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Prime #:  Contract entity: GTRC
Subprojects #: N  
Main project #:  

Project unit: CHEMISTRY  Unit code: 02.010.136  
Project director(s): BRIGGS M S  CHEMISTRY  (404)894-4001

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
Sponsor/division codes: 108 / 001

Award period: 930101 to 941231 (performance) 950331 (reports)

Sponsor amount New this change Total to date
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Funded 0.00 96,279.00  
Cost sharing amount 0.00  

Does subcontracting plan apply #: N

Title: PEPTIDE MODELS OF PROTEIN STRUCTURE & FOLDING

PROJECT ADMINISTRATION DATA

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Security class (U,C,S,TS) : U  
Defense priority rating : N/A  
Equipment title vests with: Sponsor
ONR resident rep. is ACO (Y/N): N  
NIH supplemental sheet GIT X

Administrative comments - 
REVISED NOTICE OF GRANT AWARD, DATED 3/3/95 ESTABLISHED END DATE OF GRANT AS 12/31/95 AND DELETES SUPPORT RECOMMENDATION FOR YEARS -04 AND -05.
NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 06/21/95

Project No. G-33-E26 Center No. 10/24-6-R7103-3A0

Project Director BRIGGS M S School/Lab CHEMISTRY

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH

Contract/Grant No. 5 R29 GM45616-03 Contract Entity GTRC

Effective Completion Date 941231 (Performance) 950331 (Reports)

Closeout Actions Required: Y/N Submitted

Final Invoice or Copy of Final Invoice Y 950517
Final Report of Inventions and/or Subcontracts Y 950522
Government Property Inventory & Related Certificate N
Classified Material Certificate N
Release and Assignment N
Other

Comments

Subproject Under Main Project No. 

Continues Project No. G-33-661

Distribution Required:

Y Y Y Y Y Y Y N Y Y N N

Project Director
Administrative Network Representative
GTRI Accounting/Grants and Contracts
Procurement/Supply Services
Research Property Management
Research Security Services
Reports Coordinator (OCA)
GTRC
Project File
Other

NOTE: Final Patent Questionnaire sent to PDPI.
1. Specific Aims of the Award

The long term goals of the FIRST award were the better understanding of the forces and interactions that underlie protein structure and folding. I proposed to use small synthetic peptides whose sequences incorporate those of omega loops as models for these studies. The omega loop is a recently described element of protein structure found in nearly all proteins examined. Although omega loops are short, they exhibit many characteristics of larger polypeptides. As omega loops are small, they are amenable to chemical synthesis, and can be studied in solution at atomic resolution using NMR. The combination of protein-like characteristics and small size makes omega loops a convenient system with which to study questions of protein structure and folding that are not easily approached by the study of entire proteins.

In particular, I planned to carry out experiments designed to answer the following questions:

Do peptides incorporating omega loop sequences take on loop-like structure in the absence of the rest of the protein?

What is the stability of the loop conformation? Are there different classes of loops that have different stabilities?

Are stability differences related to the functions of the loops in proteins?

Does the loop adopt one conformation or an ensemble of related conformations? How does the loop's conformation(s) in solution compare to the loop's structure in the protein context?

Does loop formation require certain specific hydrophobic or hydrogen-bonding interactions? Are there mutant sequences that strengthen or weaken specific loop-promoting interactions and/or narrow or expand the ensemble of preferred conformations?

Is loop formation context-dependent? I.e. Does stable loop formation require the presence of additional amino acids from the native protein sequence?

Can a loop sequence be driven into a helix? Can a helical sequence be driven into a loop? Are loops helix-stop signals?

What is the role of loops in stabilizing protein structure? How does deleting a loop affect protein structure and stability?

Are omega loops determinants in protein folding?

Can we confidently predict the tendency for a given sequence to form a loop?

Can we design an independently stable omega loop without homology to known loops?

To answer these questions, I proposed to:

Measure the equilibrium constant for loop formation in a diverse series of synthetic peptides incorporating omega loop sequences, and to relate functional properties and primary structure to the tendency for loop closure.

