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Two-Dimensional Arrays and Particles in Negative Staining Preparations of Fragmented Human Erythrocyte Ghosts

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Abstract

Studies of negative staining preparations of fragmented human erythrocyte ghosts from blood group A_1 have revealed 90 Å-particles with dark depressions. These particles are found in irregular distribution as well as in two-dimensional arrays.

Introduction

Particles located inside the red cell membrane and exposed by freeze-cleavage have been studied in freeze fracture replicas by electron microscopy [1-6]. Their chemical nature is unknown, although indirect evidence suggests that they are lipoproteins and it has been suggested that they are the A_1-antigen sites [7]. We developed a technique for revealing these particles by negative staining, which is distinguished for its extreme simplicity. The particles themselves now are demonstrable instead of their freeze-cleavage replicas.

Materials and Methods

2 ml unwashed red cells from blood group A_1 (freed of plasma and buffy coat) are frozen overnight at -25°, thawed and then washed 4 times with distilled water in a 20 ml centrifuge tube each time for 10 min at 3000 x g. The centrifugation steps were interrupted by 2 hr pauses for membrane swelling in extreme loose package at room temperature. After that the fragmented ghosts (vesicles) were kept at room temperature for 30 min in a 0,3 M NaCl-solution and the saline removed by gentle centrifugation at 3000 x g with distilled water. The specimens were

prepared by dipping the copper grids, coated with formvar and carbon, face downwards on the surface of the vesicle-solution. Excess fluid was removed with filter paper. The negative staining with 2% phosphotungstic acid, PTA (pH 5), to which was added a trace of bovine serum albumin, was carried out in the same way.

Results

Examination of the specimens in a Siemens Elmiskop I electron microscope revealed on each grid 90 Å-particles with dark depressions. These particles were often found to be in a 120 Å-arrangement (Fig. 1 and 2) as well as irregularly distributed (Fig. 1, insert). The arrays and particles were located in the ghost fragments ("puddle"-like spots with diffluent borders). A lattice structure could be observed under the application of a wide range of pH values (pH 4-7) [8]. Staining at pH 5 gave the clearest pictures and was routinely employed for the work reported here. The

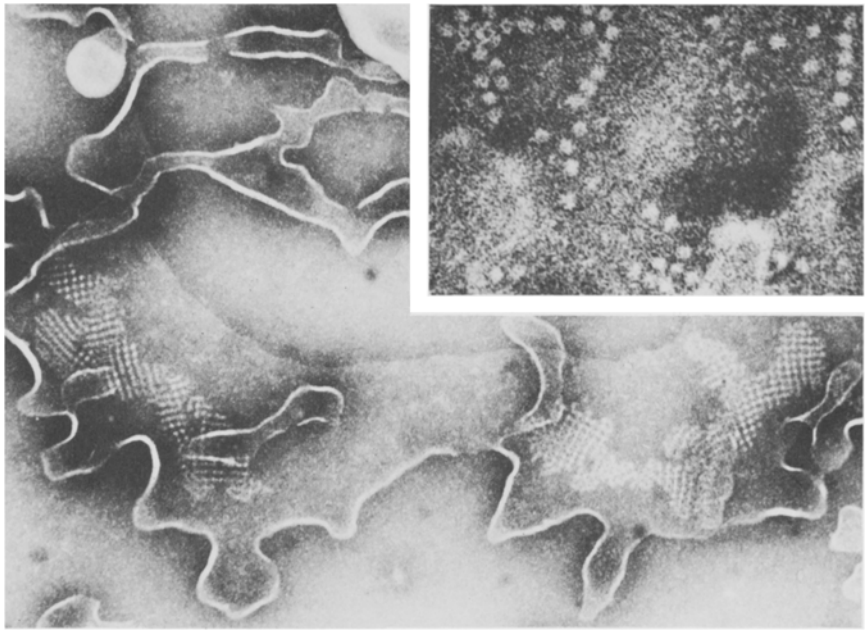

Figure 1. Appearance of fragmented human erythrocyte ghosts on carbon-coated formvar film, negatively stained at pH 5. Two array patterns composed of 90 Å-particles can be seen. The direction of the arrays changes from one area to another (see left). x 80,000. Insert: Irregularly distributed 90 Å-particles located in a ghost fragment. x 200,000.

