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Ultrastructural Changes of Erythrocyte Membranes Isolated with Colloidal Silica Solution (Ludox)

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ABSTRACT

The surface morphology of membrane fractions of human erythrocytes isolated by gradient centrifugation with a colloidal silica solution (Ludox) differed from the structure of control membranes isolated without the silica solution. The altered surface had a wrinkled appearance with a granular texture, compared with the smooth appearance of the controls. Thus the colloidal silica solution changed the ultrastructure of erythrocyte membranes. Possible mechanisms for the influence of the silica particles are suggested: secondary chemical bonds, e.g. hydrogen bonds, might well have been established between the silica particles and components of the erythrocyte membrane like spectrin of the inner surface of the membrane. Less probable is an attachment to positive sites of the outer side of the membranes as well as any association to the pitlike infoldings of the erythrocyte surface.

INTRODUCTION

Gradient centrifugation in a silica solution of an isolated membrane fraction of human erythrocytes resulted in a well defined single band comprising about 90% of the material (3). This membrane material contained some enzyme activities, among which that of glyceraldehyde-3-phosphate dehydrogenase could be detected, provided sulfhydryls were added to the medium.

Transmission electron microscopy demonstrated a particulate appearance of the membranes when silica was present. It was suggested that the particles observed were silica particles which had been attached to the membraneous structure and, furthermore, that such an attachment might have contributed to the inactivation of the enzyme observed when sulfhydryls were absent during the preparation.

An incorporation of silica particles into membranes might well influence their structural properties and the present investigation was undertaken to find out if the surface configuration of the membrane is influenced by silica particles, as judged by scanning electron microscopy.

MATERIALS AND METHODS

Special chemicals

Glutathione (reduced form) was purchased from Sigma Chemical Company, St. Louis, Mo., USA, and Dextran T40 from Pharmacia Fine Chemicals, Uppsala, Sweden. The aqueous silica sols used were Ludox HS, obtained from Du Pont de Nemours, Wilmington, Delaware, USA, and Nyacol, Colloidal Silica grade 2040 from Nyanza Inc., Ashland, Mass., USA. All chemicals were of analytical grade.

Preparation of membrane fraction

Membrane fraction was prepared as described by Ronquist (6).

Colloidal silica solutions 16% (w/v), with a pH of 7.4 and containing 65 mM NaCl, 12.5 mM KCl, 1.5% (w/v), Dextran T 40 and 1 mM gluthatione were prepared. The osmolarity of the solution was 290–300 mOsm.

Batches of silica particles of defined diameters were obtained after density gradient centrifugation according to Pertoft & Laurent (4). The following sizes were isolated: 100 Å, 250 Å and 400–500 Å. The largest particles were sampled as a gel pellet from the bottom of tubes which had been emptied of the rest of the solution. The gel pellet was dissolved in the salt solution, and pH and osmolarity were adjusted.

The silica preparation was carried out both with particles of the defined sizes and with uncentrifuged solutions with a size range from 80 to 250 Å.

One volume of the membrane fraction was added to 4 vol of isotonic silica solution that contained Dextran and sulfhydryls and was incubated for 25 min at 20°C. The material was then washed three times, each time in 10 times its volume of an isotonic salt solution containing 130 mM NaCl, 25 mM KCl, 1.5% Dextran T40 and 1 mM glutathione before preparation for scanning electron microscopy.

Preparations for scanning electron microscopy were performed in different ways to test their influence on the ultrastructure. Samples prepared both in the presence and absence of the colloidal silica solution were used. The following procedures were applied.

1. Fixation and dehydration in a graded series of ethanol, air-drying.
2. As 1, but after dehydration: ether, air-drying.
3. Fixation in a solution of 2.5% glutaraldehyde in Sörensen's phosphate buffer (pH 7.2), rinsing in distilled water, air-drying.
4. As 3, but after rinsing in distilled water: dehydration in a graded series of ethanol, air-drying.

