Letters to the Editor

A Possible Molecular Mechanism Governing Human Erythrocyte Shape

In their recent papers Gedde et al. (Gedde et al., 1997; Gedde and Huestis, 1997) reexamined various hypotheses on the mechanism of shape changes of human erythrocytes. Their careful examination suggests that the shape is determined by the cytoplasmic pH and not by the transmembrane potential (TMP) (Glaser et al., 1987). Nevertheless, the authors were unable to identify a molecular mechanism explaining their findings. They discussed five different mechanisms, one of which (cell water content) is only able to modulate shape changes. Three other mechanisms, an ionic gel model for spectrin (Stokke et al., 1986), pH-dependent intramembrane band 3 aggregation (Elgsaeter et al., 1976), and electrostatic effects of pH-titratable lipids (Gedde et al., 1995), were shown to be incapable of explaining the shape behavior of intact cells. Gedde et al. favored a mechanism in which a pH-dependent insertion of an unspecified "cell protein into the red cell inner leaflet" induces shape changes based on the bilayer couple model. The bilayer couple model is a generally accepted mechanism for erythrocyte shape changes and was proved by changing the ratio of lipids or lipophilic compounds of the two monolayers. However, under physiological conditions, transbilayer phospholipid movement is too slow to explain shape changes that occur in a matter of seconds (see Brumen et al., 1993).

Gedde et al. probably overlooked a new approach which proposes that the anion-exchange protein band 3 plays a major role in erythrocyte shape. Band 3 is a good candidate protein because many of its known characteristics match the required properties. Wong (1994) favored a mechanism in which anions transported by band 3 govern the folding and unfolding of spectrin. In contrast, Gimsa and Ried (1995) proposed a band 3 conformation-controlled bilayer couple (CCBC) model. It assumes that a conformational change of band 3 changes the ratio of inwardly and outwardly facing anion-binding sites of the intramembraneous domain of band 3. In particular, the synchronized recruitment of a certain conformation should significantly alter the membrane monolayer area ratio. This model further assumes that conformers favoring the external orientation occupy an increased volume in the external monolayer (Gimsa and Ried, 1995). This notion is backed by well-known properties of band 3 (Bamberg and Passow, 1992). Physiologically, the protein ensures the fast exchange of internal and external anions such as chloride and bicarbonate, at cycles higher than 10^4 s^{-1}. It operates by a ping-pong mechanism that exposes the transport site to the opposite sides of the membrane. This mechanism excludes a transition of the unloaded transporter and suggests that the average orientation of the binding site depends on ligand affinities, as well as on the amount of ligands available at the two membrane sides. Consequently, a lack of external anions will prevent the binding site from returning to the inside, thereby inducing a conformational change of the protein. Then a shape change according to the bilayer couple model is only retarded by the viscoelastic cell properties. There are more than 1 million copies of band 3 per human erythrocyte, and they occupy ~10% of the total membrane area. It can be estimated that the sum of all cross-sectional areas of the protein's access channels corresponds to ~1% of the total membrane area. Thus, this area is greater than that needed for a profound change in the erythrocyte shape.

Because anions, the ligands of band 3, are usually distributed according to the TMP, the CCBC model suggests an influence on the equilibrium distribution of conformers and a correlation of cell shape and TMP. An additional mechanism may be the direct recruitment of internal conformers by a positive TMP (Wyatt and Cherry, 1992); this is probably experienced in the access channel (Jennings et al., 1990). However, under physiological conditions, around neutral external pH, band 3 conformers are asymmetrically distributed, with ~90% of the transport sites facing the inside. Thus recruitment of the binding site under physiological conditions (e.g., by the lack of anions in the vicinity of a negatively charged glass surface) will involve almost all band 3 proteins and may induce a conformational change leading to a more drastic shape effect than recruitment of the internal conformation. It is known that alkaline pH changes the distribution asymmetry, so that ~80% of the binding sites face the outside (Bjerrum, 1992). In light of the CCBC model, particularly at alkaline pH, this pH dependence corresponds to the pH shape dependence of figure 1 in Gedde and Huestis (1997).

Another hint of a significant role of band 3 not discussed by the authors is that many specific band 3 inhibitors can induce shape effects at extremely low doses. Most of them by far are echinocytogenic. When their effect on the band 3 conformation was investigated, all of the echinocytogenic inhibitors were found to recruit the external conformation (4,4′-diisothiocyanatostilben-2,2′-disulfonic acid (DIDS), 4-acetamido-4′-isothiocyanatostilben-2,2′-disulfonate, furosemide, *N*-ethylmaleimide, salicylic acid, etc.). DIDS instantaneously induces echinocytes, overriding any possible correspondence with the TMP or pH. We found that even 4 nM DIDS was sufficient to form echinocytes (see also Blank et al., 1994). At our cell concentration, this DIDS concentration corresponded to an estimated concentration ratio of DIDS (in solution) to band 3 (overall content of cell suspension) of 1:1. It can be deduced that the echinocytogenic effect is specific for the inhibitory reaction of DIDS,

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