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Cloning and Structure of Different Types of Spider Silk

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Randolph V. Lewis

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Amino acid sequence from several spider silk proteins have been determined. These include: Nephila dragline (GYGPG, GQGAG, GAGQG, GYGGLG) and cocoon (GAFQ) and Araneus dragline (GPYGPGQQGP) and cocoon (FLGG, SVGLV-[I/L]A-Y-A-L). Over 18 positive clones have been identified from a Nephila silk gland library using an 18 mer probe based on the dragline protein sequence. These have been plague purified and sequencing has started. Libraries for the other silks are being constructed. Using FTIR (with microscope focus) changes in the IR region have been detected when the silk is stretched. Efforts are now underway to determine what structural features these changes correspond to.

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ANNUAL AND FINAL REPORT ON CONTRACT N00014-87-K-0079

PRINCIPAL INVESTIGATOR: DR. RANDOLPH V. LEWIS

CONTRACTOR: UNIVERSITY OF WYOMING

CONTRACT TITLE: CLONING AND STRUCTURE OF DIFFERENT TYPES OF SPIDER SILK

DATES: 1 DECEMBER 1986- NOVEMBER 30, 1968

RESEARCH OBJECTIVE: To sequence the protein(s) which compose spider silk and compare them with different types of silk. Then to express the proteins and determine their functional characteristics and structures.

PROGRESS (YEAR 2): In the past year we have sequenced several more peptide fragments from different spider silks. These include: Nephila cocoon(SAFQ), Araneus dragline(GPYGPGQQGP) and cocoon(FLGG and SVGLV[I,L]AYAL). We were unable to get fragments of more than 3 residues for swathing silk but the amino acid composition indicates it is completely different than any silk known. It has very high Ser and Ala with the Gly only about 10% which could explain the rapid and frequent fragmentation pattern.

In the cloning arena, we have isolated a number of positive clones from our Nephila major ampullate silk gland cDNA library using a probe based on the protein sequences we have. Due to presently unknown difficulties in sequencing the clones we could not identify our probe sequence in any of the clones. We therefore fragmented the clones with two restrictions enzymes to obtain fragments small enough to sequence and which contained our probe sequence. Two clones were sequenced in this fashion and both contained the correct sequence in the open reading frame and, in addition, contained other protein sequences we have obtained from this silk. These data indicate that it is extremely likely that the clones we have do encode for the dragline silk protein.

We are currently beginning to sequence the largest of these clones which is about 2 kb. In order to assess the size of the protein and the mRNA, Northern blotting is being done using our two clones as the probes. If the mRNA is larger than about 5kb we will turn to a genomic library to sequence the complete protein. The Nephila genomic library is currently

being made.

We are also constructing libraries from the whole abdomen of Nephila which will then include all of the different silk gland proteins since the other glands are too small to effectively dissect out and make libraries from. With Araneus we have constructed a major ampullate gland library and are in the process of screening it. We will construct an abdomen library for the other silk proteins and a genomic library in this species as well.

In a somewhat unusual turn, our studies of the silk using FTIR which we initially thought were nonproductive, have generated data that may play an important role in determining the structural changes of the silk proteins. Very clear changes have been observed in the dragline silk when it is stretched(see attached figure). So far we have not been able to interpret what the changes are structurally but are working in that direction. They do appear to be involved with the formation of a more regular structure when the silk stretches. The new peaks are indicative of helix like formations but aren't sufficient in and of themselves. We hope to establish a collaboration that will allow us to study these silk proteins using X-ray diffraction in order to further characterize these structural changes.

WORK PLAN (YEAR 3): We have received a renewal of this contract. In the next year we will be continuing our cloning and sequencing of the different silks. This will enable us to compare the silks at the protein level and begin to identify structurally important features. A major effort will be directed toward the factors responsible for the elasticity of the fibers. We will also begin, depending on the clones we have, to express the proteins in order to more easily study them. The FTIR studies will be expanded to other silks and intensified toward characterizing the changes observed when the silk fibers are stretched.

INVENTIONS: None.

PUBLICATIONS:

1. No publications have been submitted yet but two are being prepared.
2. Abstracts,posters, and talks were presented at the Protein Society Meetings, Rocky Mountain Biochemistry Meetings, Cornell, CNRS(France), and UW.