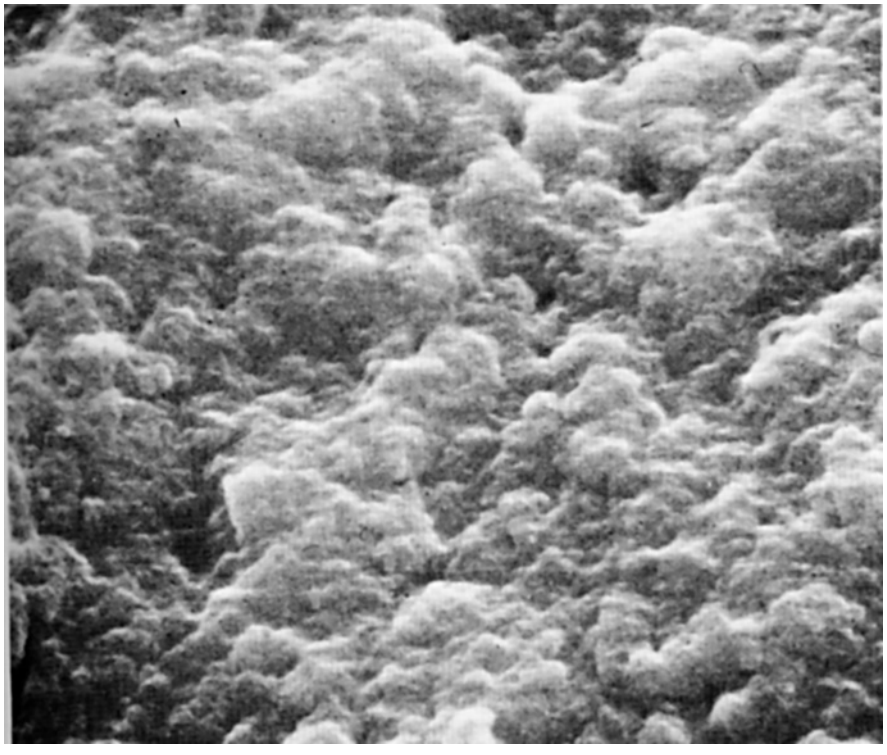

Fig. 1. Membrane fraction, prepared without the colloidal solution of silica. × 2000.

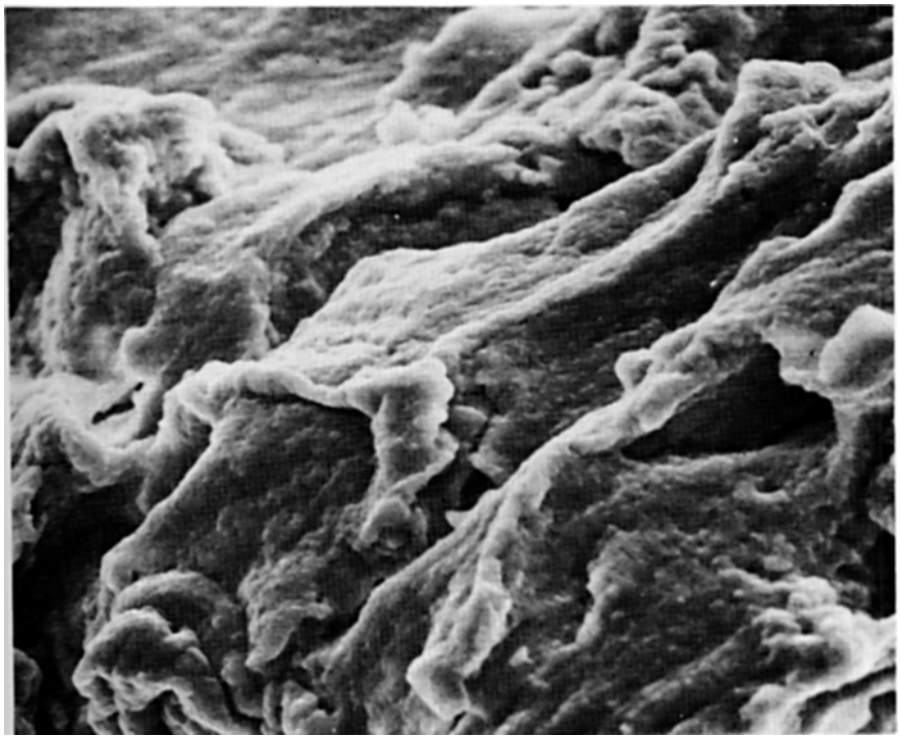

Fig. 2. Membrane fraction, prepared without the colloidal solution of silica. × 20000.

