Biochimica et Biophysica Acta, 1150 (1993) 57–62

BBAMEM 76035

Dithiothreitol stimulates the activity of the plasma membrane aminophospholipid translocator

Hoai-Thu N. Truong [1], David L. Daleke [2] and Wray H. Huestis

Department of Chemistry, Stanford University, Stanford, CA (USA)

(Received 15 April 1993)

Key words: Erythrocyte shape; Aminophospholipid translocation; Dithiothreitol

Metabolic depletion induces human erythrocytes to crenate, a shape change that is reversed when ATP is regenerated by nutrient supplementation. In the presence of the sulfhydryl reducing agent dithiothreitol (DTT), this shape reversal is exaggerated, proceeding beyond normal discoid morphology to stomatocytic forms. DTT-induced stomatocytosis does not correlate consistently with alterations in cell ATP, spectrin phosphorylation, or phosphoinositide metabolism (Truong, H.-T.N., Ferrell, J.E., Jr. and Huestis, W.H. (1986) Blood 67, 214–221). The effect of DTT on outer-to-inner-monolayer transport of aminophospholipids was examined by monitoring shape changes induced by dilauroylphosphatidylserine (DLPS). Stomatocytosis induced by transport of this exogenous lipid to the membrane inner monolayer is accelerated and exaggerated by DTT. The effect of DTT on DLPS translocation is reversible and temperature dependent, consistent with the intervention of reducing agents in the activity of the aminophospholipid translocator. These findings bear on the relationship between cell redox status and shape regulation.

Introduction

Normal human erythrocytes are biconcave discs, a morphology critical to circulatory competence. Their discoid shape can be manipulated in vitro in a variety of ways. Amphipathic molecules such as lysophosphatidylcholine or dinitrophenol convert the cells to spiked spheres (echinocytes), a process called crenation [1,2]. Crenation also ensues when cells are loaded with Ca^{2+} [3,4], depleted of Mg^{2+} [5,6], or incubated in the absence of nutrients [7]. When these conditions are reversed, as by nutrient or Mg^{2+} supplementation or removal of Ca^{2+} or amphipaths, erythrocytes recover stable discoid morphology [4,7].

Reducing agents such as dithiothreitol (DTT) compromise erythrocyte shape regulation in a complex manner. Cells recovering from metabolic crenation in the presence of DTT regain discoid shape, but then become cupped and develop the invaginations and endocytic inclusions of stomatocytes [8]. DTT-induced stomatocytosis is observed in echinocytes recovering from crenation processes involving cell metabolic machinery (ATP depletion, Ca^{2+} loading, Mg^{2+} depletion), but not in echinocytes recovering from amphipath crenation (see accompanying manuscript [35]). The extent of such 'metabolic' stomatocytosis is proportional to the original degree of echinocytosis: more severely crenated cells become more severely stomatocytic, while DTT has no evident effect on the morphology of discocytes. DTT must be present continuously to effect and maintain stomatocytosis; reduction of discocytes prior to crenation does not lead to stomatocytosis, and DTT-induced stomatocytes revert to discs on removal of the reducing agent.

These observations suggest that DTT affects the metabolic mechanism of erythrocyte shape regulation. However, several reasonable candidates for such intervention can be ruled out: DTT has no effect on ATP resynthesis or glutathione reduction, nor does it alter the phosphorylation of phosphatidylinositides or cytoskeletal proteins [8].

Another metabolically regulated cell property is the phospholipid distribution in the plasma membrane. The nonrandom distribution of the major classes of phospholipids is well established; in healthy erythro-

Correspondence to: W.H. Huestis, Stanford University, Department of Chemistry, Stanford, CA 94305, USA.

[1] Present address: Scios Nova, 2450 Bayshore Parkway, Mountain View, CA 94034, USA.

[2] Present address: Department of Chemistry, Indiana University, Bloomington, IN 47405, USA.