Determine whether loop peptides take on stable structure in aqueous environments.

Characterize the structure(s) of selected loop peptides by NMR.

Characterize the structure and activity of loop-deleted protein(s) in isolation and in the presence of synthetic loop peptides, using NMR and standard activity assays.

Describe the context dependence of loop formation by synthesizing peptides containing loop sequences plus N- and C-terminal extensions derived from the protein sequence, or from known helical "caps".

Determine sequence requirements for loop formation and stabilization.

Develop methods for predicting the occurrence and conformation of omega loops from sequence
data.
Design an independently stable omega loop.

2. Summary Statement of Progress
During the term of the award, progress was made on the first four experimental approaches listed in the specific aims. Omega loops from cytochrome c, lysozyme, triosephosphate isomerase, and superoxide dismutase were synthesized and studied by NMR and circular dichroism spectroscopies, and by the loop-closing assay described in the grant proposal. A loop-deleted variant of yeast cytochrome c was isolated and spectroscopic studies were begun. In addition, hydrogen exchange studies of the backbone protons of ubiquitin indicate that loop regions are less stably hydrogen bonded than helix and sheet regions, in both the native protein and in an alcohol-induced molten globule form.

3. Significant Experimental Results
Loops A and C from cytochrome c were synthesized with and without added terminal cysteine residues. The loops with cysteines were allowed to oxidize in the presence of oxygen in order to close the loop. The oxidized peptides had more structure in solution than the reduced peptides, as determined by the number of interresidue NOE's in NOESY spectra. However, neither the oxidized nor the reduced peptides had NMR spectra consistent with their having a solution structure identical to their structure in the protein.

The antigenic region of hen egg white lysozyme comprised of residues 64 through 80 constitute an omega loop closed by a disulfide bond in the native protein. This loop was synthesized and studied by NMR. Preliminary NOESY spectra indicated that the oxidized loop takes on somewhat native-like structure in solution. This result is consistent with prior experiments that showed that antibodies that react with the native protein also react with the oxidized form of the synthetic peptide, but not to the reduced form.

An 11 residue region of triosephosphate isomerase (TIM) that was shown to be involved in a conformational change upon substrate binding was also synthesized and studied by NMR. The work was complicated by solubility difficulties, and we found no evidence that this loop takes on any non-random structure in solution.

A zinc-binding loop from superoxide dismutase was synthesized and studied in the presence and absence of zinc. Initial one-dimensional NMR studies were encouraging, as several signals shifted and broadened upon addition of zinc. However, two-dimensional spectra were difficult to interpret and showed no clear evidence for structure formation either with or without zinc.

A strain of yeast expressing cytochrome c with loop C (residues 40 through 54 by the tuna numbering system) was obtained from a collaborator, J. S. Fetrow of SUNY Albany. The protein was isolated and preliminary circular dichroism and NMR data were obtained. The work was hampered by the instability of the protein, and no firm conclusions could be drawn from the spectra.

In a related study, we observed hydrogen exchange rates of the backbone protons of ubiquitin in both its native state and an alcohol-induced molten globule structure. Protons from the loop-like regions of ubiquitin on average had faster hydrogen exchange rates than those in alpha helix or beta sheet regions.

4. Publications, Abstracts, and Reports
One publication, one manuscript in preparation, and nine abstracts were produced during the term of this award.

Published

In preparation
Solution Structure of Synthetic Omega Loop Peptides From Cytochrome c, J. K. Barnett and M. S. Briggs, in preparation.
Abstracts


$^{13}$C NMR Model Peptide Studies of the Effects of Proline Isomerization on Ubiquitin Folding, Y. Pan and M. S. Briggs, Southeastern Magnetic Resonance Conference, Raleigh, NC, October, 1992.

Structural Analysis of the HEW Lysozyme Omega Loop Peptide by CD and 2D NMR, P. A. McIlwain and M. S. Briggs, Southeastern Magnetic Resonance Conference, Raleigh, NC, October, 1992.
