ANION TRANSPORT IN RED CELLS OF THE DOGFISH SHARK, SQUALUS ACANTHIAS

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INTRODUCTION

The transport of CO_2 in the blood of almost all vertebrates is dependent on a functioning Jacobs-Stewart cycle (Jacobs and Stewart, J. Cell. Comp. Physiol. 30: 79-103, 1947) in red cells which allows CO_2 from the tissue capillaries to be moved, predominantly as HCO_3, to the respiratory capillaries where it can then be excreted. This cycle requires three red cell proteins: 1) intracellular carbonic anhydrase to catalyze the hydration of CO_2 within the cells at the level of the tissue capillaries and the dehydration of HCO_3 within the cells at the respiratory capillaries, 2) intracellular hemoglobin to buffer the protons utilized and produced by the hydration-dehydration reactions, and 3) the anion exchange protein, capnophorin, within the cell membrane to mediate the rapid exchange of HCO_3 and Cl.

Recent work has demonstrated that the red cells of elasmobranchs are important in the normal transport and excretion of CO_2 (Swenson and Maren, Am. J. Physiol. 253: R450-R458, 1987). Whereas much research has centered around the activity of carbonic anhydrase in elasmobranch red cells, little attention has been paid to the hemoglobin buffer capacity and capnophorin activity in these cells. Cl/HCO_3 exchange has been examined in the red cells of only one elasmobranch, the smooth dogfish, Mustelus canis (Obaid et al., Am. J. Physiol. 237: R132-R138, 1979). In an effort to further define the properties of capnophorin in elasmobranch red cells, we have characterized anion transport in the red cells of the spiny dogfish, Squalus acanthias, using three experimental approaches: pH regulation in an unbuffered physiological saline, effect of a specific capnophorin inhibitor, DIDS (4,4'-diisothiocyanostilbene-2,2'-disulfonic acid), on pH regulation, and determination of the stoichiometry of Cl/HCO_3 exchange.

MATERIALS AND METHODS

Blood was drawn into a heparinized syringe from the dorsal aorta of the spiny dogfish, Squalus acanthias. The blood was quickly oxygenated, and then either washed and preincubated (see below) immediately or stored in plasma for 1-3 days at 5°C. There was no observable difference in the data obtained from blood which was used immediately after removal and that which was stored in plasma at 5°C, as long as the blood was properly washed and preincubated prior to experimentation.

Before use, the blood was centrifuged at 5000-6000 rpm for 5 min at 4°C and the plasma and buffy coat were carefully removed by aspiration. The packed red cells were then washed four times in 20 volumes of standard isotonic (960 mOsm/kg) wash medium containing 273 mM NaCl, 350 mM urea, 72 mM TMAO, and buffered to a final pH of 7.80 at 4°C with 10 mM K_2HPO_4/KH_2PO_4. The washed packed red cells were then preincubated overnight (approx. 12 hours) at 5% hematocrit and at 12°C in an isotonic preincubation medium. This procedure allowed the red cells to attain a steady state with respect to ions and water after removal of plasma catecholamines. The preincubation medium contained (mM): NaCl, 247; KCl, 6.0; $CaCl_2$, 5.0; $MgCl_2$, 3.0; Na_2SO_4, 0.5; NaH_2PO_4, 1.0;

urea, 350; TMAO, 72; glucose, 5.0; and buffered to a final pH of 7.8 at 12°C with 15 mM Tris-HCl.

The presence of a Cl/HCO_3 exchange system in red cells of S. acanthias was examined by monitoring the ability of a red cell suspension to buffer external pH (pH_o) after the addition of an acid load (0.20 meq/l HCl) to the external medium before and after the application of DIDS at a final concentration of 10^{-4} M. DIDS was added from a stock solution of 5.0 mM and prepared in unbuffered shark saline. After preincubation, red cells were washed three times at 4°C in unbuffered shark saline containing 247 mM NaCl, 6.0 mM KCl, 5.0 mM $CaCl_2$, 2.5 mM $MgSO_4$, 350 mM urea, 72 mM TMAO, 5.0 mM glucose, and 34 mM sucrose (isotonic--960 mOsm/kg). A suspension of red cells in unbuffered shark saline at 8% hematocrit was then allowed to equilibrate pH_o and temperature (12 ±1°C) while insulated on a cooling plate. The suspension was continuously stirred with a small magnetic follower, and pH_o was monitored with a Corning pH/ion meter (Model 150) connected to a dual channel Cole-Parmer chart recorder.

