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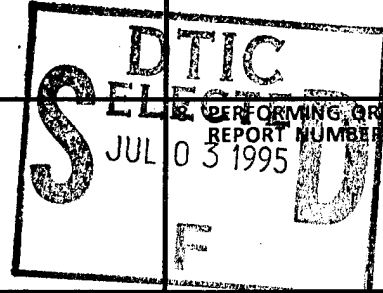
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13. ABSTRACT (Maximum 200 words)

Protein-protein and intraprotein interactions were studied in natural, recombinant and synthetic domains of proteins that form aquatic silk. Subsets of natural proteins were found to associate into discrete high molecular mass complexes thought to be the non-covalent precursors to insoluble silk. A recombinant protein mimicking a single "core repeat" from a 1000-kDa silk protein reversibly formed two intramolecular disulfide bonds; however, more than one conformation existed. A synthetic peptide encompassing all four cysteines also formed intramolecular disulfide bonds. The results obtained suggested new steps in the fiber formation pathway including properties of the recombinant protein that may render it useful as a degradable biomolecular material.

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FINAL REPORT

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The paucity and heterogeneity of natural silk proteins renders them unsuitable reagents for in-depth structural studies. To overcome this limitation, we synthesized peptides and recombinant proteins as model substrates for detailed biophysical and biochemical studies. Since spIs form the fibrous backbone of silk, we chose to study their core repeats. The "constant regions" of these repeats has four Cys residues that in synthetic peptides⁶ and recombinant proteins^{3,8,10} reversibly form two disulfide bonds. The major focus of this work was rCAS, a recombinant Constant And Subrepeat (rCAS) protein modeled after a core repeat from spIa³.

Characterization of rCAS included polyacrylamide gel electrophoresis, N-terminal amino acid sequencing, quantitative amino acid composition, quantitative sulfhydryl assays, matrix-assisted laser desorption/time-of-flight and electrospray ionization mass spectrometry and mapping disulfide-containing tryptic peptides¹⁰. As purified, there are three rCAS conformers that differ in their Cys-pairing. Denatured and reduced rCAS was subjected to refolding at pH 8 and 10 with O₂- and DTT^{ox}-mediated formation of disulfide bonds. The kinetics of refolding was monitored at different times by HPLC. While the rates of the reactions varied, in all instances, reduced rCAS passed through one disulfide-containing intermediates on the way to reforming two disulfide bonds; however, heterogeneity of Cys pairs persisted suggesting that rCAS may have more than one stable structure. The occasional appearance of rCAS dimers and trimers prompted us to investigate aggregation in solution by analytical equilibrium ultracentrifugation. At protein concentrations ranging from 0.25-1.0 mg/ml in 100 mM Na⁺, rCAS is a monomer¹⁰.

Using *in vitro* mutagenesis, a set of 20 genes was made that encompass all single, double, triple and quadruple Cys→Ala substitutions. Most also include a Phe→Trp substitution that provides an intrinsic fluorochrome at one specified site. We subcloned 19/20 mutant genes into expression vector pET3a and obtained inducible synthesis of all recombinant proteins. Selected variants are being purified and subjected to biochemical verification of their encoded amino acid substitutions by amino acid composition, sulfhydryl counting gels, mass spectrometry, and UV absorption and fluorescence spectroscopies. Preliminary data are exciting. For example, one Cys→Ala substitution alters reformation of disulfide bonds⁸ and the quantum yield of fluorescence for QUAD (Cys_{9,21,23,79}→Ala) is similar to free Trp whereas F16W exhibits a decreased yield that can be increased by denaturation. This suggests that while the sole Trp in F16W is buried, in QUAD, which lacks Cys (hence no disulfide bonds), it is exposed to the solvent. These results have set the stage for a detailed structural and thermodynamic comparison of these proteins.

D. Related Proteins

Several collaborations have enabled us to examine other Cys-containing proteins and gain experience with biophysical methods. For example, the homologue of *C. tentans* sp185 (motif Cys-X-Cys-X-Cys every 22-26 residues) has been identified in two other species of *Chironomus*². Cys-rich chorion (egg-shell) shell proteins in two Lepidopteran have been examined¹¹. Native, synthetic and recombinant proteins have been examined by a variety of spectroscopic methods. Most notably we have acquired the capacity to conduct FTIR of proteins

in solution; this offers advantages over the typical acquisition of spectra in KBr pellets. A spectral library of known proteins has been assembled to provide basis spectra from which quantitative estimates of the percentages of secondary structures can be made for unknown proteins.

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11. PARTICIPATING SCIENTIFIC PERSONNEL

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