PHOTODYNAMIC EFFECTS OF PROTOPORPHYRIN ON RED BLOOD CELL DEFORMABILITY

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Received April 1,1977

SUMMARY: Human erythrocytes exposed to visible light in the presence of protoporphyrin showed a pronounced decrease of cell deformability. The photodynamic action of protoporphyrin on red blood cells has previously been shown to cause a.o. an extensive cross-linking of spectrin. As spectrin is presumably involved in processes regulating cell shape and viscoelasticity, conceivably the decreased deformability is attributable to the cross-linking of this protein.

Iodosobenzoate evoked a similar decreased cell deformability, due to the formation of disulphide bridges by this agent. These S-S linkages could be reduced by dithiothreitol, with a concomitant change of cell deformability to normal values. Dithiothreitol had only a very limited effect on the protoporphyrin-sensitized decrease of cell deformability and protein cross-linking, indicating that the photodynamic effects cannot be rationalized on the basis of disulphide formation.

INTRODUCTION: Exposure of human erythrocytes to visible light in the presence of protoporphyrin causes extensive membrane damage. This has been demonstrated both with normal erythrocytes or ghosts in a protoporphyrin-containing medium and with red blood cells of erythropoietic protoporphyria patients containing a high intracellular concentration of protoporphyrin (1 - 4). This photodynamic effect is characterized a.o. by cross-linking of membrane proteins (3 - 5), aggregation of particles in the membrane as seen in freeze-etch electron microscopy (3), inhibition of several membrane-bound enzymes (6) and ultimately, colloid-osmotic photohemolysis (1, 7).

Spectrin has previously been shown to be especially sensitive to photodynamic cross-linking (3, 4). Since experimental evidence indicates that spectrin may be an important determinant of the visco-elastic properties of the red cell membrane (8, 9), it could be anticipated that the protoporphyrin-

ISSN 0006-291X

induced photodynamic effect might be reflected by changes of red cell deformability. This was confirmed by experimental results described in this communication.

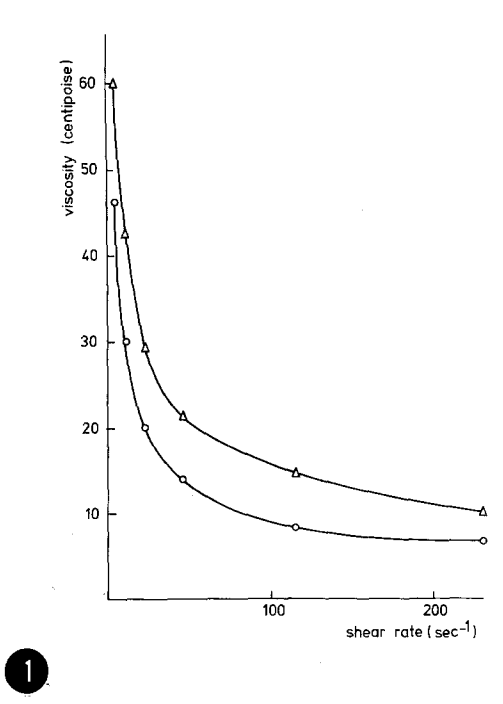
METHODS: Freshly drawn heparinized human blood was centrifuged and washed three times in buffered isotonic NaCl solution. Red cell ghosts were prepared according to the method of Weed et al. (10). Illumination of cells or ghosts in the presence of protoporphyrin was carried out as described previously (3). K^+ was measured in the incubation medium with a flame photometer.

Membrane proteins were dissolved in a solution containing 10 mM Tris, pH 8.0, 1 mM EDTA, 1% sodium dodecyl sulphate and, where indicated, 40 mM dithiothreitol. Polyacrylamide gel electrophoresis of solubilized membrane proteins was performed as described by Fairbanks et al. (11). Red cell filtration characteristics were measured according to Miller et al. (12), by filtering a 2% cell suspension through a 25 mm circular Nucleopore polycarbonate filter with a mean pore diameter of 5 microns, at a constant flow rate of 10 ml/min. The filtration pressure was recorded during 3 minutes. Red cell viscosity measurements were carried out in a cone-plate viscometer, as described by Weed et al. (13).

RESULTS: During illumination in the presence of protoporphyrin, cellular K^+ is lost to the medium. Up to 80% of the cellular K^+ is lost without any hemolysis and without change of the mean cellular volume, due to compensating Na^+ influx (7). Beyond this point the mean cellular volume increases sharply, followed by colloid-osmotic hemolysis (7). During the present investigations K^+ leakage never exceeded 20%. Therefore the experimental results were not obscured by cell swelling or hemolysis.