The equilibrium distribution of Cl and HCO_3 was determined experimentally in red cells of S. acanthias using the method of Wieth et al. (Ann. N.Y. Acad. Sci., 341: 394-418, 1980) in which the external [Cl] ($[Cl]_o$) and pH_o of a suspension of red cells in isotonic sucrose medium are monitored as the $[Cl]_o$ is increased incrementally. In short, after preincubation, cells were washed three times in an unbuffered isotonic sucrose medium at 4°C containing 447 mM sucrose, 350 mM urea, 72 mM TMAO, and 0.3 mM KCl. A small portion (5 ml) of this cell suspension was then added to 20 ml of cell-free isotonic sucrose solution to a final hematocrit of 8%. The cell suspension was allowed to equilibrate as above, and pH_o was monitored as $[Cl]_o$ was increased stepwise by addition of isotonic (525 mM) KCl. After each addition of KCl, the red cell suspension was allowed to reach a steady state pH_o and then a 200 µl sample of the cell suspension was used to amperometrically measure $[Cl]_o$ on a Buchler chloridometer.

RESULTS AND DISCUSSION

A suspension of shark red cells in unbuffered saline normally attained a pH_o of 7.4. The addition of a small acid load to this suspension caused an immediate decrease in pH_o of about 0.25 units. After approximately 8 min, the red cells were able to buffer the pH_o to an equilibrium value which was 0.10 units below the initial starting pH_o. When the exchange diffusion of anions was inhibited by the addition of DIDS (10^{-4} M) prior to the administration of another acid load, the suspension of red cells was unable to equilibrate pH_o. The finding of DIDS sensitive pH equilibration in the red cells of an elasmobranch (S. acanthias) confirms the data of Obaid et al. (Op. Cit.) who demonstrated an 80% reduction in the Cl/HCO_3 exchange flux after exposure of red cells of Mustelus canis to 0.11 mM SITS (4-acetamido-4-isothiocyano-stilbene-2,2-disulfonic acid; another capnophorin inhibitor). This finding provides evidence for the presence of a Cl/HCO_3 exchange pathway and a functioning Jacobs-Stewart cycle in the red cells of elasmobranchs.

In most red cells the Jacobs-Stewart cycle is quantitatively the most important pH equilibration pathway (Wieth et al., Op. Cit.). The presence of an electrosilent Cl/HCO_3 exchange protein in elasmobranch red cells will allow an equilibrium distribution of Cl and HCO_3 so that at steady state:

$$\frac{[Cl]_i}{[Cl]_o} = \frac{[HCO_3]_i}{[HCO_3]_o} \quad (1)$$

where subscripts "i" and "o" specify intracellular and extracellular, respectively. Any perturbation of this equilibrium will cause the two anions to exchange rapidly in order to restore equilibrium. Since the movement of Cl and HCO_3 through this exchange process is electrically silent, the equilibrium presented in equation 1 is a chemical steady state. Therefore, it will be relatively insensitive to large changes in the membrane potential (Wieth et al., Op. Cit.). Since OH and H are related to the anion ratios in equation 1 through the reaction of OH with CO_2 to form HCO_3 and the dissociation constant of water ($K' = [OH][H]$) the following equivalencies hold true:

$$\frac{[Cl]_i}{[Cl]_o} = \frac{[HCO_3]_i}{[HCO_3]_o} = \frac{[OH]_i}{[OH]_o} = \frac{[H]_o}{[H]_i} \quad (2)$$

When packed red cells of S. acanthias were added to an isotonic sucrose medium initially containing 0.3 mM KCl, the pH_o decreased to a steady state value which was in accord with equation 2. With each addition of KCl, $[Cl]_o$ and pH_o increased to new steady state values. From equation 2 above it follows that:

$$[Cl]_o\,[H]_o = [Cl]_i\,[H]_i$$

$$\log [Cl]_o = pH_o + \log [Cl]_i - pH_i$$

Since $[Cl]_i$ and pH_i remain relatively constant during this experiment:

$$\log [Cl]_o = pH_o + \text{constant} \quad (3)$$

Therefore, a plot of $\log [Cl]_o$ versus pH_o should theoretically be a line possessing a slope of 1. The experimental plot of steady state values of $\log [Cl]_o$ versus pH_o yielded a line with a slope of 1.14 ±0.10 (mean ±S.D.; r = 0.99 and N = 4). These data are in good agreement with theory, and we can conclude that the Cl/HCO_3 stoichiometry in elasmobranch red cells is 1:1. The addition of DIDS (10^{-4} M) to the suspension prevented the redistribution of Cl and H (as well as OH and HCO_3) after the addition of KCl and caused the slope of the line to greatly increase.

In summary, we have shown that the red cells of Squalus acanthias possess an appreciable DIDS-sensitive buffer capacity which is consistent with the presence of capnophorin in the membrane of these cells. We have also demonstrated that the stoichiometry of anion exchange in shark red cells is 1:1. Therefore, we can conclude that anion exchange in elasmobranch red cells possesses properties similar to those observed in red cells of mammals (Wieth et al., Op. Cit.) and teleosts (Cossins and Richardson, J. exp. Biol., 118: 229-246, 1985). (Supported by NSF PCM-8302621 to DHE.)