

- Prevelige, P. E., J. King and J. L. Silva. 1994. Pressure denaturation of the bacteriophage P22 coat protein and its entropic stabilization in icosahedral shells. *Biophys. J.* 66:1631–1641.
- Royer, C. A., A. P. Hinck, S. N. Loh, K. E. Prehoda, X. Peng, J. Jonas, and J. L. Markley. 1993. Effects of amino acid substitutions on the pressure denaturation of Staphylococcal nuclease as monitored by fluorescence and nuclear magnetic resonance spectroscopy. *Biochemistry.* 32:5222–5232.
- Silva, J. L., and G. Weber. 1993. Pressure stability of proteins. *Annu. Rev. Phys. Chem.* 44: 89–113.

Action at a Distance: Another Lesson from the Red Cell

David E. Golan* and Peter Agre†

*Departments of Biological Chemistry and Molecular Pharmacology and of Medicine, Harvard Medical School; Hematology-Oncology Division, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts 02115 USA, and †Departments of Biological Chemistry and of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 USA

Secrets initially revealed by studies of the human red cell membrane have repeatedly proven to be of general biological significance. Membrane phospholipid composition and asymmetry, the biochemistry and functional importance of the membrane skeleton, and the physiology of membrane transport proteins are all examples of this progression in experimental membrane biology. In the field of signal transduction across biological membranes, many of the molecular events that mediate signaling upon ligand binding to the exofacial domain of a transmembrane receptor protein remain obscure. One reason for this lack of understanding is the structural and functional complexity of most biological membranes, making it difficult to tease out relationships among changes in membrane biochemistry and consequent alterations in membrane structure and function. Again the red cell may have come to the rescue.

Although the membrane of the enucleated red cell is not amenable to study by the generation of site-specific mutant molecules, the wealth of available clinical material provides “natural” mutants that can be exploited with great effectiveness. In the present issue of the *Biophysical Journal*, Knowles et al. (1994) use such mutants to describe the effects of a monoclonal antibody directed against the major membrane sialoglycoprotein, glycophorin A, on membrane rigidity and transmembrane protein lateral mobility. The anti-glycophorin antibody is found to induce a marked increase in the rigidity of normal red cells as well as lateral immobilization of both glycophorin A and the major transmembrane protein, band 3. None of these effects are induced by the anti-glycophorin antibody in mutant red cells expressing a form of glycophorin A that lacks the cytoplasmic domain and, therefore, is incapable of interaction with the red cell membrane skeleton. These findings are remarkable and important for several reasons.

The first set of implications concerns the molecular mechanism by which the anti-glycophorin antibody induces transmembrane protein immobilization and membrane rigidification. The antibody presumably induces a conformational change in glycophorin A that affects the interaction between this integral protein and the red cell membrane skeleton. The conformational change, therefore, could effect transmembrane protein immobilization and membrane rigidification by: 1) inducing increased interactions between band 3 and the membrane skeleton; or 2) inducing increased interactions between glycophorin A and the membrane skeleton, thereby pulling the skeleton closer to the membrane lipid bilayer. In the first mechanism, band 3 immobilization would result from increased direct binding interactions between band 3 and the membrane skeleton, whereas in the second mechanism, increased steric interactions would be responsible for band 3 immobilization. Although lateral mobility data cannot distinguish between direct binding and steric mechanisms as causes of lateral immobilization, rota-

tional mobility measurements can shed light on this question. Rotational mobility is sensitive to the molecular environment (nanometer scale) of the rotating species, whereas lateral mobility is measured over micrometer scale distances. Band 3 rotation has been measured in normal red cells incubated with the anti-glycophorin antibody; the results show that the antibody causes rotational immobilization of band 3 (Nigg et al., 1980). These data suggest strongly that the anti-glycophorin antibody induces increased direct binding interactions between band 3 and the membrane skeleton, although concomitant increases in steric interactions cannot be ruled out.

