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Evolution of an Oxygen-Binding Hemoprotein in a Unique Environment: Myoglobin in the Hemoglobinless Antarctic Icefishes

Bruce D. Sidell

Principal Investigator; University of Maine, Orono

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Final Report for Period: 02/1996 - 01/2001**Submitted on:** 03/15/2001**Principal Investigator:** Sidell, Bruce D.**Award ID:** 9421657**Organization:** University of Maine

Evolution of an Oxygen-Binding Hemoprotein in a Unique Environment: Myoglobin in the Hemoglobinless Antarctic Icefishes

Project Participants**Senior Personnel****Name:** Sidell, Bruce**Worked for more than 160 Hours:** Yes**Contribution to Project:****Name:** Vayda, Michael**Worked for more than 160 Hours:** Yes**Contribution to Project:****Post-doc****Name:** Eppley, Zoe**Worked for more than 160 Hours:** Yes**Contribution to Project:****Graduate Student****Name:** Grove, Theresa**Worked for more than 160 Hours:** Yes**Contribution to Project:****Name:** O'Brien, Kristin**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Kristin received institutional support in the form of an Assistantship for part of last year; the balance was provided from this award. There is no option of indicating this type of funding split on the next screen.

Name: Small, Deena**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Deena was supported by an institutional fellowship during the past year; this award provided support for necessary materials and supplies for her experiments.

Name: Riemenschneider, William**Worked for more than 160 Hours:** Yes**Contribution to Project:****Undergraduate Student****Organizational Partners****Florida State University**

Dr. T.S. Moerland is employed by Florida State University and this institution released him from other duties to participate in field work during one season.

Stanford University, Hopkins Marine Station

Dr. Peter Fields was employed by Stanford University's Hopkins Marine Station during a period where he was released from regular duties to participate in field work with our project. Dr. George N. Somero of the same institution has also been collaborating with the P.I.'s laboratory on work associated with our Antarctic research.

University of Lecce, Italy

Dr. R. Acierno was employed by the University of Lecce, Italy and was released from regular duties to participate in field work with our project at Palmer Station.

University of Otago, New Zealand

Dr. Marshall participated as a field team member during our project's 1999 field season in Antarctica.

Other Collaborators or Contacts

With Graduate Student, Kristin O'Brien, we have initiated a collaboration with Dr. Stuart Egginton of the University of Birmingham (U.K.) for ultrastructural studies of oxidative skeletal muscles of Antarctic icefish and their red-blooded notothenioid relatives. Dr. Egginton has been developing complimentary information through his project with the British Antarctic Survey.

Activities and Findings**Project Activities and Findings:**

Activities in our project are a combination of field work carried out at Palmer Station, Antarctica, aboard the research vessels supporting the station (presently the ARSV L.M. Gould) and analytical work with samples at our U.S. laboratory at the University of Maine. Animal collection in the field is conducted by deploying trawls from the research vessel. Fishes captured by trawl are transferred to aquaria aboard the vessel and, in turn, transported back to aquarium facilities at Palmer Station for experiments conducted on site with fresh material and for preparation of frozen or fixed samples to return to our University of Maine laboratory.

The major objectives of our project were to:

1. Assess the functional significance at physiological temperatures (i.e. approaching 0C) of myoglobin in Antarctic notothenioid fishes.
2. Examine the pattern of expression of myoglobin protein and mRNA expression by species of the channichthyid icefishes within an evolutionary context.
3. Define the types of mutational lesions accounting for loss of myoglobin expression.

To accomplish the above objectives, we have collected Antarctic icefishes in the field, conducted experiments on-site at Palmer Station that require fresh tissue, including isolated, perfused heart experiments, protein purifications, activity measurements of enzymes of energy metabolism that are labile to freezing. With material frozen or fixed in the field, upon return to our U.S. laboratory, we have conducted further enzyme activity determinations, done extensive electron microscopy (including quantitative estimation of ultrastructures), performed stopped-flow measurements of oxygen binding kinetics with purified myoglobins, cloned and sequenced myoglobin genes from several Antarctic fish species and done molecular biological experiments aimed at identifying the specific genetic lesions that have led to loss of myoglobin production in some icefish species.

Graduate students and Postdoctoral Associates from the University of Maine have been members of our field team during each deployment to Antarctica. In addition, components of our funded project formed the bases for the graduate research theses of 4 graduate students (2 M.S., 2 Ph.D.) who have worked under the supervision of the P.I. Work supported by this grant to date has resulted in at least 18 papers already published in refereed scientific journals, 2 others submitted and under review and 2 book chapters.

