in the required proportions. For recombination I, 5-ml portions of solubilized membranes were dialyzed against 100 vol. of the buffer at room temperature. The buffer

solution was changed after 1 and 3 h of dialysis. During the first 2 h, the pH of the dialysis solution was checked and corrected with 0.1 M NaOH to a constant value. Dialysis was completed after 6 h.

The buffer of Teorell and Stenhagen [11] was used for recombinations between pH 2 and 12. Intervals of one pH unit were used between pH 2 and 8 and of two pH units between pH 8 and 12. Concentration of the buffer anions was 10 mM for the entire pH range. In control experiments with a series of different buffers (glycine, acetate, citrate and Tris buffer, all 10 mM) between pH 2 and 8, no differences with respect to density, chemical composition and structure of the recombined lipoproteins were found. As another control, the original buffer [1] 10 mM Tris-HCl, pH 7.6 with 10 mM $CaCl_2$ and 1 mM $MgCl_2$ was also used.

Density gradient centrifugation

After completion of dialysis, the precipitated lipoproteins were sedimented at 100 000 $\times g$ for 30 min in a Spinco type 40 rotor. Protein was measured in both the supernatant and sediment. In some experiments, the supernatants were collected for sodium dodecyl sulfate-gel electrophoresis. For this purpose the solutions were dialyzed against distilled water, freeze-dried and solubilized in an appropriate amount of sodium dodecyl sulfate-dithiothreitol disintegration solution [12] for gel electrophoresis. 5 ml linear gradients ranging from 20 % to 50 % sucrose (w/w in dialysis buffer) were loaded with 0.5 ml of resuspended and sonicated sediment (2 $\times$ 30 s at maximal intensity with an MSE ultrasonic disintegrator, model 7100 in an ice bath). Each sample contained 1 to 5 mg protein. Centrifugation was run to equilibrium density (10 h, 149 000 $\times g$, Spinco SW-50.1 rotor). The lipoprotein bands were isolated, washed twice in the dialysis buffer and used for sodium dodecyl sulfate-gel electrophoresis and freeze-fracturing.

Chemical analysis

Protein concentration was measured as described by Lowry et al. [13]. Electrophoretic analysis of membrane proteins was carried out in polyacrylamide gels containing 1 % sodium dodecyl sulfate and 5.6 % acrylamide [12]. Glycoproteins were stained with periodic acid Schiff reagent [14]. Densitometer tracings were made with a Kipp and Zonen densitometer model DD2 with combined integrator for the measurement of peak areas.

Electron microscopy

For thin sectioning, pellets of membranes or lipoproteins were fixed in a mixture of 3 % glutaraldehyde and 3 % acrolein in 0.1 M cacodylate buffer pH 7.2 for 2 h at 4° C and washed in the above buffer. This material was block-stained with 0.5 % aqueous uranyl acetate, dehydrated and embedded in a mixture of epon-araldite [15]. The thin sections were poststained with lead citrate [16].

For freeze-fracturing, the membrane and lipoprotein preparations were pelleted in the dialysis buffer solution containing 30 % glycerol and frozen in liquid Freon 22. Freeze-fracturing was performed on a Balzers BA 360 apparatus after a slightly modified procedure of Moor [17]. To prevent etching, fracturing was done at -110 °C and immediately followed by shadowing. Pictures were taken with a Philips EM 200 electron microscope at magnifications between 20 000 to 40 000 times.

RESULTS

Recombination at pH 7.6

As a reference point, we studied the recombined membranes prepared by the method of Zahler and Weibel [1] using the Tris buffer at pH 7.6 including Ca^{2+} and Mg^{2+}. Fig. 2 shows a thin section through this preparation which was isolated from sucrose density gradients. This material forms stacks and concentric shells of membranes which, according to the above authors, are structurally identical to the original