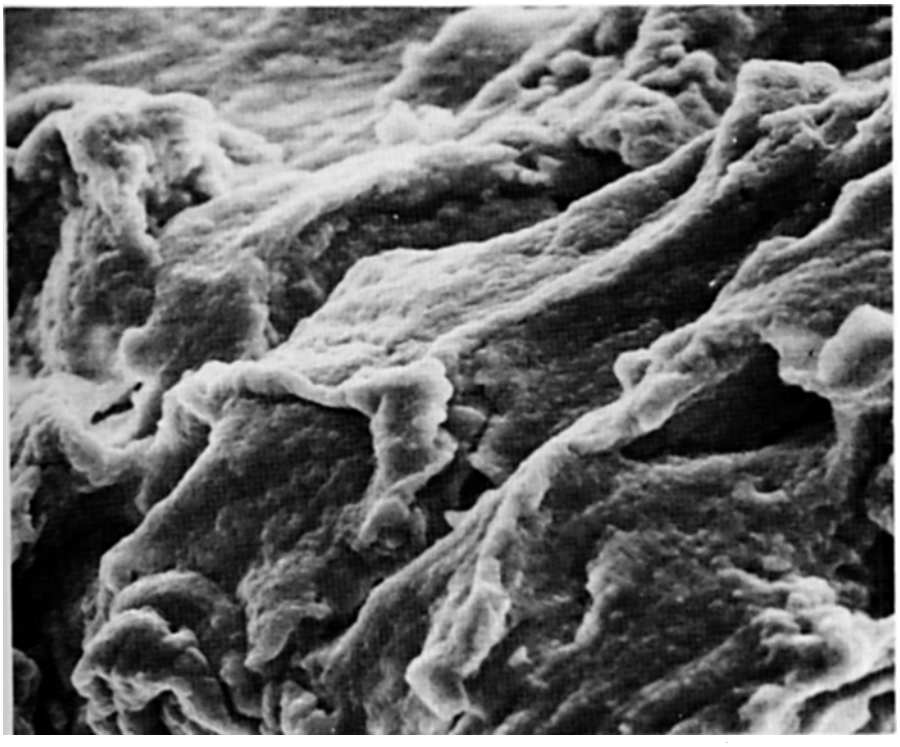

Fig. 3. Membrane fraction, prepared by gradient centrifugation in a colloidal solution of silica. ×2000.

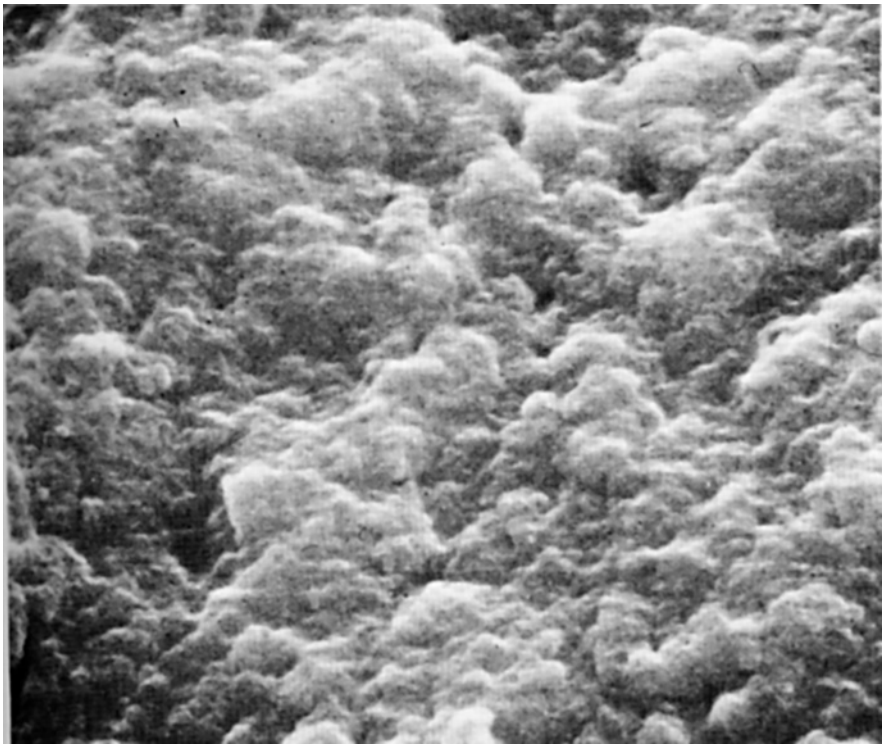

Fig. 4. Membrane fraction, prepared by gradient centrifugation in a colloidal solution of silica. ×20000.