Are glycophorin A and band 3 linked in a molecular complex? This question was first raised almost thirty years ago, and it remains unresolved to the present time. Data in favor of a glycophorin A-band 3 complex include the finding that the anti-glycophorin antibody induces rotational immobilization of band 3 (see above), and the observation that the W_r^b blood group antigen is made up of determinants from the exofacial domains of both band 3 and glycophorin A (Telen and Chasis, 1990). Data against such a complex include band 3 and glycophorin mobility measurements in a number of red cell states that show discordant values for the rates of band 3 and glycophorin translation. (If the two transmembrane proteins were tightly linked in a long-lived complex, they should manifest identical rates of translational diffusion.) Examples include: 1) normal red cells, in which glycophorin lateral diffusion rates are about 50% greater than band 3 diffusion rates (reviewed by Golan, 1989); 2) Southeast Asian ovalocytes (Liu et al., 1990; Mohandas et al., 1992) and Band 3^{PRAGUE} red cells (Jarolim et al., 1994), in which band 3 is laterally immobile, but glycophorin diffuses at control rates; and 3) anti-glycophorin antibody-treated Miltenberger V (mutant) red cells, in which lateral diffusion rates for band 3 and glycophorin A differ by a factor of about 3 (see Figs. 1 and 3 of Knowles et al., 1994). The work of Knowles et al. (1994) would appear to add to existing data favoring

Received for publication 14 March 1994 and in final form 14 March 1994.

© 1994 by the Biophysical Society
0006-3495/94/05/1271/07 \$2.00

a model in which band 3 and glycoporphin A are not linked in a tight molecular complex, but may be capable of transient lateral association in the membrane.

The third set of implications concerns the generalizability of the molecular mechanism proposed by Knowles et al. (1994). The mechanism, restated, is that receptors with short cytoplasmic tails can activate cells through cooperative actions that induce increased interactions between the cytoskeleton or membrane skeleton and other plasma membrane receptors. Early evidence for this type of mechanism came from studies on lymphocytes, in which locally bound lectins were found to immobilize receptors at locations on the cell surface remote from the site of lectin binding (Edelman, 1976; Henis and Elson, 1981). Contemporary studies on lymphocytes suggest that cell-cell adhesion (mediated by ligand-receptor binding) and cell activation are dynamically inter-related. That is, cells can be activated by ligation of certain adhesion receptors, and cell activation influences the strength of cell-cell adhesion mediated by adhesion molecules at the plasma membrane. Preliminary evidence from a number of laboratories suggests that these effects are likely to involve signal transduction pathways and cytoskeletal rearrangements that affect the lateral mobility and aggregation state of cell surface receptors.

By using the human red cell as a model system, Knowles et al. (1994) have provided clear evidence for bi-directional transmembrane signaling involving the red cell membrane skeleton. "Action at a distance," in which cell activation through one transmembrane receptor affects the structure and function of other receptors in the same membrane, is likely to be the rule rather than the exception in many, if not all, biological membranes.