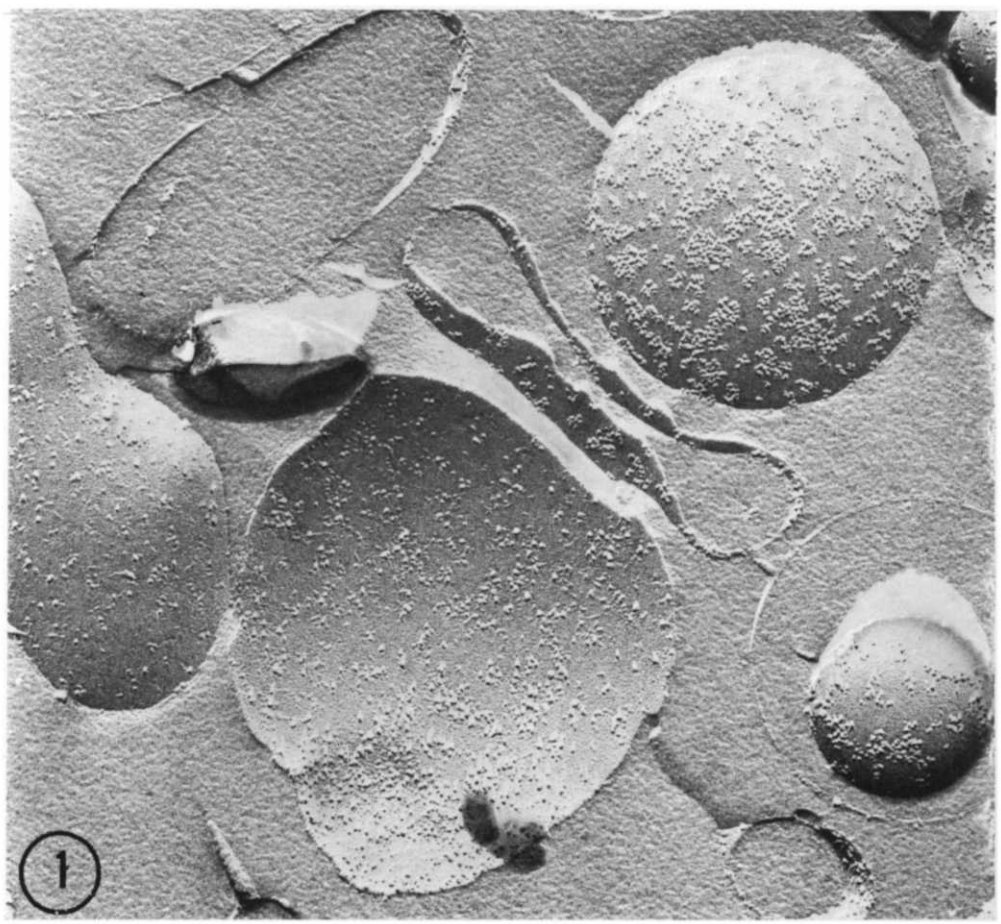
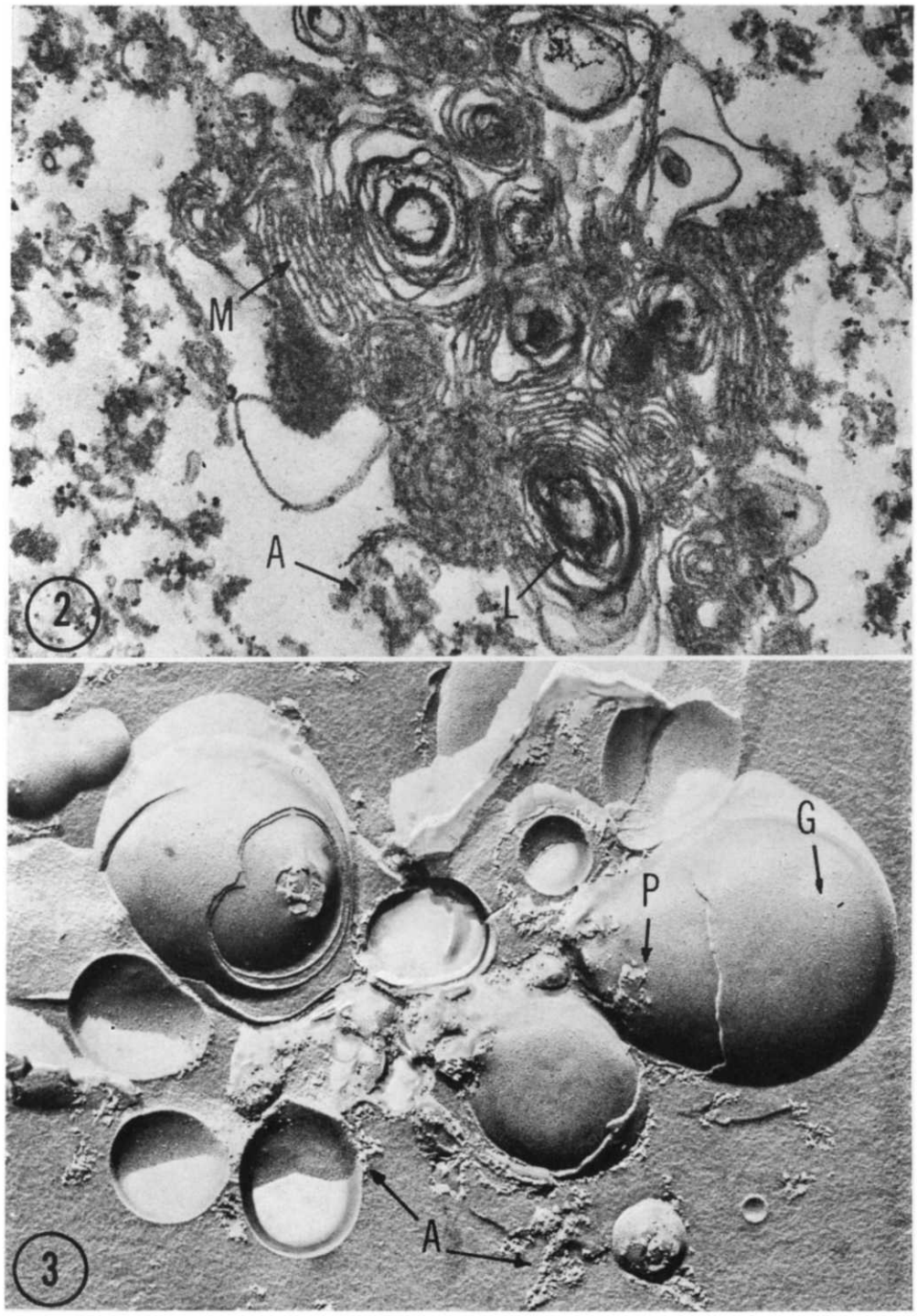


Fig. 1. Isolated erythrocyte membranes after freeze-fracturing. ×60 000.

Fig. 2. Thin section electron micrograph of recombined lipoproteins ("recombinate I", see Materials and Methods) obtained at pH 7.6 with 10 mM $CaCl_2$, 1 mM $MgCl_2$. (L), uncombined lamellar lipid within lipoprotein membranes (M). Note amount and arrangement of nonmembranous aggregates (A). ×60 000.

Fig. 3. Freeze-fracture electron micrograph of same preparation shown in Fig. 2. Fracture faces are smooth. Small granulations (G) are also present in protein free liposomes. (A), membrane bound aggregated material. (P), fracture face particles. ×60 000.



Figs. 2 and 3. For legends see page 274.

membranes. In addition to these membrane stacks, we could always find a varying amount of aggregated material which remained free or adjacent to the membrane surface (A in Fig. 2).

A typical freeze-fracture replica of these recombined membranes is shown in Fig. 3. As in Fig. 2, the membranes are organized as single vesicles or membrane whorls. Convex and concave fracture faces are devoid of particles. In some small vesicles and on a few localized regions of large vesicles, particles of the same size as those in the native erythrocyte membrane are present (P in Fig. 3). They appear in regions where aggregated material is in contact with the vesicle surface. Native erythrocyte membranes in contrast show a considerable number of particles asymmetrically arranged (Fig. 1). The aggregated material shows up as clusters of particles (A in Fig. 3) which have sizes similar to those on the fracture faces of the native membranes (around 100 Å, Fig. 1).

Membrane vesicles from total lipid extracts or of uncombined lipids floating on top of the sucrose density gradients show completely smooth fracture faces, whereas precipitated apoprotein sedimenting to the bottom of these gradients yields freeze-fracture images of nonmembranous aggregates (not shown).

These recombinates are highly resistant to mechanical and chemical treatments, e.g., sonication for 90 s at maximal intensity (MSE ultrasonic disintegrator. Model 7100), extreme pH values down to pH 2, high concentration of NaCl up to 4 M. Their structural and chemical properties are independent of various factors during recombination, e.g. temperature (from 4 °C to 40 °C), time of 2-chloroethanol exchange by aqueous buffer (from spontaneous dilution to 15 days dialysis), protein concentration (from 0.5 mg/ml up to about 100 mg/ml), presence of NaCl up to 1.5 M within the aqueous buffer. However, the properties of the recombinates were shown to be dependent on pH during recombination.