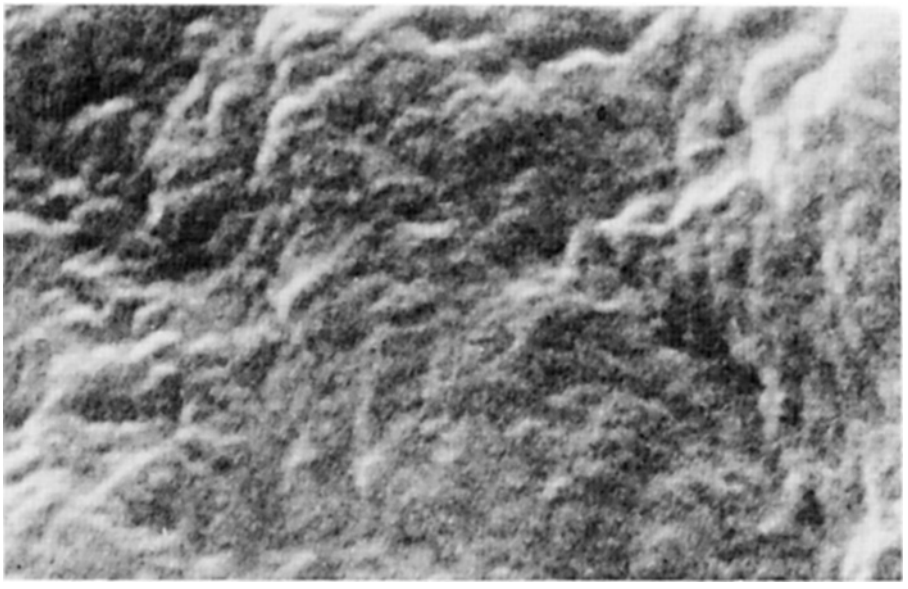

Fig. 5. Membrane fraction, prepared by gradient centrifugation in a colloidal solution of silica particles with a size of about 250 Å. ×100000.

5. Fixation in a solution of 1% osmium tetroxide in Sörensen's phosphate buffer (pH 7.2), rinsing in distilled water, air-drying.
6. As 5, but after rinsing in distilled water: dehydration in a graded series of ethanol, air-drying.
7. As 6, but after dehydration: ether, air-drying.

The samples were allowed to dry on small squares of clean glass, which then were fixed to the specimen stubs. The coating was performed with gold-palladium, and the specimens were examined in a Jeol EMU-3-scanning electron microscope.

RESULTS

Membrane fractions prepared without the colloidal silica solution demonstrated a smooth surface (Fig. 1) which in high magnification showed only minor irregularities (Fig. 2). Even where stacks of folded membranes were observed, the smooth character of the membranes remained.

Membrane fractions prepared with the colloidal silica solution were irregularly wrinkled (Fig. 3) and a granularity of the surface was evident at high magnification (Fig. 4).

Application of two batches of silica particles, with diameters of 250 Å and 500 Å, respectively, did not cause any difference in the appearance of the membrane of the two groups. At high magnification the 500 Å particles were visible (Fig. 6) but the 250 Å ones were too small to be distinguished clearly (Fig. 5).

The various preparative methods did not influence the characteristic appearance of the membranes.

DISCUSSION

The silica particles have a moderate negative charge under the present experimental conditions and can therefore react with positive groups of the membranes. An ionic bond is, however, not necessarily the only possible one between the silica particles and the membrane since the silica particles can easily establish hydrogen bonds (H. Pertoft, 1973, personal communication).