Abbreviations: DLPC, dilauroylphosphatidylcholine; DLPS, dilauroylphosphatidylserine; DTT, dithiothreitol; MI, morphological index; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.

cytes, choline phospholipids are located primarily in the outer monolayer of the membrane, while 80% of phosphatidylethanolamine (PE) and 100% of phosphatidylserine (PS) is found in the cytoplasmic monolayer [4,10]. Aminophospholipid asymmetry is maintained by headgroup specific binding to inner monolayer sites [11–15] and/or by the transport activity of an ATP-dependent aminophospholipid translocator [16–20]. In addition to the requirement for ATP, such translocation is inhibited by Mg^{2+} depletion [17] and Ca^{2+} loading [21]; thus cells subjected to these conditions eventually develop more nearly random phospholipid distributions. The studies reported here explore the connection between DTT-dependent stomatocytosis and the activity of the aminophospholipid translocator.

The technique employed to examine aminophospholipid transport is observation of cell shape changes. Prior work showed that the morphology of erythrocytes is a sensitive function of the relative lipid occupancy of the inner and outer monolayers of the plasma membrane [22]. Incorporation and translocation of exogenous phospholipids is conveniently monitored from microscopic examination of cell shape changes: outer monolayer intercalators such as phosphatidylcholine (PC) induce stable spiculation, while agents that accumulate in the membrane inner monolayer produce cupping and invagination. The activity of the aminophospholipid translocator is reflected in cell responses to exogenous PS; cells spiculate transiently, then revert to discoid and eventually stomatocytic forms [17]. Radiolabel extraction studies using both PC [22] and PS [23] have shown that these shape changes report reliably on the transbilayer location of exogenous lipids.

Materials and Methods

Materials. Penicillin G was obtained from Pfizer (New York). All other biochemicals were products of Sigma (St. Louis, MO). Other chemicals, obtained from J.T. Baker (Phillipsburg, NJ) or Fischer Scientific (Pittsburgh, PA), were at least reagent grade. Dilauroylphosphatidylserine (DLPS) was prepared enzymatically from dilauroylphosphatidylcholine (DLPC) as described [22].

Cells. Human erythrocytes were obtained from healthy donors and isolated as described [8]. Cells were used within 3 h of being drawn.

All incubations were carried out at 37°C in capped plastic tubes. In lengthy incubations, suspending buffers contained 100 μg/ml streptomycin and 100 μg/ml penicillin (added as a 1000 × concentrate) to retard bacterial growth.

Metabolic depletion and recovery. To induce ATP depletion and crenation, cells were incubated at 37°C in 4 vol of $NaCl/P_i$ buffer (150 mM NaCl, 7.5 mM Na_2HPO_4 (pH 7.4)). Shape recovery was effected by pelleting echinocytes and resuspending them at 37°C in 4 vol. of buffer, either $NaCl/P_i$ or phosphate-buffered saline (138 mM NaCl, 5 mM KCl, 1.4 mM NaH_2PO_4, 1 mM $MgSO_4$, 5 mM glucose, pH 7.4) supplemented with 10 mM glucose, 10 mM inosine, and 1 mM adenosine (final concentrations; added as 10 × concentrates), as indicated in figure legends. Stomatocyte formation was induced by adding DTT to replenishing buffers, to yield a concentration of 10 mM.

DLPS vesicle incubations. DLPS vesicles were prepared by drying a chloroform solution of the lipid with a nitrogen stream, resuspending the lipid in aqueous buffer (138 mM NaCl, 5 mM KCl, 7.5 mM Na_2HPO_4, 5 mM glucose (pH 7.4)) at the concentrations indicated in figure legends, and sonicating to clarity in a bath sonicator warmed to 10°C above the phase transition temperature of the lipid. Cells were incubated at 0°C in an equal volume of prechilled vesicle suspension for 45–60 min, conditions which result in incorporation of 95–100% of vesicle lipid into cells [6]. Suspensions were then diluted to 10% hematocrit in phosphate-buffered saline at 37°C to initiate DLPS translocation. As indicated in figure legends, some of these resuspension media contained 10 mM DTT.

