

polymerization occurs. The difference in the peak tube elution volumes for normal hemoglobin in Figure 1C relative to A and B is that different columns were poured, calibrated, and used for C and D.

Polymerization can be reversed by the addition of B-ME. In Figure 1D there is a partial but significant shift from the 160,000 molecular weight polymer to the normal 60,000 molecular weight species.

The results obtained with CuCl_2 , including polymerization, inhibition with IAA, and reversal with B-ME, were indistinguishable from those obtained when $\text{K}_3\text{Fe}(\text{CN})_6$ was used as the polymerizing agent.

Preliminary structural studies with urea gel electrophoresis indicate that pout hemoglobin is a tetramer consisting of two pairs of globin subunits.

In conclusion, pout hemoglobin has a molecular weight of about 60,000, approximately six sulfhydryl groups per tetramer, as measured by PCMB titration, and consists of two dissimilar

subunits. It is polymerized, by $\text{K}_3\text{Fe}(\text{CN})_6$ or CuCl_2 , to polymers corresponding to about two to three tetramers. Polymerization is inhibited by IAA and reversed by B-ME, suggesting that polymerization may be due to the oxidation of cysteine sulfhydryl groups and the formation of intermolecular disulfide bonds.

This work has been supported by PSC-CUNY Grants #664308 and 665168 and NIGMS-NIH MARC Program Grant #5T34GM-08182.

Literature Cited

1. Reischl, E. 1976. *Comp. Biochem. Physiol.* **55B**: 255-257.
2. Borgese, T. A., et al. 1988. *Comp. Biochem. Physiol.* **91B**: 663-670.
3. Leibovitz, L., et al. 1986. *New England Chap. American Fisheries Soc. Univ. Mass., Amherst* (Abstract).
4. Blackwell, R. Q., et al. 1971. *BBA* **243**: 467-474.
5. Adams, J. G., et al. 1987. *Hemoglobin* **11**: 435-437.
6. Tondo, C., et al. 1963. *Am. J. Genetics* **15**: 265-279.

Reference: *Biol. Bull.* **187**: 247-248. (October, 1994)

Catalase Activity in Dogfish (*Mustelus canis*) Ocular Tissues

Seymour Zigman (University of Rochester School of Medicine, Rochester, NY) and Nancy S. Rafferty

Lens epithelium contains catalase, whose enzymatic activity is strongly inhibited by UV_A exposure (1, 2). This enzyme protects the lens from H_2O_2 -related oxidative damage. A decrease in lens catalase activity would diminish its protective function. This would increase the lens' oxidative stress. The purpose of this work was to elucidate systems that protect the eye from oxidative stress.

The breakdown of H_2O_2 was measured with an O_2 electrode (Microelectrodes, Inc., MI-730), an oxygen meter (O M-7), and an XY recorder. The system was preset at 21% oxygen with H_2O . Beef liver catalase (Sigma) was the quantitative standard. Fresh ocular tissues or 50 μl of the 15,000 $\times g$ supernates of homogenates were added to Ringer's medium containing 0.88 mM H_2O_2 . The increase in O_2 with time was recorded. The same procedure was followed with 15,000 $\times g$ supernatants of lens capsule epithelium that were exposed to radiation from a Woods lamp or sunlight through a Pyrex beaker. UV-A was the predominant radiant energy reaching the cells (*i.e.*, UV_A). The protection of catalase activity against UV damage was also tested by presoaking and irradiating samples in Ringer's medium containing 10 $\mu\text{g}/\text{ml}$ of α -tocopherol or β -carotene.

Retina and iris had the most catalase activity; cornea epithelium and lens epithelium had 54-72% of the activity of these pigmented tissues. Aqueous humor had only trace activity (see Table IA). UV_A exposure (75 J/cm^2) reduced the catalase activity of whole lens to about 50% of dark controls (Table IB). Beta-carotene (10 $\mu\text{g}/\text{ml}$) was ineffective in protecting whole lens catalase activity, but α -tocopherol (10 $\mu\text{g}/\text{ml}$) was protective. Lens capsule epithelium 15,000 $\times g$ supernates were also exposed to UV_A (25 J/cm^2); some samples contained total lens-soluble protein (TSP) or purified α -crystallin (which protects proteins against

Table I

A. Catalase activities in dogfish ocular tissues		
Tissue	Whole tissue % O_2 per minute	15K g supernatant per OD ₂₈₀
Retina	2.52 \pm 16%*	2.78*
Iris	2.48 \pm 20%	3.15
Cornea epithelium	1.36 \pm 14%	2.23
Lens capsule epithelium	1.78 \pm 12%	2.01
Aqueous humor	0.02 \pm 50%	0.03
B. Effects on lens capsule epithelium catalase activity of Woods lamp UV		
Whole tissue	% of control	
Lens dark	100 \pm 18%*	
Lens UV-exposed	51 \pm 20%	
UV-exposed plus β -carotene (10 $\mu\text{g}/\text{ml}$)	51 \pm 22%	
UV-exposed plus α -tocopherol (10 $\mu\text{g}/\text{ml}$)	84 \pm 15%	
C. Effects on lens capsule epithelium catalase activity of sunlight		
15K g supernatants	% of control	
Dark	100*	
UV-exposed	44	
UV plus lens-soluble proteins	31	
UV plus alpha crystallin	96	

* Average of two experiments \pm range; *One experiment in duplicate.
Note: Supernatant O.D.₂₈₀ = 0.750; alpha-crystallin O.D.₂₈₀ = 1.0.

denaturation). Addition of TSP did not protect catalase activity, whereas Biogel A -50M purified α -crystallin did protect it (Table IC).