Our work on the molecular biology of myoglobin expression/lack of expression among the icefishes has yielded several important advances and provocative observations.

1. For 13 species of icefishes examined to date, 8 species produce myoglobin in their heart ventricle, 5 do not synthesize this protein in heart ventricle.
2. From isolated, perfused heart studies and oxygen-binding kinetic determinations, we have established that myoglobin does function in Antarctic fishes and, when present, enhances the mechanical performance of heart muscle. The oxygen-binding kinetic studies have further shown that myoglobin protein in Antarctic fishes possesses a more loose and conformationally flexible structure than that of mammals.
3. Based upon the consensus phylogeny of the icefish family, ability to produce myoglobin has been lost through at least 4 independent events during the evolution of this group.

4. Based upon our molecular biological findings, at least 3 completely discrete mechanisms have been involved in loss of myoglobin expression. Each of these specific mechanisms has been identified.
5. Electron microscopic studies have shown that significant (and presumably adaptive) differences in fine structure of heart muscle are found when comparing tissues from myoglobin-expressors and myoglobin-non-expressors. In animals lacking myoglobin, some of these differences appear to be aimed at minimizing diffusional distances for delivery of blood-borne oxygen to cellular sites of its utilization (mitochondria) and at maximizing surface areas through which this diffusion occurs.

One of the most interesting recent findings is identification of the putative mechanism accounting for lack of myoglobin expression in *Chaenocephalus aceratus*, a species where the myoglobin gene appears to be largely intact (see Small et al., 1998). During the 1999 field season, we tested our hypothesis that lack of expression is due to a 15 nucleotide insertion upstream of the core promoter region of this gene that duplicates one of the binding sites for an essential transcription factor. We injected nucleotide constructs lacking this insertion into tissues of live *C. aceratus* on-site at Palmer Station and subsequently prepared tissue samples for analysis in our CONUS laboratory to see whether any myoglobin gene product can be detected. Our results indicate that constructs containing the 15 nucleotide insertion produced no signal, while those lacking this insertion produced a positive signal, indicating gene transcription. These results are very strong evidence in support of our hypothesis that this insertion in *C. aceratus* is responsible for lack of myoglobin production in this species. Our findings from these experiments presently are being written up for submission to a scientific journal.

Project Training and Development:

Please see reprints of published scientific papers and monographs forwarded to Polar Biology and Medicine

Research Training:

Theresa J. Grove received her M.S. degree in Zoology during 1998 and was admitted into the Ph.D. program in Marine Biology. She continues her work on NSF-supported experiments in this latter program.

Deena J. Small received her Ph.D. in Biochemistry and Molecular Biology in 1998. Experimental work for this thesis was supported by our award. Small was co-advised by Sidell (P.I.) and M.E. Vayda (Faculty Associate).

Kristin M. O'Brien received her Ph.D. in Zoology in 1999. Dr. O'Brien's work covered studies on heart and skeletal muscle ultrastructure of icefishes that were components of our project.

Thomas J. Moylan received his M.S. in Zoology in 1997. Mr. Moylan's work quantified expression of myoglobin protein and mRNA in tissues of Antarctic icefishes, part of the scope of work of this project.

Dr. Zoe A. Eppley was supported as a Postdoctoral Research Associate during part of the award period. In September of 1997, Dr. Eppley left the University of Maine to accept a position of Program Director in the Integrative Biology and Neurosciences section of NSF in Washington.

Outreach Activities:

1. Presentation to the 4th grade class at Alton, ME Elementary School of a Career Series talk and slide show about the nature of a career in marine biology; this highlighted our Antarctic research.
2. Responded to several inquiries from the popular press about our USAP supported research. At least one of these appeared online at the Science Daily Web site. Another resulted in a feature article in a new nature-oriented magazine in Austria.
3. During the 1997 field season, the P.I. (Sidell) agreed to provide digital images via satellite from Antarctica to an educational Web site developed by the Gulf of Maine Aquarium that is aimed at K-12 audiences. Combined with other material, the GOM Aquarium staff developed an instructional program for primary school students that was centered around this contribution. See: <http://www.octopus.gma.org/onlocation/Antarctica/>
4. In October of 1999, I contributed an invited talk on the Physiology and Biochemistry of Antarctic Fishes to a symposium at the annual meeting of the American Society of Gravitational and Space Biology, held in Seattle, WA. Although this group represents a scientific audience, their expertise (space biology) is rather disparate from my own discipline and I consider this talk to be an outreach activity aimed at non-polar scientists.

