ANOXIA AFFECTS THE MEMBRANE CYTOSKELETON OF FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) ERYTHROCYTES

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As for many cells, e.g. muscle, glucose (Glc) transport by flounder red blood cells (RBC) is a slow process, which is enhanced by anoxia, vanadate, and Ca^{2+} ionophore [Booz and Kleinzeller, Bull. MDIBL 29:52, 1990]. The basis for such non-hormonal increases in Glc transport is unknown. In this study, we asked if anoxia also affected the protein composition of flounder RBC plasma membranes (PM), as such an action might offer a clue as to how anoxia enhances Glc transport.

Blood from the caudal vein was centrifuged to remove white cells and platelets, and RBC resuspended to a 5-10 % hematocrit in flounder Ringer's (MOPS buffer pH 7.8) with 1 mM Na-acetate. RBC were made anoxic by incubation (12°C) under N_2 for 2 h with 1 mM KCN. Control cells were incubated under air. RBC were then spun 5 min at 2000 x g, and resuspended to 30-50 % hematocrit. PM were prepared by adapting the Watts and Wheeler method [Biochem. J. 173:899, 1978]. All steps were done on ice or at 4°C. All solutions contained 0.1 mM phenyl-methyl-sulfonyl-flouride (PMSF). While vortexing, a 10-fold volume of buffer A (in mM: NaCl, 3; $MgCl_2$, 2; Tris, 8.5; pH 7.8) was added to RBC suspensions. RBC were spun 7 min at 3100 x g. Resuspension (in buffer A) and centrifugation were repeated until the pellet was hemoglobin-free. The pellet was then washed with low Mg^{2+} (0.2 mM) buffer A and spun 10 min at 3100 x g. The pellet was brought up to 15 ml with low Mg^{2+} buffer A, and homogenized with a drill-driven, tight-fitting pestle (25 passes). The homogenate was sonicated 3 x 10 s with a minicell disruptor, and spun 10 min at 3100 x g. The supernatant was spun 1 h at 100000 x g to yield PM and cytoskeletal elements.

SDS-PAGE electrophoresis was done using 100 μg of the protein dissolved in: 0.17 M Tris/HCl, pH 8.5, 8.7 % SDS, 36 % glycerol, 415 mM β-mercaptoethanol, 0.05 % bromphenol blue. Samples were boiled for 3 min and gradient gels were used to separate the proteins. Gels were stained with coomassie blue, and analysed by densitometry. Fig. 1 shows a representative gel from normoxic and anoxic cells. Comparison of banding patterns showed that anoxia caused a 58.7 $\pm$ 4.0 % (mean $\pm$ se, N = 4, $p < 0.01$) reduction in the relative amounts of band 2 protein (Table 1).

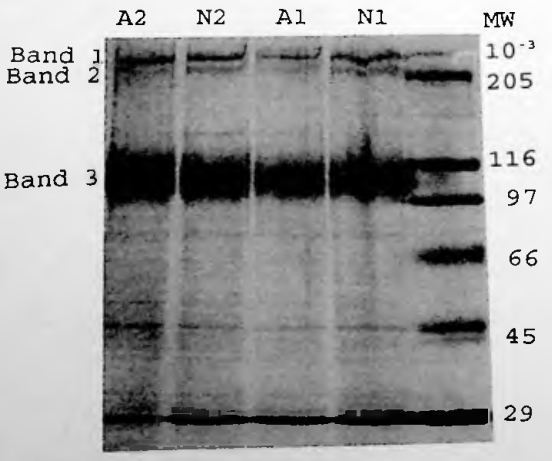

Fig. 1. SDS-PAGE of flounder red cell PM and cytoskeletal elements. N = normoxia and A = anoxia.

The molecular weight of this protein (239 kDa) and relative gel position are consistent with β-spectrin; attempts are underway to confirm this by Western blot analysis. In contrast, anoxia had no effect on other bands, such as band 1 and band 3 (Table 1).

β-spectrin is thought to play a role in maintaining membrane organization and restricting movement of PM proteins of RBC [Bennet, Physiol. Rev. 70:1029, 1990]. Our results suggest that a reduction in β-spectrin under anoxic conditions, with a lessening of the restriction on the mobility of Glc transporters, might explain anoxia-enhanced Glc uptake by flounder RBC. Such a possibility is consistent with the model of Cheung et al. [Biochim. Biophys. Acta 470:212, 1977] to explain anoxia-enhanced Glc transport by avian RBC. From kinetic studies, Cheung et al. postulated that anoxia increased the mobility of Glc transporters by causing loss of bias for its binding site to face in towards the cytosol.

Table 1. High molecular weight proteins of flounder RBC membranes

Band	MW(kDa)	Relative Area		Likely Identity
		Normoxia	Anoxia	
1	255-258	3.1±0.2	3.3±0.7	α-spectrin
2	238-238	1.7±0.2	0.7±0.2*	β-spectrin
3	100-119	24.9±3.4	25.4±2.0	anion exchanger

Mean + SE (4) * $p < 0.005$ vs normoxic control (paired t-test)

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