Morphology assay. After the intervals indicated in figure legends, aliquots of erythrocyte suspensions were fixed at 5% hematocrit in 150 mM NaCl containing 0.5% glutaraldehyde. Cells were examined by bright field microscopy at 500 × magnification. Stomatocytes were assigned a morphology score of −4 to −1, discocytes a score of 0, and echinocytes a score of +1 to +5 [4,17,23]. The average score for a field of 100 cells was designated its morphological index (MI; [2]). The absolute magnitude of shape responses to amphipaths varied with the donor, so averaging data from multiple experiments was impractical. The data shown in figures represent the averages of replicate samples in a typical experiment. Repeated blind counting of replicate samples yielded errors on the order of 0.1 MI unit, an uncertainty reflected in the size of the figure symbols.

Results

DTT effects on recovery from metabolic crenation

Erythrocytes were incubated in nutrient-free $NaCl/P_i$ to deplete ATP stores. The resulting spiculate cells (echinocytes) were pelleted and resuspended in sugar-supplemented $NaCl/P_i$ in the presence or absence of 10 mM DTT. Cells in control samples recovered and retained discoid shape, while cells incubated with DTT recovered discoid shape more rapidly, then became cupped and invaginated (Fig. 1). When the reducing agent was removed by pelleting and resuspending cells, stomatocytes reverted slowly to discoid morphology (arrows, Fig. 1).

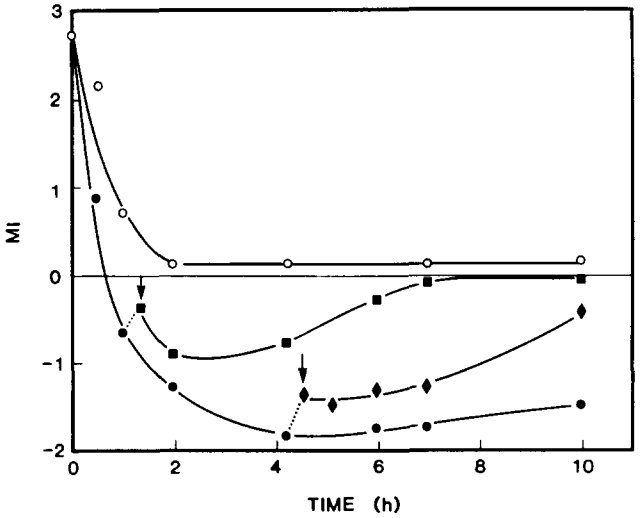

Fig. 1. Reversible DTT-induced stomatocytosis in erythrocytes recovering from metabolic starvation. Cells depleted by incubation at 37°C for 23 h were resuspended in supplemented NaCl/P_i in the absence (○) or presence (●) of 10 mM DTT. At $t = 1$ h (■) and 4.25 h (◆) after resuspension (arrows), aliquots were removed, washed free of DTT, and incubated further in DTT-free supplemented NaCl/P_i. The discontinuity in MI seen after the fourth washing step reflects the echinogenic effect of chilling.

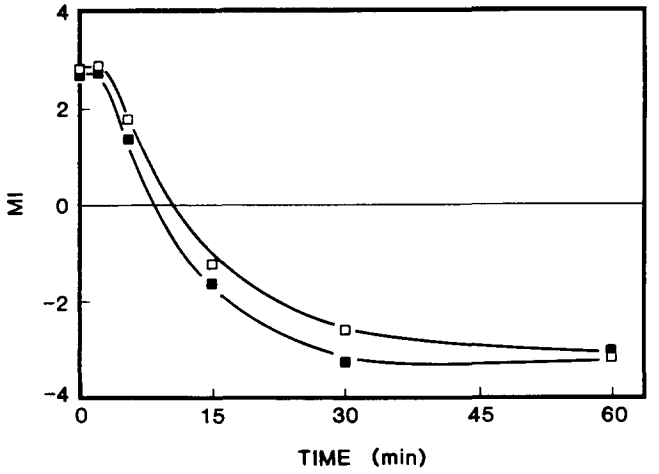

Fig. 3. DTT increases the rate of DLPS-induced stomatocytosis. Erythrocytes were pretreated with an equal volume of 250 μM DLPS for 1 h at 0°C in the absence (□) or presence (■) of 10 mM DTT. Samples were then ($t = 0$) diluted to 10% hematocrit with warmed buffer and incubated further at 37°C.