The catalase activity of dogfish ocular tissues, especially the iris and retina, appears to detoxify ocular H_2O_2 efficiently. The epithelia of cornea and lens are less efficient in breaking down H_2O_2 . It appears that α -tocopherol protects lens catalase activity from UV_A radiation, whereas β -carotene does not. In addition, α -crystallin protected lens catalase activity from UV_A -induced inhibition.

Support: National Eye Institute Grant EY 00459 (S.Z.); EY 00698 (N.S.R.) and Research to Prevent Blindness, Inc. Thanks to Bunnie R. Zigman for preparation of the manuscript.

Literature Cited

1. Zigman, S., and N. S. Rafferty. 1993. *Biol. Bull.* 185: 328.
2. Zigman, S., and N. S. Rafferty. 1994. *Comp. Biochem. Physiol.* (in press).

Reference: *Biol. Bull.* 187: 248–249. (October, 1994)

N^G -Monomethyl-L-Arginine Inhibits *Arbacia* Fertilization and Differentiation Diane E. Heck, Jeffrey D. Laskin (UMDNJ—Robert Wood Johnson Medical School), Seymour Zigman, and Walter Troll

The amino acid L-arginine is crucial to a number of metabolic processes including not only protein synthesis, but also the urea cycle and polyamine metabolism (1). Recent studies also demonstrate that L-arginine metabolites are important mediators of cellular signal transduction (2, 3). Using eggs from the sea urchin *Arbacia punctulata*, we investigated the effects of a structural analog of L-arginine, N^G -monomethyl-L-arginine (NMMA), on the fertilization process. NMMA inhibits crucial metabolic processes involving arginine metabolism that are independent of protein synthesis, in particular, the activity of the L-arginine-dependent enzyme, NADPH diaphorase (4).

We have found that following fertilization, sea urchin eggs contain NADPH diaphorase activity, as measured by oxidation of nitroblue tetrazolium (2 mM), which was inhibited by NMMA. A characteristic of the fertilization process by sea urchin eggs is an oxidative burst. This is associated with the production of high levels of reactive oxygen intermediates, in particular, hydrogen peroxide. Current studies indicate that hydrogen peroxide is required for fertilization (5). At low concentrations of L-arginine, NADPH diaphorase generates superoxide anion as well as hydrogen peroxide. Using fluorescence image analysis in conjunction with the hydroperoxy-sensitive probe 2',7'-dichlorofluorescein diacetate (DCFH-DA), we analyzed hydrogen peroxide production induced during the fertilization of sea urchin eggs (6). Eggs were immobilized on poly-L-lysine treated cell culture plates and incubated in seawater containing DCFH-DA (5 μM) in the presence and absence of amino acids. After 30 min the plates were washed three times in seawater, inoculated with sperm, and fluorescence intensity quantified with a Meridian ACAS 570 anchored cell analysis system equipped with a 5-watt argon laser; the excitation wavelength was set at 488 nm and emission monitored at 525 nm. During fertilization, untreated eggs readily produced hydrogen peroxide (Fig. 1, panel

A). We found that when eggs are fertilized, there is an approximately 10-fold increase in hydrogen peroxide over 10 min. Eggs fertilized in the presence of 1 mM L-arginine (Fig. 1, panel B) also produced significant amounts of hydrogen peroxide during the fertilization process. But, in the presence of NMMA (3 mM), hydrogen peroxide production was markedly inhibited (90–95%), an effect that could be overcome by the presence of 1 mM L-arginine (Fig. 1, panels C and D, respectively). Both eggs and, in particular, sperm contained catalase, which may also regulate hydrogen peroxide production during development.

In further studies we have found that NMMA (0.1–3 mM) inhibits the fertilization and development process, as measured by the percentage of fertilized eggs undergoing cell division 1 h after fertilization, and the formation of rotating blastulas after 20 h. These effects were reversible by equimolar concentrations of L-arginine. Taken together, these data indicate that L-arginine is required for fertilization. In further studies it will be important to determine the precise site of action of NMMA in the fertilization process.

Research support was provided in part by NIH grants ES 03647 and ES 05022.

Literature Cited

1. Stryer, L. 1975. *Biochemistry*. W. H. Freeman and Co., San Francisco. Pp. 503–527.
2. Rengasamy, A., and R. A. Johns. 1994. *Biochem. Pharmacol.* 48: 423–425.
3. Nathan, C., and Q. W. Xie. 1994. *J. Biol. Chem.* 269: 13725–13728.
4. Brecht, B. S., and S. H. Snyder. 1990. *PNAS* 87: 682–685.
5. Shapiro, B. M. 1991. *Science* 252: 533–536.
6. Heck, D. E., D. L. Laskin, C. R. Gardner, and J. D. Laskin. 1992. *J. Biol. Chem.* 267: 21277–21280.