Influence of pH during dialysis

Precipitation of apoprotein. The lipid-free erythrocyte membrane protein (apoprotein) shows a broad precipitation curve (Fig. 4a). Precipitation increases from 2 % at pH 2 to 80 % at pH 4 (percentage of protein recovered in the sediment after sedimentation at 100 000 $\times g$ for 30 min). The proteins remain largely insoluble (80–95 %) between pH 4 and 10. Above pH 10 solubility increases again and at pH 12 about 11 % of the total protein is recovered in the sediment.

Precipitation of lipoprotein recombinate. All pH dependent recombinations were done directly with solubilized membranes without prior separation of lipids and proteins (recombination I, see materials and methods). Above pH 4 the solubility of the recombinates follows closely that of the apoprotein alone (Fig. 4a). Below pH 4 however, the amount of protein recovered in the sedimentable recombinate is enhanced by the presence of membrane lipids. At pH 2 this value increases ten fold over that of the lipid-free apoprotein (from 2 % to 22 %). The visual appearance of the lipoprotein dialysates after completion of dialysis is shown in Fig. 4b. Between pH 4 and 10 heavy precipitates are formed, but below and above these values the recombinates form homogeneously dispersed suspensions.

Density gradient centrifugation. Equilibrium density gradient centrifugation shows formation of lipoprotein bands over the whole pH range from 2 to 12 (Fig. 5). The density of the recombined lipoproteins gradually increases with increasing pH.

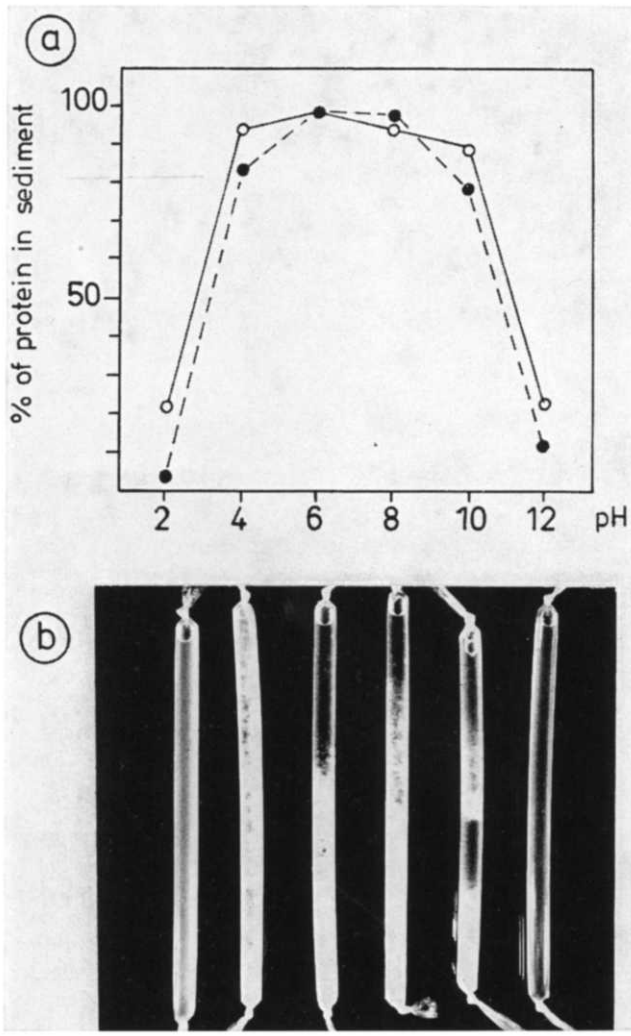


Fig. 4. (a) Precipitation of lipoprotein and apoprotein as a function of pH (pH 2 to 12). The values express the percentage of proteins sedimented after centrifugation of the dialysates for 30 min at 100 000 × *g*. ○—○, proteins precipitated in the presence of lipids (recombinate I, mean values of 6 experiments) and ●- - -●, proteins precipitated in the absence of lipids (apoproteins, mean values of 3 experiments). (b) Photograph of dialysis bags with recombined lipoproteins obtained at pH 2 to 12.

It remains at an almost constant value of 1.140 g · cm^{-3} between pH 4 and 5, which corresponds to the density of the original erythrocyte membranes (STR in Fig. 5) and to a lipid to protein ratio of 0.8. This ratio varies between 2.3 at pH 2 and 0.3 at pH 12*.

* These values are approximations obtained from the linear relationship between density and lipid percentage of the lipoprotein bands [18]. For the density of lipids a value of 1.00 g · cm^{-3} and for the proteins of 1.30 g · cm^{-3} was taken.