Temperature dependence of DTT-induced metabolic stomatocytosis

Metabolically depleted cells were incubated in nutrient supplemented buffer at 37°C or 15°C, in the presence and absence of 10 mM DTT. DTT accelerated shape recovery at both temperatures (Fig. 2, closed symbols). Final morphology attained by control cells was similar at 37°C and 15°C (MI approx. +0.2; Fig. 2, open symbols). DTT induced reversion to severely stomatocytic shapes at 37°C (MI −1.5), but not a 15°C (MI −0.1)

DTT effects on DLPS translocation

Erythrocytes were incubated with DLPS vesicles (250 μM DLPS) at 0°C, treatment that introduces the exogenous lipid into the outer membrane monolayer and crenates the cells. On subsequent incubation at 37°C, such cells become first discoid and then stomatocytic [17,23]. In the presence of DTT, this shape reversion was accelerated slightly (MI/min = −0.38 in the presence of DTT, vs. −0.30 in controls (Fig. 3)). After

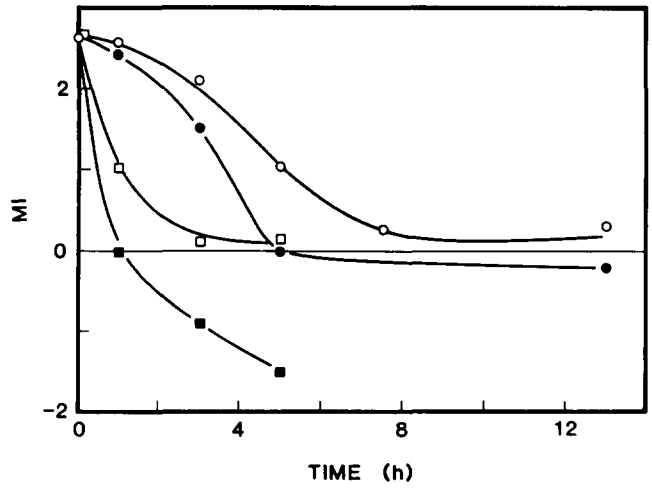

Fig. 2. Temperature dependence of DTT-induced stomatocytosis. Metabolically crenated cells were incubated in supplemented phosphate buffered saline in the absence (open symbols) or presence (closed symbols) of 10 mM DTT at 15°C (○, ●) or 37°C (□, ■). Error bars (representing reproducibility of replicate cell counting) are shown by the size of symbols.

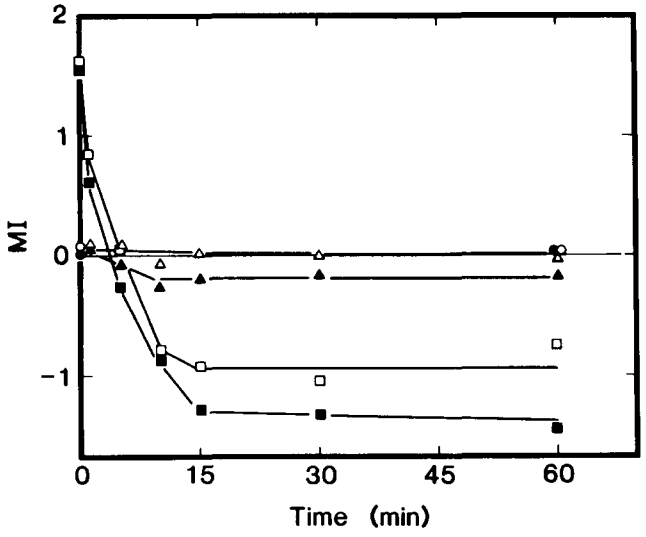

Fig. 4. For cells treated with lower concentrations of DLPS, DTT increases rate and extent of stomatocytosis. Erythrocytes were pretreated at 0°C first with an equal volume of 25 (△, ▲) or 100 uM (□, ■) DLPS for 1 h, then with 0 mM (open symbols) or 10 mM (closed symbols) DTT for 45 min. Cells were then ($t = 0$) diluted to 10% hematocrit with warmed buffer and incubated further at 37°C.