Journal Publications

- Sidell, B.D., "Intracellular oxygen diffusion: The roles of myoglobin and lipid at cold body temperature.", *Journal of Experimental Biology*, p. 1118, vol. 201, (1998).) Published
- Small, D.J., M.E. Vayda and B.D. Sidell, "A novel vertebrate myoglobin gene containing three A+T-rich introns is conserved among Antarctic teleost species which differ in myoglobin expression.", *Journal of Molecular Evolution*, p. 156, vol. 47, (1998).) Published
- Vayda, M.E., R.L. Londraville, R.E. Cashon, L. Costello and B.D. Sidell, "Two distinct types of fatty acid-binding protein are expressed in heart ventricle of Antarctic teleost fishes.", *Biochemical Journal*, p. 375, vol. 330, (1998).) Published
- Dowse, H.B., S.F. Norton and B.D. Sidell, "The estimation of the diffusion constant and solubility of O₂ in tissue using kinetics.", *Journal of Theoretical Biology*, p. 531, vol. 207, (2000).) Published
- Sidell, B.D., M.E. Vayda, D.J. Small, R.L. Londraville, M.-L. Yuan, K.J. Rodnick, Z.A. Eppley and L. Costello, "Variation in the expression of myoglobin among species of the Antarctic icefishes.", *Proceedings of the National Academy of Sciences (U.S.A.)*, p. 3420, vol. 94, (1997).) Published
- Acerno, R., C. Agnisola, B. Tota and B.D. Sidell, "Myoglobin enhances cardiac performance in the Antarctic icefish species that express the protein.", *American Journal of Physiology*, p. R100, vol. 273, (1997).) Published
- Vayda, M.E., D.J. Small, M.-L. Yuan and B.D. Sidell, "Conservation of the myoglobin gene among notothenioid fishes.", *Molecular Marine Biology and Biotechnology*, p. 206, vol. 6, (1997).) Published
- Cashon, R.E., M.E. Vayda and B.D. Sidell, "Kinetic characterization of myoglobins from vertebrates with vastly different body temperatures.", *Comparative Biochemistry and Physiology*, p. 613, vol. 117B, (1997).) Published
- Londraville, R.L. and B.D. Sidell, "Purification and characterization of fatty acid-binding protein from aerobic muscle of Antarctic icefish, *Chaenocephalus aceratus*.", *Journal of Experimental Zoology*, p. 190, vol. 273, (1995).) Published
- Londraville, R.L., J. Storch and B.D. Sidell, "Binding site polarity and ligand affinity of homologous fatty acid-binding proteins from animals with different body temperatures.", *Molecular and Cellular Biochemistry*, p. 39, vol. 159, (1996).) Published
- Londraville, R.L., "Intracellular fatty acid-binding proteins: Putting lower vertebrates in perspective.", *Brazilian Journal of Medical and Biological Research*, p. 707, vol. 29, (1996).) Published
- Sidell, B.D., E.L. Crockett and W.R. Driedzic, "Antarctic fish tissues preferentially catabolize monoenoic fatty acids.", *Journal of Experimental Zoology*, p. 73, vol. 271, (1995).) Published
- Fields, P.A. and G.N. Somero, "Hot spots in cold adaptation: Localized increases in conformational flexibility in lactate dehydrogenase A4 orthologs of Antarctic notothenioid fishes.", *Proceedings of the National Academy of Sciences, U.S.A.*, p. 11476, vol. 95, (1998).) Published
- Moylan, T.J. and B.D. Sidell, "Concentrations of myoglobin and myoglobin mRNA in heart ventricles from Antarctic fishes.", *Journal of Experimental Biology*, p. 1277, vol. 203, (2000).) Published
- O'Brien, K.M. and B.D. Sidell, "The interplay among cardiac ultrastructure, metabolism and the expression of oxygen-binding proteins in Antarctic fishes.", *Journal of Experimental Biology*, p. 1287, vol. 203, (2000).) Published
- Sidell, B.D., "Life at body temperatures below 0C: The physiology and Biochemistry of Antarctic fishes.", *Gravitational and Space Biology Bulletin*, p. 25, vol. 13, (2000).) Published
- O'Brien, K.M., H. Xue and B.D. Sidell, "Quantification of diffusion distance within the spongy myocardium of hearts from antarctic fishes.", *Respiration Physiology*, p. 71, vol. 122, (2000).) Published
- West, J.L., J.R. Bailey, V.M.F. Almeida-Val, A.L. Val, B.D. Sidell and W.R. Driedzic, "Activity levels of enzymes of energy metabolism in heart and red muscle are higher in north-temperate-zone than in Amazonian teleosts.", *Canadian Journal of Zoology*, p. 690, vol. 77, (1999).) Published
- Kawall, H.E., J.J. Torres, B.D. Sidell and G.N. Somero, "Metabolic cold adaptation in Antarctic fishes: Evidence from enzymatic activities of brain.", *Marine Biology*, p. , vol. , () Submitted
- Dunlap, W.C., A. Fujisawa, Y. Yamamoto, T. Moylan and B.D. Sidell, "The vitamin E content of notothenioid fish, krill and phytoplankton from Antarctica includes a vitamin E constituent (a-tocomonenol) functionally associated with coldwater adaptation.", *Antarctic Science*, p. , vol. , () Submitted

