VOLUME EXPANSION DECREASES THE BINDING OF BAND 3 AND BAND 4.1 IN LITTLE SKATE (*RAJA ERINACEA)* ERYTHROCYTES

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Volume expansion of skate erythrocytes results in the efflux of solutes through a permeability which appears to require band 3. Expression of trout, but not murine band 3 in Xenopus oocytes results in the development of a permeability for a number of solutes including the amino acid taurine (Fievet, B. et al., *Proc. Nat. Acad. Sci. USA*, 95:10996-11001, 1998). In the skate erythrocyte, we have previously demonstrated that a number of biochemical events occur upon volume expansion including activation of phospholipase D, tyrosine phosphorylation of band 3 and a number of other protein targets (of unknown nature), oligomerization of band 3, and increased affinity of the cytoskeletal protein ankyrin for band 3. Band 3 has many interactions with the cytoskeleton and its activity has been hypothesized to be regulated by these interactions. Erythrocyte band 4.1 is an important protein in the interaction of band 3 with the cytoskeleton. Recently, 4.1 has been shown to specifically interact with band 3 and this interaction may depend not only on the oligomeric state of band 3, but may also be influenced by other cytoskeletal proteins such as ankyrin. Therefore, the present studies were undertaken to examine the interaction of band 3 with band 4.1 and whether this interaction is altered by volume expansion.

Binding studies were performed using inside out vesicles purified from erythrocytes under isotonic and hypotonic conditions. Band 4.1 was purified from human erythocytes rather than the skate erythrocytes since the amount of human red cells available as the starting material for band 4.1 isolation was significantly greater. To confirm that skate and human band 4.1 were similar, Western blots were performed on human and skate erythrocyte band 4.1. A similar band was noted, indicating that at least some part of the sequences of these two proteins were similar. Human band 4.1 was iodinated using Bolton-Hunter reagent and binding assays of the labeled band 4.1 to erythrocyte inside-out vesicles (IOV) were performed as described in our studies of ankyrin binding to band 3 (Musch, M. and L. Goldstein, *J. Biol. Chem.* 271:21221-21225, 1996).

Binding of band 4.1 to skate erthrocyte IOV occurred at a number of different binding sites as assessed by pH sensitivity, trypsin sensitivity, and competition with a peptide specific for band 4.1 interaction with band 3 in human erythroyctes (IRRRY). When the specific interaction of band 4.1 to band 3 was analyzed by Scatchard analysis, the affinity of band 4.1 for band 3 was not altered under hypotonic conditions. However, the number of binding sites was decreased by over 50%. Binding of band 4.1 to other proteins, potentially glycophorins, was not significantly decreased by volume expansion. Band 4.1 did not alter the number of ankyrin binding sites, but the high affinity ankyrin binding sites observed during volume expansion were converted to low affinity sites by inclusion of band 4.1. Therefore volume expansion of skate erythrocytes alters the interaction of a number of cytoskeletal proteins with band 3. This interaction may be important in the participation of band 3 in the permeability increase observed in the regulatory volume decrease.

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