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Studies on the rapid self assembly of elastic tensile fibers from a natural protein polymer found in marine snails

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Abstract

1. Whelk egg case precursor proteins are stockpiled in the nidamental gland of a gravid female whelk and assembled post-fertilization into an impervious protective coating for the egg. 2. Egg case maturation is rapid and involves extensive chemical crosslinking. 3. Egg case material is quasi-elastic and completely reversible in tension, but relies primarily on nonentropic forces. 4. Fibers with the mechanical properties of whelk egg case can be electrospun from protein precursors.

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Final Project Progress Report

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Studies on the rapid self assembly of elastic tensile fibers from a natural protein polymer found in marine snails

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Project Hypotheses
1. Whelk egg case precursor proteins are stockpiled in the nidamental gland of a gravid female whelk and assembled post-fertilization into an impervious protective coating for the egg.
2. Egg case maturation is rapid and involves extensive chemical crosslinking.
3. Egg case material is quasi-elastic and completely reversible in tension, but relies primarily on nonentropic forces.
4. Fibers with the mechanical properties of whelk egg case can be electrospun from protein precursors.

Project Goals and Objectives
a) Characterize the biochemical and biomechanical properties of whelk egg case,
b) study mechanisms and microarchitecture of egg case assembly,
c) explore structure-function relationships between the macromolecular structure, elasticity and self-healing in the egg case proteins.
d) Spin fibers from egg case proteins by artificial means.

Briefly describe project methodology
Protein Biochemistry - Egg case protein precursors were isolated from nidamental glands as well as immature egg cases. Most proteins were purified by C8 HPLC and sequenced at the N-terminus by Edman chemistry. Those not sufficiently resolved were partially sequenced from transferred bands derived from SDS polyacrylamide gels. MALDI TOF mass spectrometry was used to estimate protein molecular weights. Most egg case precursors weighed from 45-55 kiloDaltons. Egg case cross-links were reduced in situ with borohydride and released by acid hydrolysis. The acid was flash evaporated and the nonvolatile residue reconstituted and separated by C18 high performance liquid chromatography. Molecular biology: RNA was extracted from the nidamental glands derived from live gravid females. Complete sequence for each protein was deduced from complete cDNAs prepared from the RNA using RT-PCR with degenerate oligonucleotides designed from unique Edman sequences followed by 3'- and 5'- RACE with nondegenerate oligonucleotides. Biophysics: Egg case protein structure was investigated by circular dichroism, FTIR, and by both small and wide angle

X-ray scattering of hydrated and dry material. Mechanics: Strips of egg case material were tested wet and dry in uniaxial tension with an MTS tensiometer. For thermomechanical testing the strips were immersed in a temperature regulated bath adjustable from 0 to 100 íC. Spinning of egg precursor fibers: Fragments of capsule polymer were subjected to mechanical tests with a micro-force device on a microscope stage that allowed simultaneous measurement of optical properties (birefringence). This allowed us to track changes in structural organization with the degree and pattern of extension. Protein was isolated from the nidamental gland of female whelks, dissolved in formic acid and spun into micron sized fibers using a Kato-Tech Electrospinning appartus.

Describe progress and accomplishments toward meeting goals and objectives
1. Protein and molecular biology progress: Five proteins were isolated from the nidamental gland and partially sequenced; mean protein mass was 50kD. Protein presence in egg capsule was confirmed. Using oligonucleotide primers based on reliable protein sequence, four complete cDNA sequences have been completed and a fifth is on the way. ExPASy sequence tools reveal the presence of extensive coiled coil trimers alternating with amorphous regions in the deduced proteins. Cross-link analysis of reduced mature egg case material reveals the presence of large condensed cross-links. Fragmentation of these reveals Biophysics progress. Circular dichroism confirms coiled coil and amorphous structure. Small and wide angle x-ray scattering of both wet and dry egg case material confirms abundant alpha and amorphous structure when unstressed. The alpha structure is lost at 15% strain; the amorphous reigns at 15-60 %; beta structure prevails at above 40% engineering strain. Egg case has orthotropic structure, that is, a 90í cross-ply construction. Each layer consists of fibers with 50 nm axial periodicity.