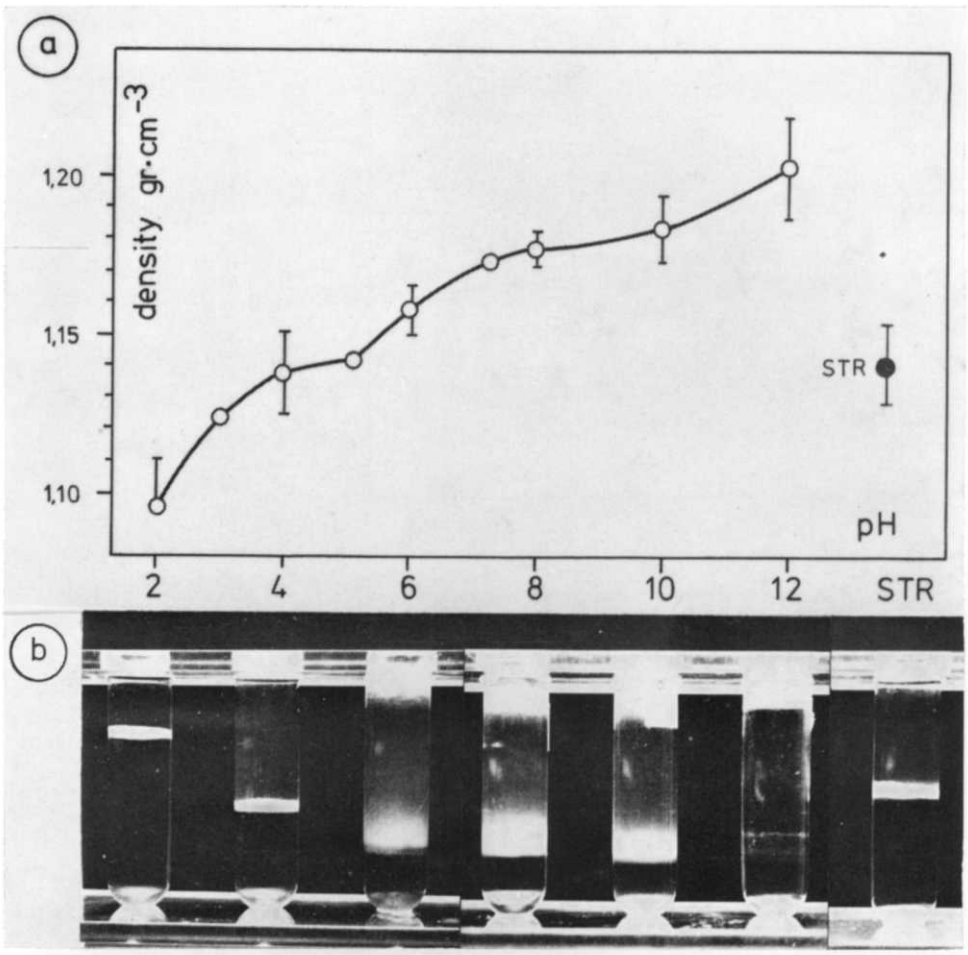


Fig. 5. Isopycnic sucrose density gradient centrifugation of recombined lipoproteins obtained at pH 2 to 12. STR, erythrocyte (stroma) membranes. The gradients range from 20 % to 50 % sucrose. (a) Increase of lipoprotein density as a function of pH. Results are expressed as mean ± S.E. (8 experiments at pH 2, 4, 6 and 4 experiments at pH 8, 10, 12). The values for pH 3, 5, and 7.6 express the mean for two experiments. (b) Photograph of centrifuge tubes with lipoprotein bands at equilibrium density and with free lipid floating on top of the gradients (pH 6 and above).

Four pH regions may be distinguished: (1) A region below pH 4 which is characterized by complete binding of the lipids but only partial binding of the proteins into the recombinate. The density increase in this region is due to an increased binding of proteins into the recombined lipoproteins. (2) A region between pH 4 and 5 where both lipids and proteins are almost completely bound into the recombinate. The density therefore remains constant and equals that of the original erythrocyte membrane. Note that this region lies within the isoelectric precipitation region of the total erythrocyte apoprotein (pH 3.5–5, ref. 6) and also coincides with pH of minimal extractability of erythrocyte membrane proteins (pH 5, ref. 19). (3) A region between pH 5 and 10 where binding of proteins into the recombinate is almost complete

whereas binding of lipids decreases with increasing pH (see increasing amounts of unbound lipids floating on top of the sucrose density gradients in Fig. 5). The density increase in this region is due to decreasing lipid binding. (4) Finally a region above pH 10 where the recombination of proteins and lipids is heavily reduced.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Between pH 4 and 10 where both apoprotein and lipoprotein are largely insoluble, the protein patterns of the recombinates are comparable to that of the original membranes (STR in Fig. 6). With increasing pH increasing amounts of aggregated proteins remain in the upper part of the gels (see indicated areas in the gel scans of Fig. 7). At pH 12 strong aggregation equally reduces the pattern of all the proteins. The tendency of selective protein binding at low pH values during recombination is shown in the gel scans of Fig. 7. From the whole spectrum of erythrocyte membrane proteins only spectrin (bands 1 and 2), components 4, 5 and 7 (after the nomenclature of Fairbanks et al., ref. 12) and the glycoproteins are present in the recombinate of pH 2, whereas components 3 and 6 are greatly reduced at this pH. They reach their normal values only above pH 3. Hemoglobin is absent below pH 4 and bound to the recombinates between pH 4 and 8. The diffuse region of protein stain between spectrin and component 3 is characteristic also for the lipid-free apoproteins obtained from 2-chloroethanol solubilized erythrocyte membranes (see Fig. 8).

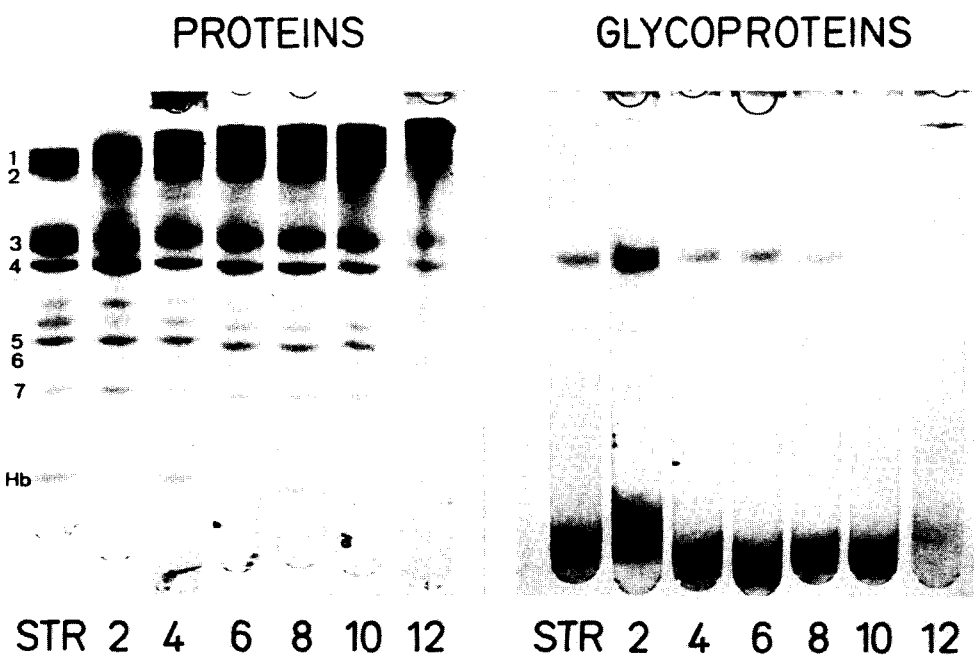


Fig. 6. Electrophoresis patterns of proteins and glycoproteins from erythrocyte (stroma) membranes (STR) and recombined lipoproteins obtained at pH 2 to 12. Labelling corresponds to that of ref. 12. Hb represents residual hemoglobin. Note the aggregated protein on top of the gels, the reduced protein pattern at pH 2 and the high amount of glycoprotein stain at pH 2. The fast moving bands in the glycoprotein stains represent glycolipids. 75 μg of protein was applied to each gel for protein staining and 225 μg of protein to each gel for glycoprotein staining. Electrophoresis was performed as described under Materials and Methods.

