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# Proteins of Oxygen-Binding and Energy Metabolism in Muscles of Antarctic Fishes: Evolutionary Adjustments to Life at Cold Temperature

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**Final Report for Period:** 04/2000 - 03/2003

**Submitted on:** 03/20/2003

**Principal Investigator:** Sidell, Bruce D.

**Award ID:** 9909055

**Organization:** University of Maine

**Title:**

Proteins of Oxygen-Binding and Energy Metabolism in Muscles of Antarctic Fishes: Evolutionary Adjustments to Life at Cold Temperature

**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:**

Named Senior Faculty Associate. Dr. Vayda has actively collaborated on work within the scope of the award. Dr. Vayda received one month of summer salary annually from the award.

**Post-doc**

**Name:** Metcalf, Victoria

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

Victoria Metcalf participated in our project as a Postdoctoral Research Assistant from May 2001 until September 2001.

**Graduate Student**

**Name:** Grove, Theresa

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

This award supported a Graduate Research Assistantship for Theresa Grove and also funded the cost of materials and supplies for her Ph.D. research which was within the scope of the award. Dr. Grove successfully defended her Ph.D. thesis entitled 'Characterization of fatty acyl CoA synthetase in notothenioid fishes: Examining substrate specificity and structure' in July 2002.

**Name:** Magnoni, Leonardo

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

This award provided partial support of materials and supplies for Leonardo Magnoni's M.S. thesis research. Mr. Magnoni successfully defended his M.S. thesis entitled 'Antarctic notothenioid fishes do not display metabolic cold adaption in hepatic gluconeogenesis' in August 2002.

**Name:** Winnard, Paul

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

Paul Winnard was a graduate student working under the supervision of Dr. Michael E. Vayda (named senior faculty associate in this award) at the University of Maine. This award supported the materials and supplies for Dr. Winnard's Ph.D. thesis project, which was within the scope of our funded research. Dr. Winnard successfully defended his Ph.D. Thesis entitled 'Cold temperature adaptation of muscle creatine kinase from an Antarctic teleost (*Chaenocephalus aceratus*)' in October 2001.

**Name:** Hendrickson, Jamie

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

Ms. Hendrickson joined our laboratory in September 2001 and her Ph.D. thesis research has been partially supported by this award.

**Undergraduate Student****Technician, Programmer****Other Participant****Research Experience for Undergraduates****Organizational Partners****Other Collaborators or Contacts**

We are completing collaboration with Dr. Stuart Egginton, Department of Physiology, University of Birmingham, England on a manuscript describing ultrastructure of oxidative skeletal muscles in Antarctic fishes. This work was published:

O'Brien, K.M., C. Skilbeck, B.D. Sidell and S. Egginton (2003). Muscle fine structure may maintain the function of oxidative fibres in haemoglobinless Antarctic fishes. *J. exp. Biol.* 206: 411-421.

**Activities and Findings****Research and Education Activities:**

Our award focused on three primary objectives:

1. Isolation and characterization of fatty acyl CoA synthase from oxidative muscles of channichthyid icefishes, coupled with the cloning and sequencing of the gene for this protein.
2. Analysis of the expression of myoglobin gene promotor constructs that were injected into oxidative muscle of non-myoglobin expressing species of icefishes.
3. Kinetic characteristization of creatine kinases from glycolytic muscles of Antarctic fishes and the cloning and sequencing of the gene(s) for this enzyme.

**Findings:**

Results of our studies are summarized below and in the published work that acknowledges support from this award (see appropriate section of report). Enumeration refers to each of the objectives mentioned above.

1. Graduate student, Theresa Grove, successfully cloned and sequenced the full length cDNA for fatty acyl CoA synthase (FACS) from oxidative skeletal muscle of three species of Antarctic fishes, *Chaenocephalus aceratus*, *Notothenia coriiceps* and *Gobionotothen gibberifrons*. Race PCR technique was employed for this work. Predicted proteins are 698 amino acids in length, with 66-67% identity to mouse and human. Part of the fatty acid binding pocket of the protein is encoded by a 25-amino acid consensus sequence or 'signature motif', which is common to all FACS. This region from three notothenoids has 76% (*N. coriiceps*) to 80% (*G. gibberifrons* and *C. aceratus*) identity to the consensus sequence. In all three species examined, the non-polar Ile(21) of the consensus sequence is changed to a polar Thr(552) residue. This amino acid difference may significantly impact preference for long chain unsaturated fatty acids. Additional amino acid substitutions in this binding motif may also play a role in determining the enzyme's specificity for both chain length and degree of saturation of its fatty acyl substrates.

We modified successfully a spectrophotometric assay for activity of FACS so that it is suitable for use with relatively crude preparations of the enzyme. This assay was used to monitor the chromatographic separation of the enzyme during purification steps. Partially purified enzyme was subject to kinetic analyses to determine substrate specificities, kinetic constants and structural characteristics of the enzyme that have been shaped by evolution of the species at chronically and severely cold body temperature. Results from this work are presently in the final stages of analysis and we anticipate producing a manuscript for journal submission within the next 3 months.

