

Publication

Band 3-glycophorin A association in erythrocyte membrane demonstrated by combining protein diffusion measurements with antibody-induced cross-linking

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A new approach to the study of molecular protein interactions in biological membranes is presented. The technique is based on measuring the rotation of a membrane protein in the presence and absence of specific antibodies directed toward a purported complex partner. As a first illustration of the method, the putative association of band 3 with glycophorin A in the human erythrocyte membrane was investigated. The rotational diffusion of band 3 was strongly reduced following cross-linking of glycophorin A with divalent antibodies. However, little or no effect on band 3 rotation was produced by monovalent antiglycophorin A Fab fragments, antispectrin or nonspecific antibodies, ruling out major effects on band 3 mobility due to steric hindrance, unspecific antibody adsorption, or transmembrane interactions involving spectrin. It is concluded that immobilization of band 3 by antiglycophorin A antibodies is directly caused by cross-linking of a preexisting band 3-glycophorin A complex in the human erythrocyte membrane.

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