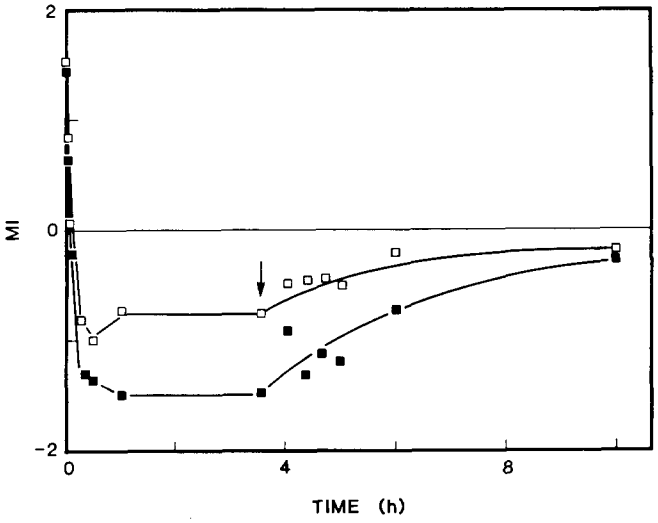

Fig. 5. Removal of DTT reverses its effect on DLPS-induced stomatocytosis. Erythrocytes were pretreated as in Fig. 4 first with 100 μM DLPS, then 0 (□) or 10 (■) mM DTT. Samples were diluted to 10% hematocrit in warmed buffer and incubated for 3.5 h at 37°C. Cells were then pelleted (arrow), washed four times in 10 vols. phosphate-buffered saline, and reincubated at 37°C in supplemented phosphate-buffered saline.

treatment with 250 μM DLPS, the eventual extent of stomatocytosis was similar (MI −3) in control and DTT-treated samples.

For cells exposed to lower concentrations of DLPS, DTT increased the extent of stomatocytosis (Fig. 4). At concentrations ≤ 25 μM, DLPS had minimal effect on cell morphology, but subsequent addition of DTT induced slight cell cupping (Fig. 4, triangular symbols).

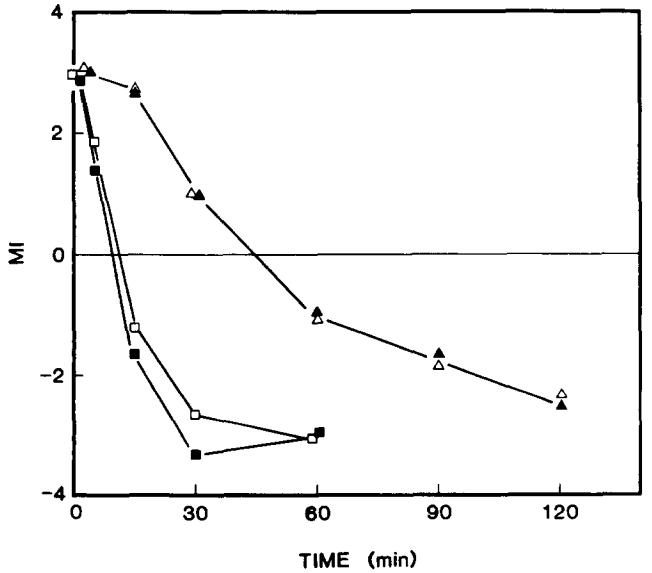

Fig. 6. DTT activates DLPS translocation at 37°C, but not at 15°C. Erythrocytes were pretreated with DLPS as in Fig. 3, then diluted to 10% hematocrit and incubated at 15°C (△, ▲) or 37°C (□, ■) in the absence (open symbols) or presence (closed symbols) of 10 mM DTT.

Cells pretreated with 100 μM DLPS reverted to moderately advanced stomatocytes (MI −1; open squares), a shape change accelerated and enhanced (MI −1.6; closed squares) by DTT.

Recovery from DTT-induced stomatocytosis

Cells were treated with DLPS (100 μM), then incubated at 37°C in the presence or absence of 10 mM DTT (Fig. 5). DTT-treated cells reverted to an MI of −1.4, while controls reverted to MI −0.9. After 3 h, control and DTT-treated cells were pelleted and washed to remove DTT, then resuspended in supplemented phosphate-buffered saline and incubated further. Both control and DTT-treated cells reverted to discoid shape, attaining the same morphology after 7 h.