2. These experiments were conducted in collaboration with named faculty associate, Dr. Michael Vayda. We were extremely fortunate that preliminary experiments with promotor constructs from the myoglobin genes of both myoglobin-expressing (Mb+; *Chionodraco rastrospinosus*) and myoglobin-nonexpressing (Mb-; *Chaenocephalus aceratus*) yielded definitive information about the nature of the specific lesion that has led to lack of Mb production in *C. aceratus*. These injections of promotor constructs attached to a reporter gene (luciferase) were done during our 1999 field season with the original aim of determining suitable experimental conditions.

Results from these experiments have shown definitively that a 15-nucleotide insertion that occurs well upstream of the normal transcription start site of the gene in *C. aceratus* is responsible for lack of Mb expression in this species. Results from these experiments were published in *Journal of Experimental Biology* in early 2003 (See Small et al., 2003 in listing of published work.)

3. Graduate student Paul Winnard successfully cloned and sequenced the full length cDNA for 3 different creatine kinase enzymes that are expressed in glycolytic muscle of the icefish, *Chaenocephalus aceratus*. Based upon analyses of sequence comparisons, we definitively identified one of these forms as the mitochondrial form of the enzyme and identified the other two forms as both being muscle-type isoforms. This finding is highly unusual and, to our knowledge, the only report of two different muscle-type forms of creatine kinase being expressed in skeletal muscle of any vertebrate animal. Our sequence analyses further indicated that these two muscle-type forms are not allelic variants but represent the products of two discrete genes.

Partial purification and preliminary kinetic analyses of the muscle type enzyme was performed. We established that the pH optimum for this enzyme from Antarctic icefishes is substantially more alkaline than that displayed by homologous forms of the enzyme from warmer bodied vertebrate animals. This is consistent with the considerably more alkaline physiological pH of body fluids in Antarctic fishes than those observed in warmer-bodied species. Results of these experiments indicate that muscle-specific forms of creatine kinase from *C. aceratus* display significant catalytic activity at cold physiological temperature and at the intracellular pH characteristic of icefish muscle. These characteristics enable the enzyme to contribute to sustaining energy charge of glycolytic skeletal muscle of Antarctic species during burst swimming activity. Results of these experiments have been published in *Comparative Biochemistry and Physiology* in early 2003 (See Winnard et al, 2003 in listing of published work.)

#### **Training and Development:**

Graduate thesis research of students, Theresa Grove, Paul Winnard and Leonardo Magnoni has been supported by this award. Each successfully defended their graduate thesis research, leading to award of the Ph.D. degree to Dr.s Winnard and Grove in October 2001 and August 2002, respectively and the M.S. degree to Mr. Magnoni in August of 2002.

#### **Outreach Activities:**

The P.I. has provided information to the Educational officer at the University of Maine's Sea Grant office for incorporation into an educational Web site on marine research that is aimed at an audience of K-12 students. This information was used to generate a specific set of pages that highlight our USAP-sponsored research.

#### **Journal Publications**

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

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-tococomonoenol) functionally associated with coldwater adaptation.", *Comparative Biochemistry and Physiology*, p. 299, vol. 133B, (2002). Published

Kewall, 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. 279, vol. 140, (2002). Published

Small, D.J., M.E. Vayda and B.D. Sidell, "The myoglobin gene of the Antarctic icefish, *Chaenocephalus aceratus*, contains a duplicated TATAAAA sequence that interferes with transcription.", *Journal of Experimental Biology*, p. 131, vol. 206, (2003). 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. C. Skilbeck, B.D. Sidell and S. Egginton, "Muscle fine structure may maintain the function of oxidative fibres in haemoglobinless Antarctic fishes.", *Journal of Experimental Biology*, p. 411, vol. 206, (2003). Published

Eastman, J.T. and B.D. Sidell, "Measurements of buoyancy for some Antarctic notothenioid fishes from the South Shetland Islands.", *Polar Biology*, p. 753, vol. 25, (2002). Published

Grove, T.J. and B.D. Sidell, "Myoglobin deficiency in the hearts of phylogenetically diverse temperate-zone fish species.", *Canadian Journal of Zoology*, p. 893, vol. 80, (2002). Published

Hazel, J.R. and B.D. Sidell, "Triacylglycerol lipase activities in tissues of Antarctic fishes.", *Polar Biology*, p. 517, vol. 25, (2002). Published

Sidell, B.D., "Life at body temperatures below 0 C: The physiology and biochemistry of Antarctic fishes.", *Gravitational and Space Biology Bulletin*, p. 25, vol. 13, (2000). Published

Winnard, P., R.E. Cashon, B.D. Sidell and M.E. Vayda, "Isolation, characterization and nucleotide sequence of the muscle isoforms of creatine kinase from the Antarctic teleost, *Chaenocephalus aceratus*.", *Comparative Biochemistry and Physiology*, p. 651, vol. 134B, (2003). Published

### Books or Other One-time Publications

#### Web/Internet Site

**URL(s):**

<http://www.ume.maine.edu/~marine/sidell.htm>

[http://kodiak.asap.um.maine.edu/sms/faculty/faculty\\_indiv.php?faculty\\_id=39](http://kodiak.asap.um.maine.edu/sms/faculty/faculty_indiv.php?faculty_id=39)

**Description:**

These sites are the P.I.'s home pages and provide a description of our NSF-supported research activities, citations of sample publications and a link to a C.V. for the P.I. containing a comprehensive bibliography of the P.I.'s work.