The influence of illumination in the presence of protoporphyrin on the viscosity of 80% red blood cell suspensions is depicted in fig. 1. As the viscosity of suspensions with a hematocrit in excess of 60 is determined by the visco-elastic properties of the cells (14), these results indicate a decreasing cell deformability with increasing illumination periods. With ghosts illuminated in the presence of protoporphyrin a similar decrease of deformability was observed, as shown in fig. 2.

The influence of the photodynamic process on the filterability of red cells is shown in fig. 3. The initial pressure was significantly increased.



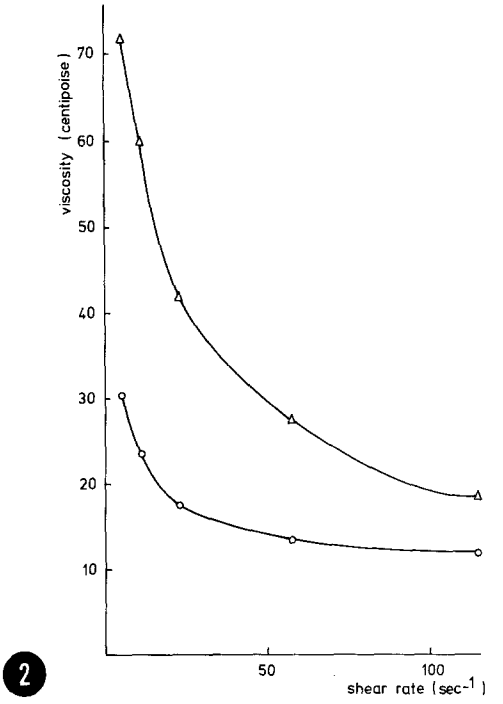


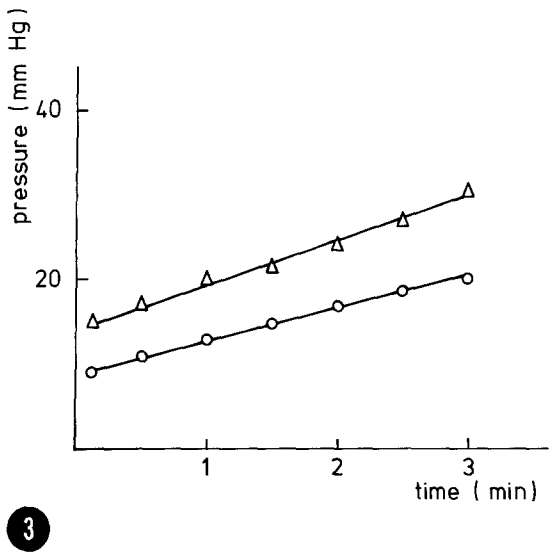
Fig. 1. Photodynamic effect of protoporphyrin on the viscosity of 80% red blood cell suspensions.
0 — 0 control, kept in the dark;
Δ — Δ 15 min illuminated in the presence of 0.17 mM protoporphyrin.

Fig. 2. Photodynamic effect of protoporphyrin on the viscosity of 80% ghost suspensions.
0 — 0 control, kept in the dark;
Δ — Δ 15 min illuminated in the presence of 0.17 mM protoporphyrin.

The subsequent rate of pressure rise during filtration was not, or only slightly, augmented. This photodynamically induced decrease of cell deformability was not influenced by dithiothreitol, except after very short illumination periods. In this initial stage dithiothreitol evoked a partial reversal of the decreased cell deformability.

A similar decrease of cell deformability could be evoked by incubation of the cells with o-iodosobenzoate (fig. 4). This decrease was completely reversed by subsequent treatment of the cells with dithiothreitol.

Electrophoretograms of solubilized membrane proteins are shown in fig. 5. Following treatment of the cells with o-iodosobenzoate there was an increase



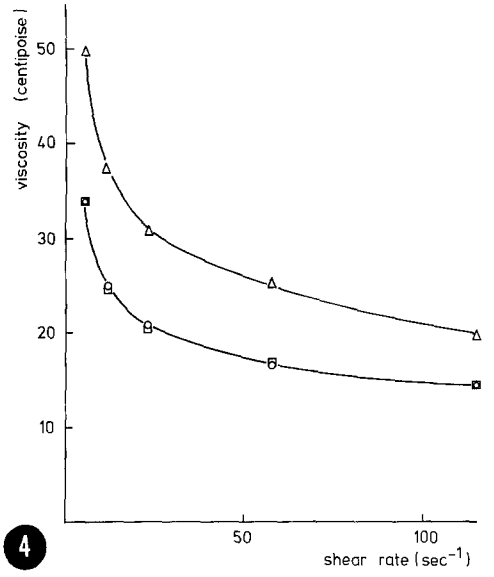


Fig. 3. Pressure-time curves of 2% red blood cell suspensions.
0 — 0 control, kept in the dark;
Δ — Δ 15 min illuminated in the presence of 0.17 mM protoporphyrin.