Temperature effects on reversion from DLPS-induced stomatocytosis

Cells were treated with DLPS (250 μM), then incubated with or without DTT at 15°C and 37°C (Fig. 6). At 37°C, the resulting stomatocyte formation was accelerated by DTT. At 15°C, reversion was not significantly different for control and DTT-treated cells.

Discussion

This study was prompted by the apparent correlation between conditions that compromise transbilayer phospholipid asymmetry and conditions that make erythrocytes susceptible to DTT stomatocytosis [8]. Of an array of metabolically regulated processes examined (protein and lipid phosphorylation, cell glutathione and ATP levels), only phospholipid asymmetry and/or transbilayer aminophospholipid transport are affected consistently by the echinogenic conditions that lead to DTT stomatocytosis. Diminished aminophospholipid asymmetry is reported in calcium-loaded red cells [28], resealed ghosts [24], and ATP-depleted cells [29]; (however, PS scrambling is not found in ATP-depleted cells tested by some assays [30,31]). ATP depletion, magnesium depletion, and calcium loading all inhibit the outer-to-inner-monolayer transport of exogenous aminophospholipids [16–18,25]. Each of these treatments generates echinocytes that cannot regain stable discoid morphology in the presence of DTT.

Several reports implicate protein sulfhydryl functions in the activity of the aminophospholipid translocator: transport of exogenous PS and spin- or fluorescent-labeled PS analogs is inhibited by sulfhydryl oxidizing agents and alkylating agents [17,20,26,32], and oxidant inhibition is reversed by DTT in erythrocytes [17,6] and platelets [27]. If DTT were to stimulate the activity of the aminophospholipid transporter in recovering cells, more rapid and extensive transport of PE and/or PS would be expected to generate stomatocytosis by inner-monolayer expansion.

Effect of DTT on exogenous aminophospholipid translocation

On exposure to short acyl chain PS homologs at 0°C, where the aminophospholipid translocator is inhibited, metabolically replete erythrocytes crenate as the foreign lipid accumulates in the outer membrane monolayer. On warming to 37°C, such echinocytes become discocytic and then stomatocytic as the excess PS is translocated to the inner membrane leaflet [17]. In the presence of DTT, this shape change is accelerated and (at low PS concentration) enhanced. Since DTT has no independent effect on the shape of metabolically replete cells, this enhanced stomatocytosis is consistent with DTT stimulation of PS transport.

In support of this correlation, DTT affects both cell shape and exogenous PS transport in a reversible and temperature dependent manner. On removal of the reducing agent, 'metabolic' stomatocytes and PS-treated cells revert to discoid shape (Ref. 8; see also Fig. 1) with similar time-courses ($t_{1/2} = 2$–3 h). This shape reversion occurs on a time scale similar to the reversion seen when DLPS-treated cells are mixed with exogenous receptor vesicles [25]. Both may reflect passive flip of the short acyl chain exogenous PS to the outer monolayer. DTT-induced 'metabolic' stomatocytosis and stimulated PS transport are also affected similarly by temperature; at 15°C, DTT has minimal effect on DLPS translocation in metabolically replete cells (Fig. 6), and DTT has less stomatogenic effect in crenation recovery (Fig. 2).

(In contrast, Connor and Schroit [26] report no difference in net transport of fluorescently labeled PS in the presence and absence of DTT. The conditions used in that study correspond to a phospholipid-to-cell ratio similar to our experiments using 25 μM DLPS. The effect of DTT was found be small under those conditions (Fig. 4)).

