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FREEZE-FRACTURE ELECTRON MICROSCOPY OF HUMAN ERYTHROCYTES LACKING THE MAJOR MEMBRANE SIALOGLYCOPROTEIN

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Summary

Human erythrocytes of blood group En (a–), a rare homozygous condition involving a complete lack of the major sialoglycoprotein of the cell membrane (glycophorin A), were compared with erythrocytes from normal (En (a+)) individuals by freeze-fracture electron microscopy. No decrease in number, or variation in morphology, of the intramembranal particles of En (a–) cells was detectable. These results show that the erythrocyte sialoglycoprotein is not essential for the maintenance of the integrity of the intramembranal particles of the human erythrocyte membrane.

A wide variety of biological membranes contain particles 5—10 nm in diameter which can be visualized in the hydrophobic region of the lipid bilayer by freeze-fracture electron microscopy. In the case of the human erythrocyte, a number of reports have suggested that the intramembranal particles contain the two major penetrating proteins, band-3 and the major sialoglycoprotein, (glycophorin A) [1—4]. The sialoglycoprotein carries most of the erythrocyte surface *N*-acetyl neuraminic acid (sialic acid), the MN blood group antigens, and receptors for certain lectins. It has recently been shown that a rare modification of the erythrocyte membrane occurs in individuals of blood group En (a–), a homozygous condition involving a deficiency of MN antigens, and the complete lack of the major sialoglycoprotein [5,6,10]. It was therefore of interest to study the morphology of freeze-fractured cell

Abbreviations: PF, plasmatic fracture face; EF, exoplasmatic fracture face.