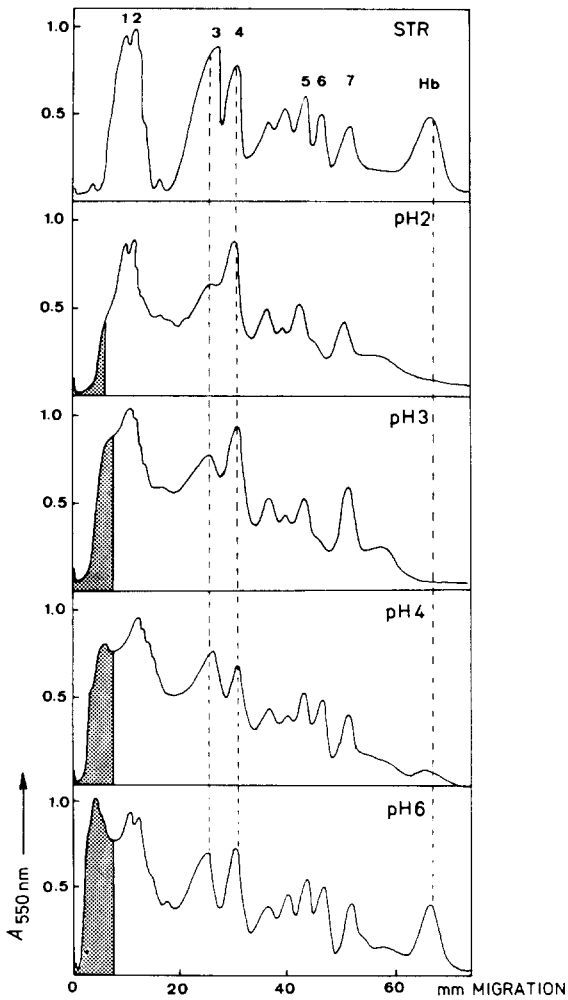


Fig. 7. Densitometer tracings of proteins from original erythrocyte membranes (STR) and pH dependent recombinates (pH 2 to 6) separated by polyacrylamide gel electrophoresis. To each gel 75 μg of protein was applied. Labelling of the protein bands as in Fig. 6. Dashed lines show pH dependent incorporation of band 3 protein and hemoglobin (Hb). With increasing pH increasing amounts of aggregated proteins remain on top of the gels (dotted areas).

The periodic acid Schiff stained gels in Fig. 6 show a preferential incorporation of the "major glycoprotein" (ref. 12, or "glycophorin", ref. 20) into the recombinates at pH 2. Two to three fold enrichment can be measured densitometrically compared with the quantity in the native membrane or in the recombinates obtained over the

Fig. 9. Freeze-fractured recombinates obtained by dialysis against buffers ranging from pH 2 to 12. The respective lipoprotein bands from the sucrose density gradients were washed and frozen in buffer containing 30 % glycerol. (P), fracture face particles seen especially at pH 2. (A), nonmembranous aggregates dominating at pH 12. × 40 000.