REFERENCES

- Edelman, G. M. 1976. Surface modulation in cell recognition and cell growth. *Science*. 192: 218-226.
- Golan, D. E. 1989. Red cell membrane protein and lipid diffusion. *In* Red Blood Cell Membranes: Structure, Function and Clinical Implications. J. C. Parker and P. C. Agre, editors. Marcel Dekker, New York. 367-400.
- Henis, Y. I., and E. L. Elson. 1981. Inhibition of the mobility of mouse lymphocyte surface immunoglobulins by locally bound concanavalin A. *Proc. Natl. Acad. Sci. USA*. 78:1072-1076.
- Jarolim, P., H. L. Rubin, S. C. Liu, M. R. Cho, V. Brabec, L. H. Derrick, S. Yi, S. T. O. Saad, S. Alper, C. Brugnara, D. E. Golan, and J. Palek. 1994. Duplication of 10 nucleotides in the erythroid band 3 (AE1) gene in a kindred with hereditary spherocytosis and band 3 protein deficiency (Band 3^{PRAGUE}). *J. Clin. Invest.* 93:121-130.
- Knowles, D. W., J. A. Chasis, E. A. Evans, and N. Mohandas. 1994. Cooperative action between band 3 and glycoporphin A in human erythrocytes: immobilization of band 3 induced by antibodies to glycoporphin A. *Biophys. J.* 66:1725-1732.
- Liu, S. C., Z. Sen, J. Palek, D. E. Golan, D. Amato, K. Hassan, G. T. Nurse, D. Babona, T. Coetzer, P. Jarolim, M. Zaik, and S. Borwein. 1990. Molecular defect of the band 3 protein in Southeast Asian ovalocytosis. *N. Engl. J. Med.* 323:1530-1538.
- Mohandas, N., R. Winardi, D. Knowles, A. Leung, M. Parra, E. George, J. Conboy, and J. Chasis. 1992. Molecular basis for membrane rigidity of hereditary ovalocytosis: a novel mechanism involving the cytoplasmic domain of band 3. *J. Clin. Invest.* 89:686-692.
- Nigg, E. A., E. Bron, M. Girardet, and R. J. Cherry. 1980. Band 3-glycoporphin A association in erythrocyte membranes demonstrated by combining protein diffusion measurements with antibody-induced cross linking. *Biochemistry*. 19:1883-1892.
- Telen, M. J., and J. A. Chasis. 1990. Relationship of the human erythrocyte Wr^b antigen to an interaction between glycoporphin A and band 3. *Blood*. 76:842-848.
- of muscle contraction, a major goal has been the direct electron microscopic (EM) visualization of this complex before and after the power stroke in which force is generated. It is widely accepted that the post-power stroke state is represented well by the tightly associated rigor complex that occurs in the absence of nucleotide or in the presence of MgADP. Under these conditions, EM shows clearly that isolated myosin heads (S1) in solution bind to actin uniformly and stereospecifically at an angle of ~45°. A much more elusive complex is the weak-binding pre-force state that predominates in the presence of ATP.
- A report by Walker et al. (1994) in this issue addresses this problem through EM experiments on solutions of actin and S1 during the steady state of the ATP hydrolysis reaction. By performing the experiments at very low (2 mM) ionic strength, which strengthens the binding of S1 to actin, they were able to obtain conditions in which up to 70% of the heads were bound to actin. To capture the short-lived (~1 ms) actin-S1-ATP complex, they froze the samples very rapidly (~10⁵ s⁻¹), producing a hydrated complex in vitreous ice, which they then examined directly (without staining) by cryoelectron microscopy. The resulting micrographs show a very *disordered actin-S1 complex in which the S1 molecules appear to bind to actin with a wide range of attachment angles*. In contrast, samples prepared in the absence of ATP, or after waiting long enough that the ATP was converted to ADP, showed the usual uniform attachment of S1 at ~45°.
- The observation of angular disorder of weakly attached S1 agrees with most (e.g., Frado and Craig, 1992) but not all (Pollard et al., 1993) previous EM studies of the actin-S1 complex during ATP hydrolysis. The present study by Walker et al. (1994) is important because the techniques used are designed to prevent artifacts that might have affected previous studies. Most previous studies showing disorder did not employ rapid freezing, and the samples were stained before electron microscopy (Frado and Craig, 1992). The present study indicates that the disorder

Angular Disorder of Weak-Binding Actomyosin Cross-Bridges

David D. Thomas

Department of Biochemistry, University of Minnesota Medical School, Minneapolis, MN 55455. Tel.: 612-625-0957; Fax: 612-624-0632; E-mail: ddt@ddt.biochem.umm.edu.

Since a large-scale structural change within the actin-myosin complex (cross-bridge) is predicted to accompany force generation in most models

Received for publication 16 March 1994 and in final form 16 March 1994.

Address reprint requests to Dr. David D. Thomas, Dept. of Biochemistry, Millard 4-225, University of Minnesota Medical School, Minneapolis, MN 55455. Tel.: 612-625-0957; Fax: 612-624-0632; E-mail: ddt@ddt.biochem.umm.edu.

© 1994 by the Biophysical Society
0006-3495/94/05/1272/07 \$2.00