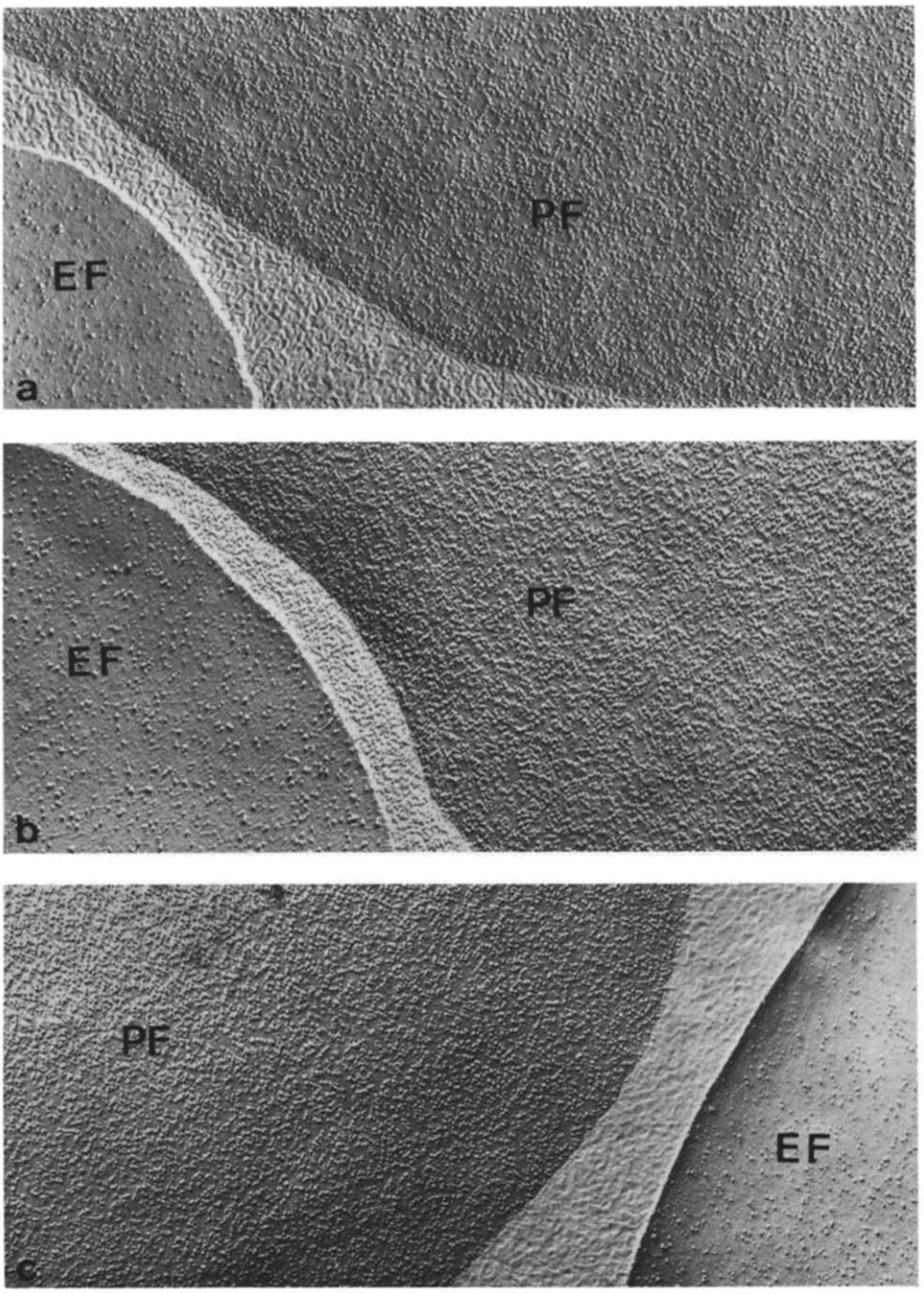

Fig. 1. Freeze-fractured membranes of intact human erythrocytes with different contents of the major sialoglycoprotein (× 50 000). The two different aspects of the plasmatic fracture face (PF) and the exoplasmatic fracture face (EF) are readily recognized by their characteristic difference in density of intramembranal particles. No significant difference in the number of intramembranal particles is detectable between the three blood samples from a: En(a–) = *EnEn* donor V.B.; b: $En^{a}En$ donor I.S. (daughter of V.B.); and c: normal $En^{a}En^{a}$ control.

membranes from such individuals, as well as from relatives heterozygous for the same condition, in comparison with controls from normal En (a+) persons.

The following blood samples were available for study: (1) Three samples from Finnish donors [7,8]: V.B. and G.W., both En (a–), *EnEn* homozygous, and I.S., the En^aEn heterozygous daughter of V.B. (2) Nine samples from an English family [9] comprising: M.E.-P., the propositus and two siblings, En (a–), genotype EnM^k; further siblings and children of genotypes En^aEn or En^aM^k. (3) En (a+) control blood, En^aEn^a homozygous. It should be pointed out that a considerable reduction (approximately 50%) of the amount of the major sialoglycoprotein is seen in heterozygotes, whether they are denoted En^aEn or En^aM^k [6]. The genetical complexities involved have been reviewed recently [6,10]. The blood samples from V.B., G.W. and I.S. were 3—4 days old and had not been frozen, whereas all other samples had been stored frozen at −20°C in 20% glycerol before study.

For freeze-etching, the fresh blood was washed three times in cold phosphate buffered saline (pH 7.4). Before the last centrifugation, glycerol was added dropwise to a final concentration of 20%. The pelleted cells were then mounted on specimen plates and frozen in liquified Freon 22. The blood which had been stored frozen was thawed, centrifuged and frozen in Freon 22 as above. The preparation of freeze-etching replicas was done according to standard methods in a Balzers apparatus.

Fig. 1 shows electron micrographs of freeze-fractured erythrocytes from V.B., homozygous *EnEn* (Fig. 1a) and I.S., heterozygous En^aEn (Fig. 1b), and of normal En^aEn^a erythrocytes (Fig. 1c). The plasmatic (PF) and exoplasmatic (EF) fracture faces of the membranes are readily recognized, the former carrying considerably more intramembranal particles than the latter. The regular arrangement, the size and number of intramembranal particles was very similar in all three samples. In the replica of the other Finnish homozygous *EnEn* donor (G.W., not shown) the particles in the PF fracture face appeared slightly more clustered than that of the corresponding control. The significance of this is not clear. The particle density found on each fracture face of the never-frozen *EnEn*, En^aEn and normal erythrocytes is given in Table I. Cells from blood which had been stored frozen gave poor freeze-etching replicas (not

TABLE I

PARTICLE DENSITIES IN FRACTURE FACES OF En(a–) AND NORMAL ERYTHROCYTES

The results are expressed as the particle count ± standard deviation, with number of different cells of each replica which were counted in brackets. At least five areas of appropriately exposed membrane of each cell were counted and the results were averaged to obtain the particle count for that cell in the replica.