The inside of the erythrocyte membrane being favoured for the binding of silica particles is suggested by the findings of Steck et al. (7). They prepared a membrane fraction from human erythrocytes in an alkaline buffer (pH 8.2) of low ionic strength and reported that the membranes appeared to bud spontaneously into the ghost interior which led to an accumulation of many small vesicles within each parent ghost. Moreover, a high percentage of these vesicles were morphologically inside out when divalent cations were absent during the preparation. Because of the very close similarity between this preparative procedure and the one we used, most of the membranes we observed in the scanning electron microscope ought to be inside out

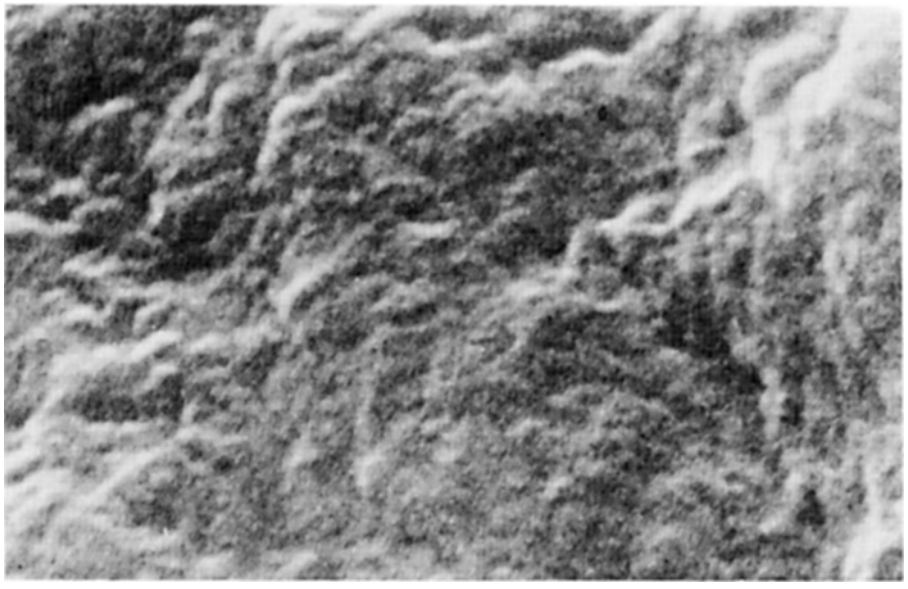

Fig. 6. Membrane fraction, prepared by gradient centrifugation in a colloidal solution of silica particles with a size of about 500 Å. ×100000.

too and therefore having an exposed inside for the silica particles. One of the major proteins of the inside surface of the erythrocyte membrane has recently been characterised and named spectrin (2). These authors also suggested that about 85% of the total SH-groups of the membrane is localized in the spectrin molecules. Because of the protective function exerted by SH-reagents like glutathione and dithiotreitol (cf. 3) in the membrane *vis-à-vis* silica particles, it is reasonable to assume that the SH-groups of spectrin might have interacted directly or indirectly with the particles giving rise to the morphological change observed.

The outer surface of the membrane on the other hand could have attracted the silica particles, since positive groups are probably present on all mammalian cells in spite of their net negative charge. Although relatively little is known about the location and distribution of positive groups on the cell surface, it would be theoretically possible for the silica particles to adhere to the cell surface between fixed anionic groups. If such were the case, one would expect that for steric reasons particles of a certain diameter would be favoured. But the qualitative result was the same whatever the particle size within the diameter range 80–500 Å.

Another possible site for binding the silica particles is the small pitlike infoldings of the erythrocyte surface (1). The pits have an outer diameter of about 300 Å—compared with 80 Å to 500 Å of the silica particles—and might well differ in charge properties from their surrondings. They seem to be sufficiently frequent to account for a change in the appearance of the erythrocyte surface—if the silica particles are bound in the pits. Here too one would expect a favouring of a certain size of the silica particles fitting the size of the pits. The results were the same, however, with particles within the diameter range 80–500 Å.

These two possibilities for binding the silica particles seem therefore to be less probable. Instead, hydrogen bonds could have been established between the silica particles and components on the inner side of the membrane. These bonds might well have produced some modification of the membrane structure giving rise to the observed change of the morphological appearance.

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