Fig. 4. Influence of iodosobenzoate on the viscosity of 80% ghost suspensions.
0 — 0 control;
Δ — Δ incubated with 1 mM iodosobenzoate (20 min, 37°C)
□ — □ incubated with 1 mM iodosobenzoate (20 min, 37°C) and subsequently with 10 mM dithiothreitol (45 min, 37°C).

in staining intensity in the high molecular size region (over 200,000 daltons), predominantly at the expense of spectrin. After subsequent treatment of the cells with dithiothreitol the normal protein pattern was restored. Illumination in the presence of protoporphyrin caused the formation of high molecular protein material, again primarily at the expense of spectrin, as described before (4). Dithiothreitol treatment of the illuminated cells had no influence on this protein modification, except after very short illumination periods, when a decrease of staining intensity in the high molecular size region and an increase of the spectrin staining could be observed.

DISCUSSION: There is growing interest in the subject of the photodynamic action of protoporphyrin on human erythrocytes. Illumination of red blood

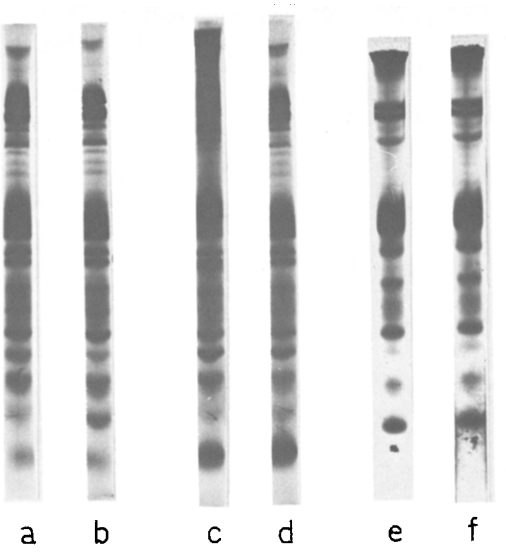


Fig. 5. Electrophoretic pattern on SDS acrylamide gels of membrane proteins.
a. control, solubilized in the absence of dithiothreitol;
b. control, solubilized in the presence of dithiothreitol;
c. incubated with iodosobenzoate (1 mM, 20 min, 37°C), solubilized in the absence of dithiothreitol;
d. incubated with iodosobenzoate (1 mM, 20 min, 37°C), solubilized in the presence of dithiothreitol;
e. 15 min illuminated in the presence of 0.17 mM protoporphyrin, solubilized in the absence of dithiothreitol;
f. 15 min illuminated in the presence of 0.17 mM protoporphyrin, solubilized in the presence of dithiothreitol.

cells in the presence of protoporphyrin has many, presumably interrelated, effects on membrane structure and function (1 - 7).

As shown previously, unsaturated fatty acid side chains (2) and cholesterol (15) are not photooxidized in intact red blood cells during short-term experiments. It also appeared that sugar residues and neuraminic acid are not susceptible to photooxidation by protoporphyrin (4). It seems likely, therefore, that the changes of cell deformability described in this paper should be attributed to photooxidation and cross-linking of membrane proteins.

After an illumination period of 15 minutes protein cross-linking was clearly visible on electrophoretograms. A high molecular protein complex had

been formed at the expense of spectrin, band 4.1 and band 6 (fig. 5). Apparently these proteins are already partly cross-linked before the other membrane proteins are involved and before the functional and structural integrity of the membrane is grossly affected.

It seems unlikely that the decreased cell deformability would be caused by photooxidation of band 6, which has been identified as the monomeric form of glyceraldehyde-3-phosphate dehydrogenase (16). The function of the polypeptides in band 4.1 is not known. Therefore, a causal relationship between band 4.1 photooxidation and decreased cell deformability cannot be excluded with certainty.

Several lines of evidence indicate that spectrin is an important determinant of red blood cell deformability (8, 17). This strongly suggests that the described changes in cell deformability are causally related to the photodynamic cross-linking of spectrin.

This notion is supported by the experiments with o-iodosobenzoate. This mild oxidizing agent causes SH-oxidation to disulphides (18). As shown in fig. 5, spectrin is sensitive to SH-oxidation by this agent, whereas bands 4.1 and 6 are not affected. The iodosobenzoate-induced spectrin cross-linking is also accompanied by decreased cell deformability. In contrast to the photodynamic effects, both iodosobenzoate-induced phenomena are completely reversed by dithiothreitol. These results strongly support the idea that spectrin plays an essential role in the regulation of red cell deformability.

ACKNOWLEDGEMENTS: The authors are grateful to Miss K. Christianse and Miss A.J. Hak for their skilful technical assistance. This work was carried out under auspices of the Netherlands Foundation for Biophysics and with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

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