Books or Other One-time Publications

- Sidell, B.D. and M.E. Vayda, "Physiological and evolutionary aspects of myoglobin expression in the haemoglobinless Antarctic icefishes", (1998). *Book*, Published
 Editor(s): Portner, H.O. and R.C. Playle
 Collection: Cold Ocean Physiology
 Bibliography: Cambridge University Press, Cambridge
- Small, Deena J., "Gene expression and molecular evolution among the Antarctic Channichthyid icefishes.", (1998). *Thesis*, Deposited in the Graduate School
 Bibliography: Ph.D. Thesis, University of Maine
- Vayda, M.E., D.J. Small and B.D. Sidell, "Expression of the myoglobin gene in Antarctic channichthyid fishes.", (1997). *Book*, Published

Editor(s): Battaglia, J., J. Valencia and D.W.H. Walton
 Collection: Antarctic Communities: Species, Structure and Survival.
 Bibliography: Cambridge University Press, Cambridge

O'Brien, K.M., "The influence of oxygen-binding protein expression on muscle structure and metabolism in Antarctic fishes.", (1999). *Thesis*, Successfully defended and deposited in the Graduate School.
 Bibliography: Ph.D. Thesis, University of Maine

Grove, T.J., "Myoglobin deficiency in the hearts of four fish species found in the Gulf of Maine.", (1998). *Thesis*, Successfully defended and deposited in the Graduate School
 Bibliography: M.S. Thesis, University of Maine

Moylan, T.J., "Quantification of myoglobin and myoglobin mRNA in heart ventricle of Antarctic fishes.", (). *Thesis*, Successfully defended and deposited in the Graduate School.
 Bibliography: M.S. Thesis, University of Maine

Web/Internet Sites

URL(s):

<http://www.ume.maine.edu/~marine/sidell.htm>
<http://ww.octopus.gma.org/onlocation/Antarctica/>
<http://www.seagrant.umaine.edu/Education/resource.htm#discover>

Description:

<http://www.ume.maine.edu/~marine/sidell.htm> -- This site is the P.I.'s home page and provides a description of our NSF-supported research activities, citations of sample publications and a link to a C.V. for the P.I. and a comprehensive bibliography of the P.I.'s work.

<http://www.octopus.gma.org/onlocation/Antarctica/> -- This site is a K-12 educational site that was integrated with reports from the P.I. from the field in Antarctica to develop an educational program for primary school students.

<http://www.seagrant.umaine.edu/Education/resource.htm#discover> -- This site (that will become active shortly) is also aimed at K-12 audiences and will be a component of a larger educational effort being developed by the University of Maine's Sea Grant office.

Other Specific Products

Contributions

Contributions within Discipline:

Within the fields of adaptational biochemistry and physiology, our findings have identified changes in the structure of myoglobin protein that lead to improved functional performance in binding and release of oxygen at cold body temperature. These findings contribute to our fundamental understanding of how this important oxygen-binding protein works and have supported the paradigm that evolutionary adaptation to cold temperature results in selection for protein structures that maintain greater conformational flexibility at cold temperature.