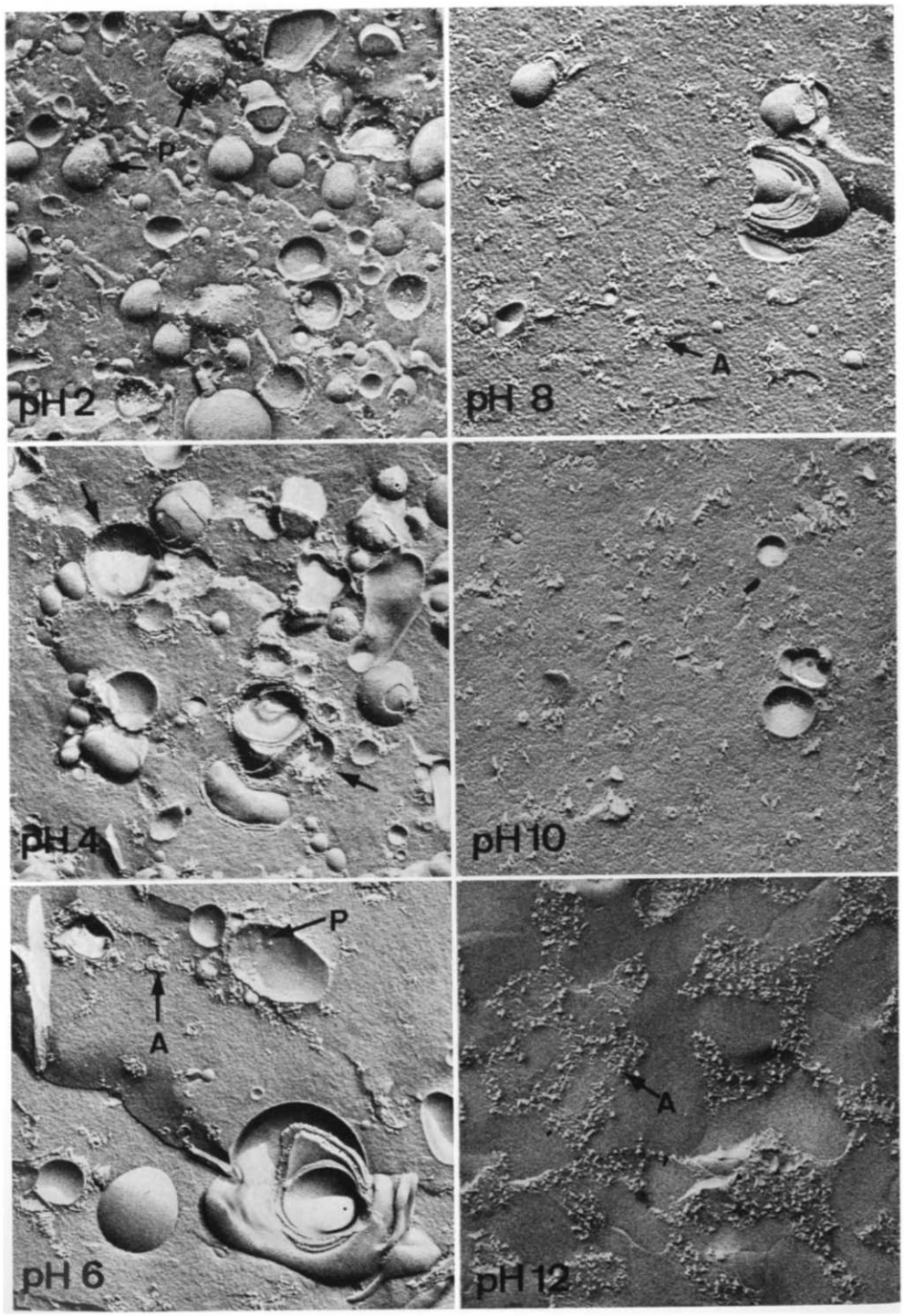


Fig. 9. For legend see page 280.

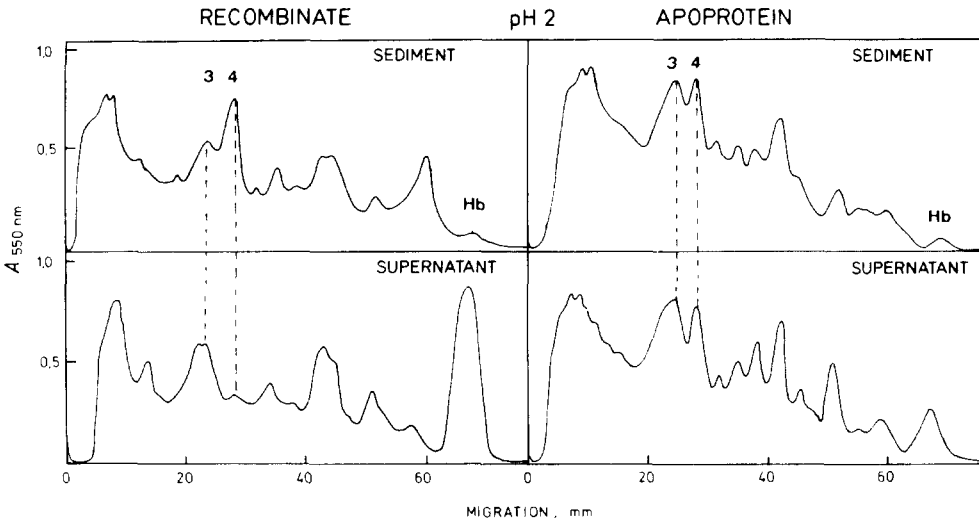


Fig. 8. Densitometer tracings of proteins recovered in sediment and supernatant after centrifugation at 100 000 × *g* for 30 min. Electrophoresis and labelling as in Fig. 6. About 20 % of the protein was recovered in the lipoprotein recombinate and only 2 % in the lipid free apoprotein. The distribution of band 3 and band 4 proteins between sediment and supernatant is indicated by dashed lines.

pH range 4 to 8. The "major glycoprotein" is virtually absent from the recombinates at pH 10 and 12.

Fig. 8 shows gel scans of sedimentable and unsedimentable recombined lipoproteins obtained at pH 2 and sedimented at 100 000 × *g* for 30 min. About 75–80 % of the total protein is found in the supernatant of the recombined lipoprotein (Fig. 4). Sediment and supernatant show a complementary protein pattern with respect to components 3 and 4. Control experiments with apoprotein alone (Fig. 8) show no differenties in the protein composition between supernatant and sediment at pH 2. This is also true over the entire pH range tested (not shown).

Electron microscopy. The pH dependence of the structures of the lipoprotein bands isolated from sucrose density gradients (Fig. 5) are shown in Fig. 9. A continuous change from a membrane to a nonmembrane structural appearance can be observed with increasing pH. Recombination at pH 2 yields unilamellar membrane vesicles that are partly studded with fracture face particles. As shown in Fig. 10, these particles have greater variability in size and shape and occur less frequently than in the native membrane. They are equally distributed over convex and concave fracture faces. There are practically no free nonmembranous aggregates besides these membrane vesicles. Similar results are obtained with pH 3 recombinates (not shown). At pH 4 the membrane vesicles are organized into more complicated whorls or large sheets. They are surrounded by particulate aggregated material (arrows in Fig. 9, pH 4). As the pH and lipoprotein density increases, increasing amounts of non-

Fig. 10. Higher magnification of freeze-fractured recombinate obtained at pH 2. Note vesicles with fracture face particles (P). × 72 500.

