PROTEOLYTIC DIGESTS OF SKATE (Raja erinacea) ERYTHROCYTE BAND 3 DO NOT REVEAL NEW DIDS BINDING SITES UNDER HYPOTONIC STRESS

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Under hypotonic stress, skate (Raja erinacea) erythrocytes bind twice as much dihydroDIDS (H_2DIDS) to a 100kDa protein recognized by an antiserum to the human erythrocyte anion exchanger. Increased H_2DIDS binding can be stimulated by decreasing medium osmolarity or by inclusion of permeant solutes such as ethylene glycol. However the mechanism of increased H_2DIDS binding is not understood.

The present studies addressed whether increased H_2DIDS binding could be attributed to an additional site for binding. DIDS binding has been characterized in the human erythrocyte anion exchanger to occur through covalent linkage to a lysine. The primary structure of the skate anion exchanger is not known. However, by covalent labeling of the protein with [3H]-H_2DIDS and limited proteolysis, additional sites distinct from the site available under isoosmotic conditions may be elucidated.

Skate erythrocytes were isolated, washed, and labeled with [3H]-H_2DIDS in medium of either 940 or 460 milliosmoles/liter. Plasma membranes were isolated and proteins separated on 10% SDS-PAGE. A one cm band corresponding to the region of the 100kDa anion exchanger was cut and laid into the well of a second SDS-PAGE (15%). Varying the amounts of (0.1-10μg) of proteases (trypsin, chymotrypsin, staph V8 protease, pronase, and pepsin) were added and the samples allowed to enter the stacking gel. When the samples were one cm above the junction of the stacking and separating gel, the power was cut off for 30 minutes to allow proteolysis to occur. The samples were then run through the gel, the gel stained to visualize major bands, and the gel lanes cut into slices to determine the location of [3H]-labeled pieces (parts of the anion exchanger to which the [3H]-H_2DIDS had been covalently bound.

Depending on the time and amount of protease present, a differing pattern of peptide fragments was observed. Chymotrypsin (0.1μg) digestion resulted in a radiolabeled peak of 28kDa, whereas 1μg produced a radiolabeled peak of 22kDa. Using 1μg pronase, a labeled peptide at 26kDa was observed, 1μg V8 protease produced a 33kDa fragment, and with trypsin (0.1-10μg) depending on the digestion time, peptides of 34, 23, 14kDa were observed. In all cases, the peptides isolated from the anion exchanger of hypotonically stressed cells (460 mOsm/liter medium), although of identical size, had 55-70% more [3H]-H_2DIDS bound.

In conclusion, no differential pattern could be found for proteolytically-derived peptides from the skate erythrocyte anion exchanger labeled with [3H]-H_2DIDS, although as previously found, increased binding was observed. A new site which was located on a distant part of the protein does not appear to explain the increase in DIDS binding under hypotonic conditions.

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