Erythrocyte		Particles/μm^2 in	
		PF face	EF face
V.B.	*EnEn* homozygote En(a–)*	2700 ± 200 (6)	1200 ± 100 (3)
I.S.	En^aEn heterozygote*	3200 ± 250 (3)	1300 ± 250 (3)
X.	En^aEn^a control*	3200 ± 200 (6)	1200 ± 100 (6)
G.W.	*EnEn* homozygote En(a–)**	2900 ± 200 (4)	1100 ± 100 (3)
Y.	En^aEn^a control**	3200 ± 200 (4)	1100 ± 100 (4)

*Samples shown in Figs. 1a—c.
**Micrographs not shown.

shown); the membrane fragments observed in these samples displayed the intramembranal particles on both fracture faces in a distribution similar to that of the normal controls. However, these replicas from frozen cells were not of a sufficiently high quality for reliable particle counts.

According to the estimates of Steck [2], an erythrocyte ghost contains 9.4×10^5 copies of band-3 molecules (mol. wt. approx. 100 000), which are probably in a dimeric form in the membrane, and $5 \cdot 10^5$ copies of the sialoglycoprotein (mol. wt. 31 000), while there are approximately $5 \cdot 10^5$ intramembranal particles ($3000/\mu m^2$) present on the PF face of a membrane. This would be compatible with the assumption that each particle is composed of two band-3 molecules and one sialoglycoprotein (total mol. wt. 230 000). If this were the case, the sialoglycoprotein would contribute only 13% to the weight of the particle and its absence would be unlikely to result in a detectable change in the spherical diameter of the intramembranal particles. It should be noted in considering these estimates that it is not known whether the intramembranal particles represent the membrane intercalated segments (i.e. the apolar domain of the polypeptide chain) or the entire protein molecules. Although the intramembranal particles appear to be fairly homogeneous in size, there is no evidence that they are all chemically identical. If the sialoglycoprotein was located in a population of intramembranal particles distinct from, and of a similar size to, those containing band 3, absence of the sialoglycoprotein would result in a 13% reduction in the particle density of the PF fracture face. Although the data in Table I show a reduction in the particle density of the PF fracture face of the sialoglycoprotein deficient cells, this reduction is within the range (±20%) reported for the particle density on the PF face of normal erythrocytes [11].

Our results show that there is little or no difference in freeze-fracture morphology between erythrocytes which lack the major sialoglycoprotein and normal erythrocytes. Clearly the protein cannot be essential for the integrity of the intramembranal particles of the human erythrocyte. This is interesting in the light of several reports relating the intramembranal particles appearing on the PF face with the sialoglycoprotein and/or the band-3 component [4, 12—14]. However, the evidence localizing the sialoglycoprotein in the intramembranal particles is mainly indirect and is based on the association of various characteristics of the isolated glycoprotein with these particles. Though none of these properties have been shown to be exclusively associated with the sialoglycoprotein, blood group A antigenic determinants [15], influenza virus and phytohemagglutinin receptor sites [3] and anionic sites [13] have been utilized as markers for this protein species. However, evidence is now accumulating that the antigens defining the ABO(H) blood groups are not present on the sialoglycoprotein [16—18]. Similarly, phytohemagglutinin binds to band-3 and other membrane components in addition to the sialoglycoprotein [19], while sialoglycolipids [20] and minor periodate-staining glycoproteins [2] also contribute anionic sites to the erythrocyte surface. Thus there is no unequivocal evidence to localize the sialoglycoprotein to the intramembranal particles. It has also been suggested that the other major erythrocyte glycoprotein (band 3) may not be entirely located in the intramembranal particles [20—24].

Further studies will be needed before the composition of the membrane particles of human erythrocytes can be defined. While an unambiguous interpretation of our results is not possible at this stage, the approach used here should be useful in resolving the composition of the intramembranal particles and in developing a detailed model of the architecture of the erythrocyte membrane.

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