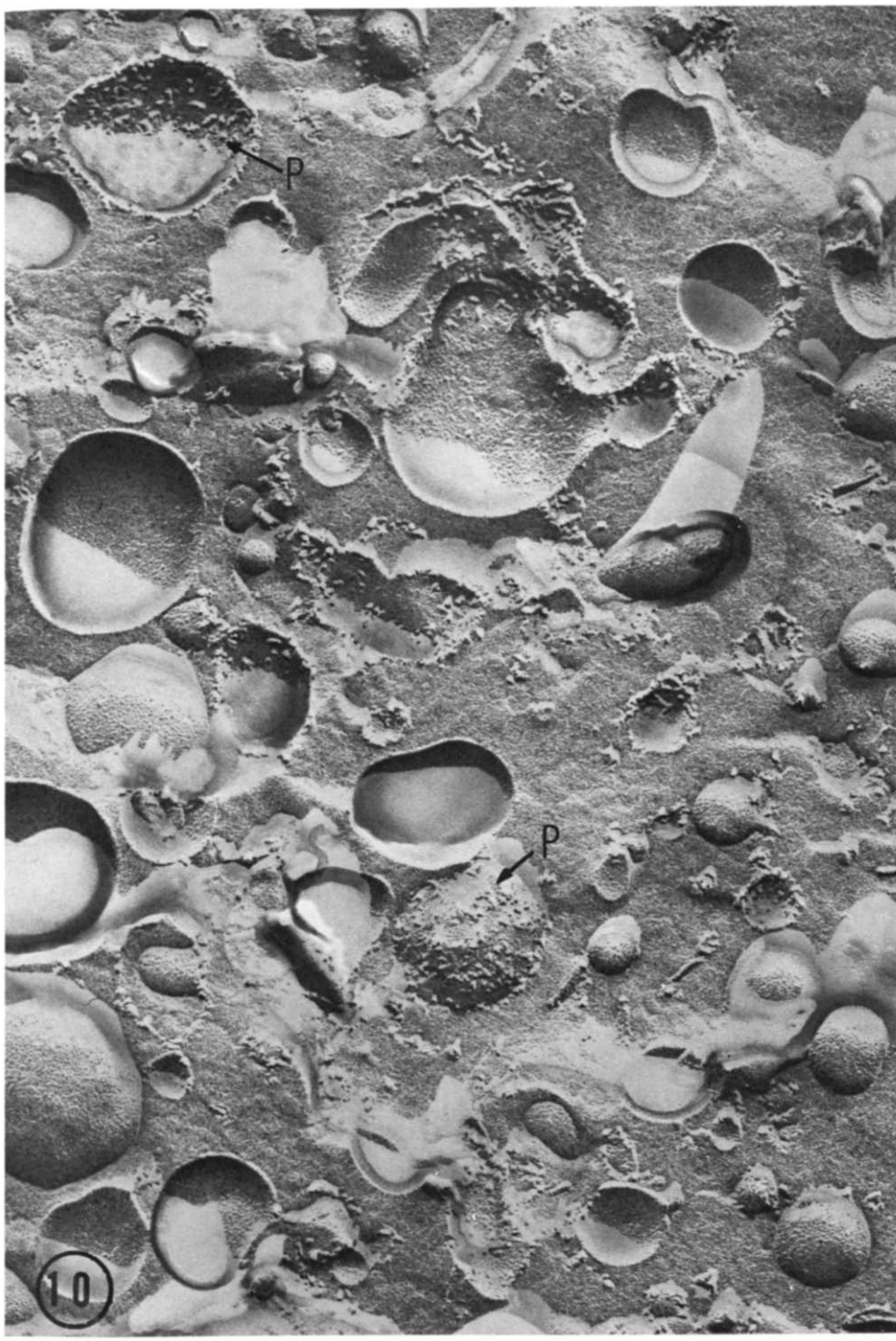


Fig. 10. For legend see page 282.

membranous aggregates are formed and simultaneously the number of membrane vesicles decreases. Particles on the fracture faces are less frequent and are seen in regions where aggregated material is in contact with the vesicle surface (arrows in Fig. 9, pH 6). The recombined lipoproteins at pH 12 no longer form membrane vesicles. They are of lipoprotein nature (see sucrose density gradients of Fig. 5) and contain particles of the same size as those seen in the fracture faces of the vesicles. It follows that reconstitution of membrane vesicles with similar structure as the native erythrocyte membrane occurs best between pH 2 and 4.

DISCUSSION

The following points emerged from this study:

(1) Erythrocyte membrane proteins and lipids isolated by 2-chloroethanol recombine into lipoprotein structures over the pH range 2 to 12.

(2) The precipitation of proteins in the presence of total erythrocyte lipids is greatly enhanced at pH values below 4.

(3) The recombinate above pH 4 is a random mixture of the proteins and lipids present during recombination. Freeze-fracture electron micrographs show that the membrane structures formed at this pH range do not bear much similarity to the native erythrocyte membrane.

(4) Recombinates resulting from dialysis below pH 4 show accumulation of several membrane proteins, especially of the "major glycoprotein". Freeze-fracture electron micrographs show membranes and to a certain extent fracture face particles which are however more heterodisperse than those of the native erythrocyte membrane.

(5) Recombination at pH 12 does not give rise to membrane structures.

Precipitation of erythrocyte membrane apoprotein

The proteins isolated from erythrocyte membranes by 2-chloroethanol are highly insoluble between pH 4 and 10. This is in contrast to the behaviour of erythrocyte membrane proteins which have been isolated either by *n*-butanol [6] or by acetic acid [7, 8] and which are largely soluble above pH 5. The isoelectric precipitation region of total erythrocyte membrane apoproteins isolated by *n*-butanol lies between pH 3.5 and 5[6]. Insolubility of these proteins is therefore mainly a result of precipitation due to charge neutralization, whereas for apoproteins isolated with 2-chloroethanol aggregation seems to be the result mostly of hydrophobic interaction as shown by the large pH region of precipitation.

Precipitation of membrane apoprotein in presence of lipids

Three general tendencies are observed with increasing pH during recombination:

(1) increased density of the recombined lipoproteins

(2) increased formation of nonmembranous aggregates

(3) decrease of the amount of the "major glycoprotein" incorporated into the recombinate.

The parallel increase with pH of the amount of nonmembranous aggregates and the density of the recombined lipoproteins suggests that pH alters the ability of the proteins to bind lipids. At low pH, little of the protein actually recombines

with the lipid, but all of the lipid present is bound to this protein. This capacity is gradually lost as pH increases and between pH 5 and 6, the lipid binding capacity of the proteins is much the same as in the intact erythrocyte membrane. At high pH, lipid binding capacity is almost completely lost. This can be interpreted as an increase with pH of protein-protein interactions at the expense of lipid-protein interactions which leads to the increased proportion of nonmembranous aggregates within the recombinates. The contradiction between the morphological heterogeneity of the recombinates and the demonstration of sharp bands after equilibrium density gradient centrifugation particularly at low pH values points to coprecipitation of different recombination products formed during dialysis. Thus, dialysis of a mixture of membrane proteins and lipids dissolved in 2-chloroethanol at pH 2 against aqueous media is a complex process whereby several factors such as concentration of 2-chloroethanol, pH and solubility of proteins and lipids are changing constantly.

The pronounced stimulation of protein precipitation in the presence of lipids between pH 2 and 4 points to a specific interaction between lipids and proteins in this pH region. This is particularly true for the "major glycoprotein" which shows increased recombination with total erythrocyte lipids at low pH values. It suggests the postulate that an initial electrostatic attraction between the positively charged lipids (isoelectric precipitation point at pH 3.5, ref. 6) and the still negatively charged sialic acid groups of the glycoprotein [21, 22] occurs between pH 2 and 3.5.