Mechanics progress. Hydrated mature whelk egg case exhibits extension to 100%; yield occurs at ~10% engineering strain. There is spontaneous recovery of initial length and stiffness, but stiffness and hysteresis decrease with increasing strain. Extensive thermomechanical analysis has shown for the first time that force decreases with increasing T, which is the inverse of entropic elasticity. The quasi elastic behavior of whelk egg case resembles models of hard keratin if a first order alpha to beta transition is assumed. Reversible extension can be calculated at an enthalpy of 1 kcal/mol res assuming an average protein mass of 50 kDa. We have determined that samples of the capsules exhibit high birefringence at rest, indicating the presence of a highly ordered sub-structure, consistent with the model of alpha-helical protein chains. Furthermore, this birefringence increases linearly with extension until the yield point whereupon it falls linearly to below the initial level during the yield plateau. This is consistent with the alpha helices unwinding with strain. Finally, all structural changes revealed by birefringence are recovered when the samples are unloaded.

Electrospinning attempts have been successful and we can now produce uniform fibers of approximately 1 micrometer diameter. We are developing a technique to crosslink and mount these fibers on the micro-force tester in order to compare mechanical properties with native material.

Project modifications
Extensive modifications to our plans for molecular biology, biochemistry, processing biology were necessitated by the utter invalidity of the protein and cDNA sequences provided by a previous graduate student, Scott Rapoport at SIO-UCSD. To date, we have had no explanation for this. Extensive basic research had

to be completely renavigated by Wasko and others to provide quality data for downstream analyses. Probably over 2 years were lost in redoing critical experiments.

Project outcomes
Primarily databases associated with the cDNA and amino acid sequences of precursor proteins. Sequences were submitted to GenBank and, upon publication of research papers, will be available to all by way of NCBI BLAST and SwissProt searches.

Impacts of project
Impact predictions are premature because the most significant research results will not be published until after the termination of this grant. We suspect that egg case material will increasingly be regarded as a uniquely novel paradigm for elastomers. We have made substantial progress in understanding the molecular structure that gives rise to the recoverable yield of this polymer. Shadwick's results are consistent with the mechanics and X-ray diffraction studies by the Waite lab.

Benefits, commercialization and application of project results
The benefit is that several companies have expressed interest in our research by inviting seminar presentations. These have included Ashland Chemical, Hughes Research (HRI), and 3M. The downside is that we have advanced the research to the point where they don't need to partner with us. It's cheaper and easier for them to carry on entirely in-house. This may seem disappointing at a first approximation, however, if these companies are actually serious about a greener way to make tougher bio-inspired materials, we all profit in the end.

Economic benefits generated by discovery
none

Issue-based forecast capabilities
none

Tools, technologies and information services developed
none

Publications
Conference papers, proceedings, symposia
Title: Recoverable-yield in a proteinaceous elastomer from marine whelks
Authors: Scott Wasko, J. Herbert Waite
Date: April '07
Conference Title: First International Conference on Self-Healing Materials
Location: Noordwijk, Netherlands

Title: Recoverable-yield in an extensible, proteinaceous material found in gastropod egg capsules
Authors: Scott Wasko, J. Herbert Waite
Date: March '08
Conference Title: Materials Research Society Spring Meeting '08
Location: San Francisco

Title: Characterization of a self-healing, biological, encapsulating material
Authors: Scott Wasko, A. Miserez, J. Herbert Waite
Date: September '09 (abstract submitted and approved)

Conference Title: 22nd European Conference on Biomaterials
Location: Lausanne, Switzerland

Didier, W., Shadwick, R.E. and Gosline, J.M. ╥Biomechanical characterization of whelk egg capsules╨.

