ACTIVATION OF SYK TYROSINE KINASE IS NOT REQUIRED FOR OLIGMOERIZATION OF LITTLE SKATE (*Raja erinacea*) ERYTHROCYTE BAND 3

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Upon volume expansion, skate erythrocytes, like many other cells, swell and then undergo loss of cell solutes to accomplish a regulatory volume decrease (RVD). Water from the cell must accompany these effluxed solutes, thereby accomplishing the volume decrease. The solutes which are lost are numerous, but include electrolytes (K and Cl), sugar alcohols (sorbitol), and non-metabolized amino acids (taurine and _-alanine). The efflux pathways for these solutes may be numerous and are currently unresolved. Is skate erythrocytes, the anion exchange protein band 3 appears to play an important role in the formation/regulation of the volume activated efflux pathway for taurine. First, pharmacologic inhibition of band 3 with stilbenes inhibits swelling-activated taurine efflux. Secondly, species which lack band 3 (hagfish and lamprey) do not demonstrate a swelling-activated taurine efflux.

In the skate erythrocyte, a number of biochemical events occur upon volume expansion which involve band 3. Notably, band 3, which may normally be present in the membrane as a dimer, is found to a much greater degree in a tetrameric form. Additionally, the interaction of band 4.1 with band 3 decreases greatly upon volume expansion and ankyrin binding to band 3 is of greater affinity. The cytoplasmic N-terminal domain of band 3 also becomes tyrosine phosphorylated to a greater degree, suggesting that tyrosine kinases may play a role in these events. Of note, two tyrosine kinases, syk and lyn, become activated in the skate erythrocyte after volume expansion and the syk inhibitor piceatannol nearly completely blocks the swelling-activated taurine efflux.

The question arises whether syk activation is required for oligomerization of band 3. To address this, cells were pretreated with piceatannol and then volume expanded. Band 3 oligomerization was then determined in these cells using our previously published technique (JBC 269:19683-19686, 1994). Whereas only 10% of the band 3 in the isoosomtic erythrocyte is in the tetrameric form, nearly 40% is found so 5-15 min after volume expansion. This increase in tetrameric form of band 3 was not inhibited by preincubation with piceatannol, strongly suggesting that tyrosine kinase activity is not required for oligomerization. To determine if oligomerization is the signal for induction of tyrosine kinase activation was addressed by incubating cells under isoosmotic conditions with pyridoxal-5-phosphate or DNDS. These agents cause oligmerization of band 3 to a lesser extent than hypotonic exposure (resulting in volume expansion). Both PLP and DNDS were able to activate syk, demonstrating that oligomerization appears to be an important signal in activation of tyrosinekinases and subsequent steps in the activation of the taurine efflux. At present, we do not know those amino acid motifs in skate band 3 which mediate this effect, but as skate band 3 has been recently been cloned, mutagenesis studies and expression in a heterologous system may allow us to address this question.

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