direction of the arrays often changes from one area to another (Fig. 1) giving the impression that the assembly starts from a number of points at the same time [9]. Some fragmented vesicles observed in sideview gave evidence of a broken line (Fig. 2). Beyond that, numerous movable ring-like 300 Å-micelles, fibrils and some rectangular 120 Å-networks composed of fibrils, situated on places where the array patterns were disintegrated by the successive preparation, were observed [10].

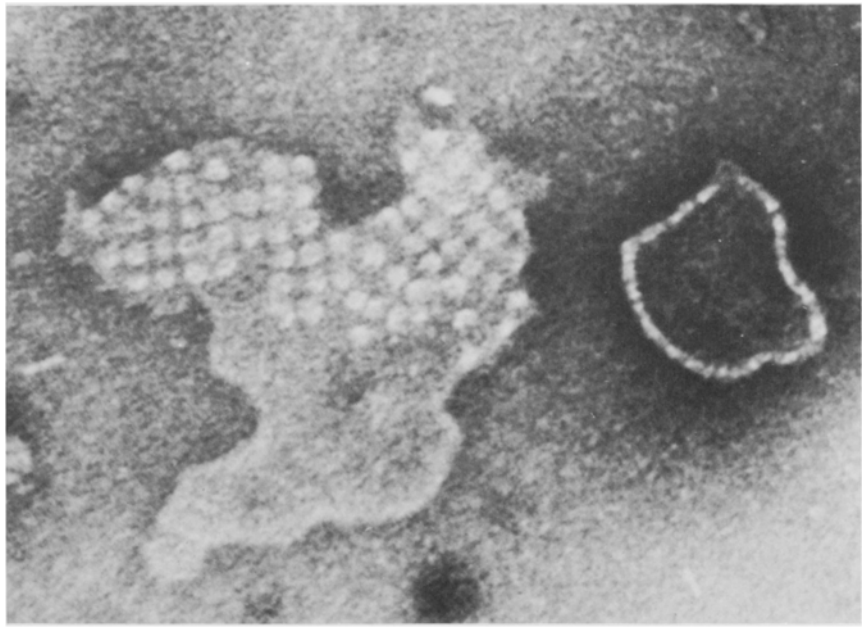

Figure 2. 90 Å-particles with dark depressions rectangularly arranged in a 2-dimensional array. The vesiculated ghost fragment on the right-hand side, observed in sideview, gives evidence of a broken line. x 300,000.

Discussion

For electron microscopy the fact that only partial solubilization of the membrane is achieved is no disadvantage, but allows the early stages of mild disruption to be studied. Separation is accomplished by stepwise disruption of the membrane employing solvents, which reduce or eliminate ionic interactions [11-18]. The high molecular weight polypeptide chains are inaccessible to externally applied reagents in the intact red blood cell. In disrupted ghosts on the other hand, inorganic

cations are removed by repeated washings in distilled water, whereby the phospholipid-cholesterol bilayer of the membrane is partially disintegrated. This treatment is a prerequisite for the resolution of the particles by electron microscopy. At the present time it is not known whether the arrays represent the native conformation of some membrane components or a reaggregation of the liberated membrane particles. According to most of our observations we prefer the latter version. Self-assembly of a surface component of bacterial outer membranes was recently described [9]. The dark depressions in the centres of the particles are filled with PTA. It is possible that they represent parallel hydrophilic pathways ("pores") [19-20]. It is interesting that the number of particles if regularly distributed over the erythrocyte surface–$(10^4/120)^2 \times 145 = 10^6$ (surface area = $145\mu m^2$ [21])–is approximately equal to the number of A_1 blood group antigen sites [22].

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