We also have been able to exploit the unique matrix of +/- expression of both circulating hemoglobin and intracellular myoglobin within the Notothenioid suborder of Antarctic fishes to examine how loss of either or both of these normally important oxygen-binding proteins affects both gross and fine structure of aerobic muscle tissues. We have found very significant differences among these species in densities of mitochondria and in intrinsic mitochondrial morphologies. These differences appear to be related to the presence or absence of oxygen-binding proteins. The overall findings have contributed to our understanding of the pathway for oxygen movement in animal systems.

Finally, our molecular biological work has established that multiple events and mechanisms have been responsible for losses in ability to express myoglobin that have occurred during the evolution of the Antarctic icefish family. These results have significant implications with respect to our understanding of evolutionary processes affecting fishes in the Southern Ocean surrounding Antarctica (see next section).

Contributions to Other Disciplines:

Our findings have significant implications to understanding the unique evolutionary history of species in the Southern Ocean surrounding Antarctica and, thus, provide further insight into how evolution by natural selection works to shape characteristics of modern animals.

Our findings based upon both oxygen-binding kinetics and perfused heart work clearly indicate that, when present, myoglobin is functional

and enhances the mechanical performance of hearts. Current population genetics theory would suggest that any event leading to the loss of expression of such a functional protein would disadvantageous and result in negative selection pressure against animals possessing this mutation and, their ultimate elimination from the population. Despite this prediction, we are confronted with our observations of at least 5 different icefish species that have lost the ability to produce myoglobin in their hearts (by several different mechanisms). In other words, an apparently disadvantageous trait has persisted through evolutionary time.

We have speculated that the most likely explanation for the apparent conundrum described above is a combination of two factors: First, the loss of myoglobin was probably not lethal at the level of individual animals because absolute oxygen demand by Antarctic fishes is not great due to their very cold body temperature and generally low levels of activity and because the Southern Ocean has a very high oxygen content that is well-mixed throughout the water column. Second, the very diverse and cosmopolitan diversity of fish species that existed around the continent ca. 45 MYA went through a dramatic crash in species diversity that occurred sometime between the mid-Tertiary and present. This dramatic drop in species diversity left the ancestral lineages of present day notothenioids isolated in a vast ocean with probably very little niche competition that would lead to selection against non-lethal deleterious traits. Thus, the unique characteristics of the Southern Ocean combine with the unique evolutionary history of the notothenioid suborder to explain the outcome of this unusual natural experiment.

Contributions to Human Resource Development:

The following University of Maine graduate students have had their graduate thesis research supported wholly or partially by this award:

Grove, Theresa -- currently a Ph.D. student in the P.I.'s laboratory and a previous participant in 2 Antarctic field seasons.

O'Brien, Kristin -- awarded the Ph.D. degree in Zoology and a participant in 2 Antarctic field seasons; currently a Postdoctoral Fellow at the University of Colorado, Boulder.

Moylan, Thomas -- awarded the M.S. degree in Zoology and a participant in 3 Antarctic field seasons; currently a Research Assistant at the University of California, Santa Barbara.

Small, Deena -- awarded the Ph.D. degree in Biochemistry and a participant in 2 Antarctic field seasons; presently a Postdoctoral Research Associate at Maine Medical Center Research Institute.

During the award period, my laboratory also has employed at least 6 different undergraduate student workers who have assisted us in routine laboratory procedures.

For approximately 26 months of the award period, Dr. Zoe A. Eppley occupied a position of Postdoctoral Research Associate in the P.I.'s laboratory and was supported by this award. Dr. Eppley left the University of Maine in late August 1998 to accept a position as Program Director at the National Science Foundation.

During the award period, approximately 6 additional graduate students supervised by either the P.I. (Sidell) or named Faculty Associate (Vayda) benefitted directly or indirectly by our OPP-sponsored project. In some cases, equipment purchased by this award was available for use in their projects that were funded from other sources; in all cases, they derived benefit from the enhanced intellectual atmosphere of our laboratories because of our OPP-funded project.

Finally, activities from our project were the bases for at least 2 WWW sites aimed at outreach to K-12 students and the public at large (see previous sections) and also formed the basis for talks given to local public schools. All were designed to educate these students/constituents about USAP science and its role in the larger scientific enterprise.

Contributions to Science and Technology Infrastructure:

Please see other sections above.

Beyond Science and Engineering:

Categories for which nothing is reported:

Any Product

Contributions: Beyond Science or Engineering