### Other Specific Products

#### Contributions

**Contributions within Discipline:**

We 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 species in the densities of mitochondria, intrinsic mitochondrial morphologies and tissue-level architectures. These differences are strongly correlated with 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.

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 evolution of the Antarctic icefish family. Most recently we have identified an unique specific mutation in the region of the myoglobin gene, well upstream of the core promoter region, that is responsible for silencing expression of this gene in *Chaenocephalus aceratus*. These results have significant implications with respect to our understanding of evolutionary processes affecting fishes in the Southern Ocean surrounding Antarctica.

Results of our experiments with creatine kinase have revealed that Antarctic fishes express two forms of the enzyme in skeletal muscle, unlike the single form expression that characterizes all other vertebrate animals. In addition, this work showed that CK from Antarctic fishes has been evolutionarily modified to function under physiological conditions of very cold body temperature and intracellular pH that is more alkaline than that of warmer bodied animals.

**Contributions to Other Disciplines:**

Our recent work, combined with results from previous awards, have led us to hypothesize that the most likely explanation for multiple losses of myoglobin expression during the evolution of the Antarctic icefish family is dependent upon 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 mixture 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. This view is equally consistent with

understanding why species lacking either myoglobin in their tissues or hemoglobin in their circulating blood have persisted, despite the obvious negative physiological impacts of these mutations.

Ancillary work supported by our project has revealed:

1. That the nucleotide base compositions of non-coding sections of genes in fishes (including Antarctic fishes), i.e. introns, are biased toward high A+T content when compared to warmer bodied non-teleosts. This observation is consistent with the view that high A+T content of teleost genes may be more reflective of the ancestral condition and that the progression toward greater G+C content in introns of endothermic homeotherms may have been driven by pressure to stabilize DNA structure at warmer body temperature. (See Winnard et al., 2002).
2. The existence of an unusual vitamin E derivative in tissues of Antarctic fishes and phytoplankton, which may function as an antioxidant, protecting against peroxidation of membrane lipids, exclusively at cold body temperature. (See Dunlap et al. 2002).
3. That brain tissue of Antarctic fishes displays enzymatic hallmarks of metabolic cold adaptation, a longstanding and continuing controversy in polar biology. (See Kawall et al., 2002).
4. That Antarctic fish species display a range of buoyant masses compared to seawater but that the pattern observed in 13 different species suggest that neutral buoyancy is rare in notothenioid fishes and may be confined to a single nototheniid clade. (See Eastman and Sidell, 2002).

#### **Contributions to Human Resource Development:**

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

Grove, Theresa J. -- Participant in the 2001 Antarctic field season. Dr. Grove successfully defended her Ph.D. thesis, 'Characterization of fatty acyl CoA synthetase in notothenioid fishes: Examining substrate specificity and structure' in August 2002.

Magnoni, Leonardo -- Participant in the 2001 Antarctic field season. Mr. Magnoni successfully defended his M.S. thesis, 'Antarctic notothenioid fishes do not display metabolic cold adaptation in hepatic gluconeogenesis' in August 2002.

Winnard, Paul -- Ph.D. student in the laboratory of named Faculty Associate, Michael Vayda. Dr. Winnard successfully defended his Ph.D. thesis, 'Cold-temperature adaptation of muscle creatine kinase from an Antarctic teleost (*Chaenocephalus aceratus*)' in October 2001.

Hendrickson, Jamie -- Ms. Hendrickson joined our laboratory in September 2001 and is currently conducting thesis research toward the Ph.D. in marine biology here at the University of Maine.

During the award period, my laboratory has also employed an undergraduate student worker to assist us in routine laboratory procedures.

Postdoctoral Research Associate, Dr. Victoria Metcalf, was active in our laboratory during 6 months of 2001.

#### **Contributions to Resources for Research and Education:**

The P.I. has given informal talks to local public schools that have been based upon our Antarctic research.

A local public school student has interviewed the P.I. as part of a 'career planning exercise'. The results of the interview will be communicated to the student's 8th grade class in the form of a report from the student on careers in marine biology.

The P.I. has presented several invited research seminars at other Universities and also presented several invited papers at national and international meetings, all of which highlight the results of our NSF-sponsored research and clearly acknowledge NSF support.

#### **Contributions Beyond Science and Engineering:**

##### **Categories for which nothing is reported:**

Organizational Partners

Any Book

Any Product

Contributions: To Any Beyond Science and Engineering