TRAINING ACTIVITIES: Two graduate students, one part-time graduate student, and two undergraduates are working on this project. Ming Xu should finish his PhD this spring and Zhengyu Dong should complete is MS this spring as well.

Women or minorities- 3
Non-citizens - 2 (China)

AWARDS: I was selected as the first University of Wyoming President's Lecturer.

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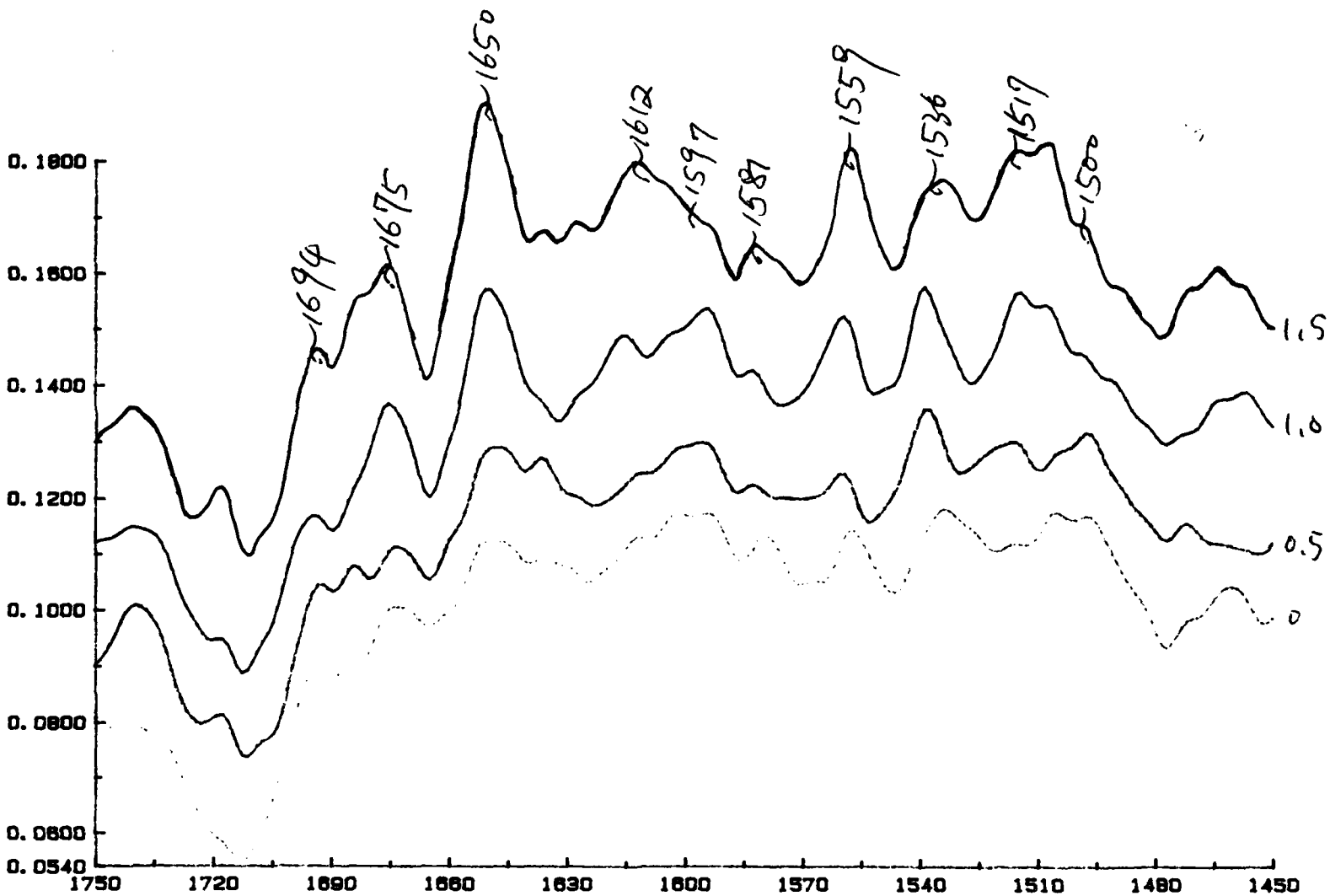

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