HYPOTONICITY INDUCES FORMATION OF TETRAMERS OF BAND 3 IN LITTLE SKATE (*RAJA ERINACEA*) ERYTHROCYTES

Mark W. Musch[1] and Leon Goldstein[2]
[1]Department of Medicine and the College, University of Chicago, Chicago, IL 60637
[2]Department of Physiology, Brown University, Providence, RI 02912

Previous studies have implicated band 3 in the volume activated transport of taurine and other organic solutes in fish erythrocytes (for review, Goldstein and Musch, J. Exp Zool.268:133-138 , 1994). During volume expansion, band 3 shows an increased ability to bind the stilbene disulfonate DIDS and an increase in phosphorylation of the band 3 protein. These results suggest that band 3 may undergo structural modification in volume expanded erythrocytes. Since band 3 has recently been demonstrated to exist in different oligomeric states in the erythrocyte membrane (Salhany et al., J. Biol. Chem. : 17688-17693, 1990; Casey and Reithmeier, J. Biol. Chem. 266:15726-15737, 1991), we tested the effects of volume expansion on band 3 oligomerization in the skate erythroycte membrane.

We used the crosslinking agent BS^3 to determine the ratio of band 3 monomer, dimer, and tetramer. Cells were incubated in media of varying osmolarities (940, 660, 460 mosm/liter) or with the inclusion of a permeant solute (200mM ethylene glycol replaced 100mM NaCl) with [3H]-H_2DIDS (0.5μM) for 60 min. Plasma membranes were then isolated and resuspended at a protein concentreation of 10μg/ml. BS^3 was added (5mM) and the reaction was allowed to proceed for 30 min at room temperature. The membranes were diluted in Tris-buffer to quench remaining BS^3, pelleted at 100,000 x g and solubilized for analysis on 5% SDS-PAGE. Acrylamide gels were run at 200V below 13°C to prevent smearing. The gels were removed and 2mm slices cut between 90-500kDa. The slices wre solubilized and radioactive [3H]-H_2DIDS in each slice quantified by liquid scintillation spectroscopy. As controls, cells were incubated with pyridoxal-5-phosphate (PLP)(2mM) or DNDS (0.5mM) in the presence of [3H]-H_2DIDS and then membranes isolated and reacted with BS^3. These two agents are known to promote the shift in distribution of band 3 into the tetrameric form from the SDS-resistant dimer to an SDS-resistant tetramer.

In control erythrocytes, the distribution of [3H]-H_2DIDS was 38 ± 6 in to monomeric form, 54 ± 12 in the dimer, and 8 ± 3 in the tetrameric form. Upon volume expansion with hypotonic media, the total amount of [3H]-H_2DIDS bound increased 65-80% and the distribution shifted. 22 ± 4 % was in the monomeric form, 31 ± 10 in the dimeric form, and 47 ± 12 in the tetrameric form (n=3 for all experiments). A similar pattern of distribution was observed when erythrocytes were volume expanded under isoosmotic conditions using the permeant solute ethylene glycol. When cells were incubated with PLP or DNDS under isoosmotic conditions, the amount of binding was not changed, however, 51 ± 16 was in the tetrameric form, 20 ± 7 % in the dimeric form, and 29 ± 11 % in the monomeric form.

In summary, volume expansion of skate erythocytes causes the formation of an allosteric form of the band 3 protein which exists in the tetrameric form. The formation of this conformation of the band 3 protein may be important in the stilbene-sensitive flux of taurine which is stimulated under volume expanded conditions; however, the precise molecular mechanism(s) is unknown.

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