The stomatocytic response of recovering metabolic echinocytes and DLPS-treated cells differ in time-course. At 37°C, the DLPS-induced shape change proceeds with a half-time of 15 min (10 min in the presence of DTT; Fig. 3), while recovery from ATP depletion at that temperature exhibits a typical half-time of 60 min. This difference likely reflects that, while the DLPS-treated cells are metabolically replete, recovery from metabolic depletion must be supported by ATP resynthesis. The aminophospholipid translocator exhibits an apparent K_m for ATP of 1.25 mM [16]. ATP levels in cells recovering from metabolic depletion rise from 0 to 0.5 mM [8] but do not return to control level (1.4 mM; [33]). The rate of aminophospholipid translocation is decreased at ATP concentrations below 0.5 mM [17,18,34]. Cells containing only 13% of control level ATP remain essentially discoid [4]. If such cells are supplemented with nutrients and treated with DLPS, they become stomatocytic with a half-time of 1.2 h [17]. Under these conditions, aminophospholipid translocation and DTT-induced metabolic stomatocytosis occur on similar time scales.

Models for DTT-induced stomatocytosis

The above observations indicate that DTT stimulates exogenous PS transport in red cells, and the effects of DTT on metabolic shape regulation are consistent with activation of the aminophospholipid translocator, or increase in inner monolayer aminophospholipid binding sites, or both. However, simple models do not readily account for some aspects of these findings. If both amino and choline phospholipids randomize across the bilayer in proportion to crenation, active transport of PS and PE in recovering cells (which is much faster than the passive flip-flop of PC and sphingomyelin) would produce stomatocytosis even in the absence of DTT. This effect, if present at all, is barely detectable. If aminophospholipids alone were to scramble, DTT activation of the selective transporter (e.g., by decreasing the K_m for PS or PE) would result in stomatocytosis that would reverse on DTT removal. However, since a substantive fraction of PE resides in the outer monolayer of control discocytes, such a mechanism would also induce their stomatocytosis. This is not found. A selective DTT effect on the K_m for PS could not alone produce stomatocytosis, since removal of all PS from the outer monolayer merely restores the status quo ante (hence discoid shape).

A DTT-induced increase in the number of inner monolayer PS/PE binding sites also would produce stomatocytosis, but again several details of the cell response do not support any simple mechanism. Such sites would have to be cryptic in healthy discocytes but susceptible to exposure in crenated cells, and only in proportion to the degree of metabolic depletion/calcium loading/magnesium depletion [8]. In that event, DTT would not be expected to increase stomatocytosis in otherwise healthy PS-treated cells, unless the activity of the transporter were also enhanced. A model invoking increased inner monolayer binding sites does account for the concentration dependence of exogenous PS-induced stomatocytosis: at low $[PS]_{ex}$, DTT increases both the rate and extent of stomatocytosis, while at higher $[PS]_{ex}$, DTT-treated cells change shape more rapidly but achieve the same final state as controls. This suggests a saturable DTT-sensitive mechanism, but it is difficult to reconcile with the reversibility of the effect and its proportionality to extent of crenation.

In sum, these findings reveal a redox-sensitive aspect of red cell shape regulation that becomes evident only in cells under metabolic stress, and that apparently invokes aminophospholipid transport and sequestration mechanisms. The difficulty in reconciling the

details of this response with individual biochemical mechanisms suggests that the shape of these cells is not controlled by any single metabolic process. Rather, it is at least a second-order function of superimposed phenomena, including (but not limited to) reticular protein interactions, phosphoinositide metabolism, and transbilayer aminophospholipid distribution.

Acknowledgement

This work was supported by a grant from the USPHS (HL 23787).

