Parallel Decrease of Erythrocyte Membrane Deformability and Spectrin Solubility at Low pH

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Reduction of pH over the range 6.0–4.5 results in a decrease of erythrocyte deformability in parallel with the induced progressive sphericity of cells. At low deformation rates employed, increase of hemoglobin viscosity was not significant. A decrease of membrane elasticity was detected in cells when sphering, the major determinant of cellular deformability, was prevented at pH 5.0 by hyperosmotic medium. The pronounced change of deformability and the reduced elasticity occurred at calculated intracellular pH values at which solubility in vitro of extracted erythrocyte spectrin is also markedly reduced. The parallel decrease of deformability and spectrin solubility supports the hypothesis that spectrin aggregation may contribute to regulation of erythrocyte deformability through effects on shape and membrane elastic characteristics.

Reduction of pH induces increase in bulk viscosity of blood and causes reduction of cellular deformability of individual erythrocytes. Fragmentation of cells with loss of membrane area increases sphericity and results in decrease of cell deformability, as does sphericity resulting from metabolic depletion. Modification of the intrinsic membrane structure by protein crosslinking agents and heating to 50°C alters the erythrocyte membrane's viscoelastic properties, and the heat affects the resistance of the membrane to areal expansion.

The present study was designed to examine the relative effect of reduced pH on membrane elasticity as one determinant of overall cellular deformability and to correlate elastic behavior with known pH-modulated properties of the membrane protein spectrin. The results indicate that there is an increase in sphericity, and a detectable reduction in membrane elasticity with reduced pH, in parallel with pH-induced aggregation of spectrin. No effect on cellular deformability as a result of increase of hemoglobin viscosity was observed, but a major influence of pH-induced sphericity on deformability was confirmed.

MATERIALS AND METHODS

Erythrocytes were obtained without anticoagulant by finger stick and diluted in filtered Tris-NaCl buffer (Tris 5 mM) containing 63 mg/dl albumin. Buffer tonicity was 306 mosm. Experiments were performed at room temperature, 24°C ± 2°C. Deformation of randomly selected cells suspended in buffer in a microscope stage chamber was performed by controlled aspiration of membrane into a glass micropipette and characterized as length of deformed membrane as a function of force or length of deformed membrane in response to standard force, using methods.
RESULTS

The deformation response of the erythrocyte membrane to force (Fig. 1) did not change greatly over pH range 5.5–9.0, either at low extensions ($\lambda = 1–3$), where response reflects membrane elastic behavior, or at large forces, which produce the added factor of tension in the membrane as the portion of cell remaining outside the pipette assumes a spherical shape. A distinct change in stress-strain behavior occurred at pH 5.0, with decreased response at low force (deformation less than unity, Fig. 1) and a marked increase in force required to produce extensions greater than 1–2 $\mu$m. Since pH-induced progressive sphericity is thought to contribute to decreased cellular deformability below pH 6.0, cells were examined at pH 5.0 and 6.0 in buffers made hypertonic with NaCl to prevent sphericity. This allowed excess membrane and unambiguous measurement of membrane elasticity. At applied pressure of 2000 dyn/cm$^2$, deformation was identical to that in isotonic medium over the pH range. This finding indicates that progressive sphericity alone does not account for the pH-induced change in response. The change in slope at small deformations at pH 5.0 suggests pH modification of membrane elasticity.

As pH is lowered, the viscosity of hemoglobin increases; to provide control for this, cells were deformed at pH 7.0 in medium of 600 mosm tonicity. The observed response did not differ from that in isotonic medium at deformation rates of 1 and 5 $\mu$m/sec, showing that increased hemoglobin viscosity does not affect deformation significantly at these rates.

The effect of pH on the solubility in vitro of spectrin, a major extrinsic protein of the membrane, was correlated with pH effect on erythrocyte deformability (Fig. 2). In this representative experiment the membrane was deformed at the small pressure of 2000 dyn/cm$^2$, which produced a maximum extension at pH 7.3. The plotted solubility data in vitro are those of Elgsaeter et al., which previously described. To achieve uniformity, one pipette of internal diameter 1.0 $\mu$m, calibrated by scanning electron microscope standards, was used for all experiments over the entire range of pH. Observations were recorded by a light microscope-video system, and membrane deformation was determined from direct mensuration on a cathode ray tube monitor. Deforming force was derived from pressure indicated by a variable-reluctance differential pressure transducer in a closed hydraulic system in which pressure was controlled by a precision micrometer drive syringe. The rate of aspiration was less than 1 $\mu$m/sec to avoid potential viscous effects.
have been confirmed in this laboratory. The intracellular pH was calculated from hemolysis data. The decrease in deformability clearly occurred in the same range as marked reduction of spectrin solubility, suggesting potential contribution of reduced spectrin solubility to the detected decrease of deformability. No direct quantitative relation is implied: the parallel slopes of the two variables depend on the scaling.

**DISCUSSION**

Low pH induces a decrease in cellular deformability of the erythrocyte. Among the factors contributing to the cell deformation response—relative sphericity, membrane viscoelastic properties, and viscosity of the intracellular contents—the relative sphericity appears to be the major determinant of the pH-induced decrease. The effect of the induced sphericity is evident in the stress-strain relationship: The relatively spherical cell deforms only slightly before additional deforming force induces tension in the membrane, seen as a leftward shift of the curve in Fig. 1. This emphasizes the rheologic advantage of the normal biconcave shape in the normal erythrocyte at pH 7.4: The cell's volume configuration is readily adapted within the flexible membrane, which has excess area over the minimum to enclose the cell volume.

In the present experiments a consistent small decrease in elastic response at low pH is observed. The elastic property of the membrane is characterized by the initial behavior of extension at low pressures and the resulting deforming forces. The contribution of pH-related membrane changes to deformation behavior is small compared to the pH-induced shape change (relative sphericity), to judge from the small change of the initial slope of the response in Fig. 1; however, the persistence of the membrane effect in nonsphered cells in hypertonic medium of low pH supports the interpretation that a definite change in membrane properties has occurred. The experiment in hypertonic medium at pH 7.0, where no membrane change is expected but intracellular viscosity is increased, indicates that any pH-induced change in viscosity of the intracellular contents has no appreciable effect on deformability at low deformation rates. At high rates the viscous effects of hemoglobin could not be distinguished from membrane behavior.
The parallel between the pH-induced sphericity, decrease of membrane elasticity, and decrease of spectrin solubility suggests that the state of spectrin might be related to membrane properties. Such a relationship would fit the predictions of the hypothesis that extrinsic membrane proteins form a contractile system, which accounts for the disc-to-sphere transformation observed in intact cells and contractility in ghosts, and that such proteins in a network arrangement determine the elastic behavior of the membrane. The definitive experiments to test this hypothesis critically will require more sensitive techniques.

REFERENCES

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