Title: Electrospinning; a new technique for materials design.
Authors: Corbett, C.M. and Shadwick, R.E.
Date: 2007
Conference Title: Canadian Society of Zoologists annual meeting

Title: Characterization of a self-healing, biological, encapsulating material
Authors: Scott Wasko, A. Miserez, J. Herbert Waite
Date: September '09 (abstract submitted and approved)
Conference Title: 22nd European Conference on Biomaterials
Location: Lausanne, Switzerland

Title: Electrospinning; a new technique for materials design.
Authors: Corbett, C.M. and Shadwick, R.E.
Date: May, 2007
Conference Title: Canadian Society of Zoologists annual meeting
Location: Montreal

Title: Biomechanical characterization of whelk egg capsules
Authors: Didier, W., Shadwick, R.E. and Gosline, J.M.
Date: 2007
Conference Title: Canadian Society of Zoologists annual meeting
Location: Montreal.

Peer-reviewed journal articles or book chapters
Title: Reversibly labile, sclerotization-induced elastic properties in a keratin analog from marine snails: whelk egg capsule biopolymer (WECB).
Authors: Rapoport, H.S. and Shadwick, R.E.
Date: 2007
Journal Name: Journal of Experimental Biology
Issue/Page Numbers: 210,12 -26.

Title: Synchrotron based dynamic structural and mechanical analysis of whelk egg case (tentative)
Authors: Wasko, S.S., Gupta, H., Fratzl, P and Waite, J.H.
Date: 2009
Journal Name: not known yet
Issue/Page Numbers: none

Title: Large Reversible Deformation of an Encapsulating Bioelastomer Correlated with Secondary Structural Transitions
Authors: Miserez, A., Wasko, S.S., Carpenter, C., and Waite, J.H.
Date: 2009
Journal Name: Submitted
Issue/Page Numbers: none yet

Title: Novel elastomeric coiled coil proteins from whelk egg case
Authors: Wasko, S.S., and Waite, J.H.
Date: 2009
Journal Name: In preparation

Issue/Page Numbers: none yet
Title: Mechanisms of sclerotization
Authors: Rubin, D.J., Miserez, A., and Waite, J.H.
Date: 2009
Journal Name: Advances in Insect Physiology (invited)
Issue/Page Numbers: none yet.

Workshops and presentations
http://jeb.biologists.org/cgi/content/full/210/1/i
Date of publication/broadcast: 2007
Headline or topic: ╥Inside JEB╙ WHELK WONDERMATERIAL by Laura Blackburn

Students
Scott S. Wasko
UC Santa Barbara
BioMolecular Science & Engineering
Degree program enrolled in: PhD in Biochemistry
Theses/dissertation title: Biochemistry and Biomechanics of Whelk Egg Case
Supported by Sea Grant funds? [x] yes [] no
Start date 03/01/2006
End date 06/30/2007

Todd Schneberk
UC Santa Barbara
MCDB
Degree program enrolled in: Master's in molecular biology
Theses/dissertation title: none
Supported by Sea Grant funds? [x] yes [] no
Start date: 07/01/2007
End date: 04/30/2008

Daniel J. Rubin
UC Santa Barbara
MCDB
Degree program enrolled in: Master's in molecular biology
Theses/dissertation title: Cross-linking in Marine Scleroproteins
Supported by Sea Grant funds? [X] yes [] no
Start date: 04/30/2008
End date: 08/31/2008

How many student volunteers were involved in the project? 3

Cooperating organizations
International organizations
Max Planck Institute of Colloids and Interfaces, Golm, Germany; technical assistance (synchroton based analysis of whelk egg case)

Academic Institutions
Marine Biological Laboratory, Woods Hole, technical assistance (gravid whelk collection, lab space for RNA extraction)

International implications
Co-PI is Robert Shadwick, a Professor in the Zoology Department at UBC (2006)

Awards

Robert Shadwick was appointed Canada Research Professor at UBC (2006)
Scott Wasko won a UC GREAT (Graduate Research and Education in Adaptive bio-Technology Training) Fellowship from the UC Biotechnology Research & Education Program (2007)
H. Waite was elected as a Fellow to the American Academy for the Advancement of Sciences (2009)
H. Waite received the Award for Excellence in Adhesion Science sponsored by 3M & Adhesion Society (2009)

Keywords

whelk egg capsule, Busycon canaliculus, nidamental gland, precursor protein, alpha to beta transition, nonentropic elasticity, self-healing, electrospinning