References

1 Deuticke, B. (1968) Biophys. Biochim. Acta 163, 494–500.
2 Fuji, T., Sato, T., Tamura, A., Wakatsuke, M. and Kanaho, Y. (1979) Biochem. Pharmacol. 28, 613–620.
3 Kuettner, J.F., Kreher, K.L., Rao, G.H.R., Eaton, J.W., Blackshear, P.L. and White, J.G. (1977) Am. J. Pathol. 88, 81–94.
4 Ferrell, J.E., Jr. and Huestis, W.H. (1984) J. Cell Biol. 98, 1992–1998.
5 Szasz, I., Hasitz, M., Breuer, J.H., Sarkade, B. and Gardos, G. (1978) Acta Biol. Acad. Sci. Hung. 29, 1–17.
6 Daleke, D.L. (1986) Ph.D. thesis, Stanford University.
7 Nakao, M., Nakao, T. and Yamazoe, S. (1960) Nature (Lond.) 187, 945–946.
8 Truong, H.-T.N., Ferrell, J.E., Jr. and Huestis, W.H. (1986) Blood 67, 214–221.
9 Van Deenen, L.L.M. (1981) FEBS Lett. 123, 3–15.
10 Op den Kamp, J.A.F. (1979) Annu. Rev. Biochem. 48, 47–71.
11 Haest, C.W.M. and Deuticke, B. (1975) Biochim. Biophys. Acta 401, 468–480.
12 Mombers, C., Verkleij, A.J., De Gier, J. and Van Deenen, L.L.M (1979) Biochim. Biophys. Acta 551, 271–281.
13 Marinetti, G.V. and Crain, R.C. (1978) J. Supramol. Struct. 8, 191–213.
14 Choe, H.R., Williamson, P., Rubin, E. and Schlegel, R.A. (1985) Cell Biol. Int. Rep. 9, 597–606.
15 Middelkoop, E., Lubin, B.H., Bevers, E.M., Op den Kamp, J.A.F., Comfurius, P., Chiu, D.T.-Y., Zwaal, R.F.A., Van Deenen, L.L.M. and Roelofsen, B. (1988) Biochim. Biophys. Acta 937, 281–288.
16 Seigneuret, M. and Devaux, P.F. (1984) Proc. Natl. Acad. Sci. USA 81, 3751–3755.
17 Daleke, D.L. and Huestis, W.H. (1985) Biochemistry 24, 5406–5416.
18 Tilley, L., Cribier, S., Roelofsen, B., Op den Kamp, J.A.F. and Van Deenen, L.L.M. (1986) FEBS Lett. 194, 21–27.
19 Martin, O. and Pagano, R.E. (1987) J. Biol. Chem. 262, 5890–5898.
20 Connor, J. and Schroit, A.J. (1987) Biochemistry 26, 5099–5105.
21 Bitbol, M., Fellmann, P., Zachowski, A. and Devaux, P.F. (1987) Biochim. Biophys. Acta 904, 268–282.
22 Ferrell, J.E., Jr., Lee, K.-J. and Huestis, W.H. (1985) Biochemistry 24, 2849–2857.
23 Daleke, D.L. and Huestis, W.H.(1989) J. Cell Biol. 108, 1375–1385.
24 Williamson, P., Algarin, L., Bateman, J., Choe, H.-R. and Schlegel, R.A. (1985) J. Cell. Physiol. 123, 209–214.
25 Bitbol, M.P. and Devaux, P.F. (1988) Proc. Natl. Acad. Sci. USA 85, 6783–6787.
26 Connor, J. and Schroit, A.J. (1988) Biochemistry 27, 848–851.
27 Bevers, E.M., Tilly, R.H.J., Senden, S.M.G., Comfurius, P. and Zwaal, R.F.A. (1989) Biochemistry 28, 2382–2387.
28 Chandra, R., Joshi, P.C., Bajpai, V.K. and Gupta, C.M. (1987) Biochim. Biophys. Acta 902, 253–262.
29 Haest, C.W.M., Plasa, G., Kamp, D. and Deuticke, B. (1978) Biochim. Biophys. Acta 509, 21–32.
30 Middelkoop, E., Van der Hoek, E.E., Bevers, E.M., Comfurius, P., Slotboom, A.J., Op den Kamp, J.A.F., Lubin, B., Zwaal, R.F.A. and Roelofsen, B. (1989) Biochim. Biophys. Acta 981, 151–160.
31 Connor, J. and Shroit, A.J. (1990) Biochemistry 29, 37–43.
32 Zachowski, A., Favre, E., Cribier, S., Herve, P. and Devaux, P.F. (1986) Biochemistry 25, 2585–2590.
33 Beutler, E. (1984) in Red Cell Metabolism. A Manual of Biochemical Methods (3rd Edn.), Springer-Verlag, New York.
34 Calvez, J.Y., Zachowski, A., Hermann, A., Morrot, G. and Devaux, P.F. (1988) Biochemistry 27, 5666–5670.
35 Truong, H.T.N., Daleke, D.L. and Huestis, W.H. (1993) Biochim. Biophys. Acta 1150, 51–56.