In parallel with the electrophoretic results, electron microscopy reveals an increase of particle numbers with decreasing pH. The fact that these particles are more heterodisperse in size and shape than those of the native erythrocyte membrane means that one has to be careful in postulating a simple correlation between particle number and amount of glycoprotein. It is possible that the low pH in general leads to increasingly denatured membrane proteins which may then be incorporated into the lipid bilayers. Also one has to consider that no such interrelation has been found for band 3 protein, one of the spanning proteins which also seems to be associated with the fracture face particles of the native membrane [23]. This protein shows decreased incorporation into the recombinate at low pH which suggests that it has a higher isoelectric point than the "major glycoprotein".

In addition, our results show that peripheral proteins located at the surface of the erythrocyte membrane, such as spectrin and component 4, are incorporated into the recombined lipoproteins at all pH values. This points to hydrophobic interaction with lipids which may be a consequence of the above mentioned denaturing effect of low pH and of denaturing caused by 2-chloroethanol. A similar binding property, however, has been demonstrated for spectrin when it is recombined with lipid monolayers and bilayers [8, 24, 25]. The recombination studies apparently contradict the binding and solubility properties of spectrin in the native membrane [26].

Our studies in the above bulk recombination system confirm those made with the monolayer system [5, 27]. Morse [27] showed that the velocity of penetration of 2-chloroethanol-extracted erythrocyte membrane apoprotein into monolayers of erythrocyte lipid was maximal between pH 2 and 3.5. Moreover, Wehrli and Morse [5] have shown reappearance of particles similar to those in the native erythrocyte membrane when apoprotein was interacted with monolayers of erythrocyte lipid, then transformed into bilayers and freeze-fractured.

In contrast to the above described recombination procedure, the system of Zwaal and van Deenen [6] using *n*-butanol isolated erythrocyte membrane proteins only leads to protein-lipid interaction in a narrow pH region between pH 3.5 and 4.5 and this only after treatment of the proteins by neuraminidase. Isolation of membrane proteins by n-butanol decreases their hydrophobicity as shown by their increased water solubility and their restricted binding of lipids at neutral pH. 2-Chloroethanol in contrast shows an opposite effect by increasing the hydrophobicity of all membrane proteins particularly of the peripheral components, e.g. spectrin, leading to hydrophobic interactions with lipids when transferred to aqueous systems. 2-Chloroethanol as a solvent has some useful characteristics, such as causing (i) full dissociation of lipids or detergents from proteins [28], offering the possibility of isolating lipid free apoprotein, (ii) monomerization of the membrane components [28], (iii) retention of the α-helix content of the membrane proteins [29] and (iv) retention of preferential lipid binding capacity [30]. However from the data of this work we come to the conclusion that the method is not suitable for studying recombination with the aim of membrane reconstitution at physiological pH. Retention of lipid binding capacity does not imply that the binding product even when morphologically similar to membranes should be comparable to biological membranes.

ACKNOWLEDGEMENTS

We gratefully acknowledge the help of Philip D. Morse, Department of Biophysics, Pennsylvania State University, during the preparation of the manuscript. This research was supported by SNSF grant No. 3.780.72.

REFERENCES

1 Zahler, P. and Weibel, E. R. (1970) Biochim. Biophys. Acta 219, 320–338
2 Branton, D. (1966) Proc. Natl. Acad. Sci. U.S. 55, 1048–1056
3 Wehrli, E., Mühlethaler, K. and Moor, H. (1970) Exptl. Cell Res. 59, 336–339
4 Branton, D. and Deamer, D. W. (1972) Membrane Structure, Protoplasmatologia, Springer Verlag, Vienna and New York
5 Wehrli, E. and Morse, P. D. II. (1974) J. Supramol. Struct. 2, 71–78
6 Zwaal, R. F. A. and Van Deenen, L. L. M. (1970) Chem. Phys. Lipids 4, 311–322
7 Schubert, D., Poensgen, J. and Werner, G. (1972) Hoppe-Seyler's Z. Physiol. Chem. 353, 1034–1042
8 Schubert, D. (1973) Hoppe-Seyler's Z. Physiol. Chem. 354, 781–790
9 Barzilay, M., Condrea, E., Ben-David, E. and DeVries, A. (1973) Biochim. Biophys. Acta 311, 576–593
10 Dodge, J. T., Mitchell, C. and Hanahan, D. J. (1963) Arch. Biochem. Biophys. 100, 119–130
11 Teorell, T. and Stenhagen, E. (1938) Biochem. Z., 416–419
12 Fairbanks, G., Steck, T. L. and Wallach, D. F. H. (1971) Biochemistry 10, 2606–2617
13 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
14 Zacharius, R. M., Zeel, T. E., Morrison, J. H. and Woodlock, J. J. (1969) Anal. Biochem. 30, 148–152
15 Hess, W. M. (1966) Stain Technol. 41, 27–35
16 Reynolds, E. S. (1963) J. Cell Biol. 17, 208–213
17 Moor, H. (1969) Int. Rev. Cytol. 23, 391–412
18 Parsons, D. F. (1967) Can. Cancer Conf. 7, 193

19 Hoogeveen, J. Th., Juliano, R., Coleman, J. and Rothstein, A. (1970) J. Membrane Biol. 3, 156–172
20 Marchesi, V. T., Tillack, T. W., Jackson, R. L., Segrest, J. P. and Scott, R. E. (1972) Proc. Nat. Acad. Sci. U.S. 69, 1445–1449
21 Haurowitz, F. (1973) The Chemistry and Function of Proteins, p. 119, Academic Press, New York
22 Nicolson, G. L. (1973) J. Cell Biol. 57, 373–387
23 Pinto da Silva, P. and Nicolson, G. L. (1974) Biochim. Biophys. Acta 363, 311–319
24 Sweet, C. and Zull, J. E. (1970) Biochem. Biophys. Res. Commun. 41, 135–141
25 Juliano, R. L., Kimelberg, H. K. and Papahadjopoulos, D. (1971) Biochim. Biophys. Acta 241, 894–905
26 Steck, T. L. and Yu, J. (1973) J. Supramol. Struct. 1, 220–232
27 Morse, P. D. II (1974) J. Supramol. Struct. 2, 60–70
28 Zahler, P., Wallach, D. H. F. and Lüscher, E. F. (1967) Protides of the Biological Fluids, Vol. 15, 69–78
29 Wallach, D. F. H. and Zahler, P. (1966) Proc. Nat. Acad, Sci. U.S. 56, 1552–1559
30 Kramer, R., Schlatter, Ch. and Zahler, P. (1972) Biochim. Biophys. Acta 282, 146–156