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None.

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18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

Nuclear Magnetic Resonance, Molecular Recognition, NOESY Spectral Simulation, Structure Refinement, Software Development

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The work originally proposed for contract #N00014-88-K-0202 has been continued and brought to a conclusive point where the methods can be used by other workers. The most recent software developments are the incorporation of model-free order parameters into NOESY simulations and the addition of routines for comparing computer-extracted distances (or cross-rates) with the expectation ranges based on the full spectrum of torsional freedom so as to generate precision estimates for NOE-distances from a single NOESY spectrum. These methods have been used to define the conformational states of a variety of polypeptide and drug molecule systems including: prostaglandin and steroid analogs, rigid and flexible endothelin analogs, and a small protein allergen.

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S/N 0102-LF-014-6603

ANNUAL PROGRESS REPORT

GRANT #: N00014-91-J-1393 R&T Code: 441n002

PRINCIPAL INVESTIGATOR: Niels H. Andersen

INSTITUTION: University of Washington

GRANT TITLE: NMR Elucidation of Molecular/Macromolecular Complex Stereochemistry

REPORTING PERIOD: 01 June 1991 → 31 March 1992

AWARD PERIOD: → 31 March 1992

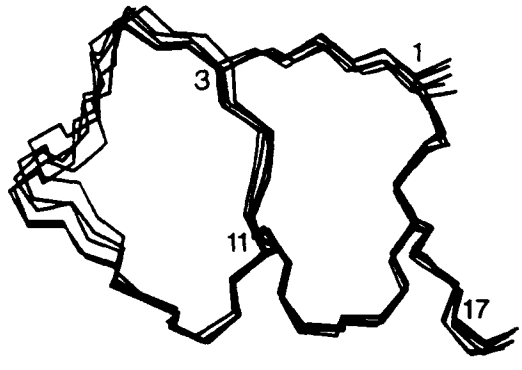
OBJECTIVE: To develop methods and software for quantitative analysis of NOESY data and the extraction of experimental constraints for the refinement of structures of biomacromolecules and their complexes. as a means for studying molecular recognition phenomena.

ACCOMPLISHMENTS (last 12 months):

1) The DISCON algorithm for the model-free extraction of accurate cross-rates (and estimates of their experimental precision) from a single NOESY data set has been fully implemented in user-friendly software. The theoretical basis of the method and validation tests are the subject of two papers that are now in press. These papers include demonstrations of: a) the use of DISCON-derived rates (and distances) to ascertain that a multi-conformer equilibrium must be assumed and to define major conformer structures, and b) the superiority of DISCON (*versus* nOe growth rate estimates followed by BKCALC distance adjustment) for deriving constraints for DNA structure refinement.

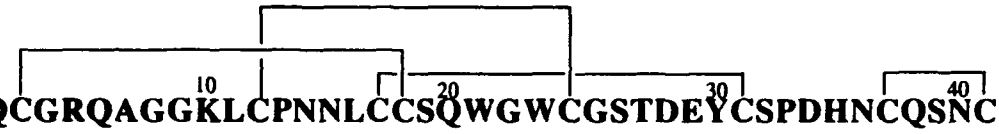
2) The incorporation of model-free order parameters in NOESYSIM, our program for calculating the expectation NOESY spectrum for a structural and dynamic model and the experimental conditions, including the non-ideal effects of incomplete return to equilibrium magnetization values during truncated preparatory delays.

3) The structure elucidations of endothelin-1, an endogenous vasoconstrictor = 1/15.3/11–**C S C S S L M D K E C V Y F C H L D I I W**. and a number of both more rigid and more flexible analogs that retain biological activity have been completed and reported. All permissable bioactive conformers are α-helical from K9→C15. In the case of the more rigid analogs in which the $Cys^{3,15}$ residues were replaced by ββ-Me_2-Cys (=Pen), the helical region extends to Leu^{17} and the backbone conformation is well defined from residue 1→18. The backbone conformation over that region is shown to the right. In the case of the native form of endothelin-1, four additional contributing conformers were located. In this conformational mixture, NH Δδ/ΔT-values near zero do not correspond to persistent H-bonds.



A novel analytical paradigm for using Δδ/ΔT-values in conjunction with exchange rate and solvent induced shift data to locate and characterize loci of conformational motion has been developed.

4) A high resolution NMR structure has been obtained for hevein



this is the first solution structure for a member of the agglutinin-toxin fold family of proteins which retains the diagnostic disulfide linkage pattern. Parallel refinements were carried out with tight bounds obtained using the DISCON algorithm and with these converted to 'conservative' bounds as commonly used in protein structure elucidation. Only when tight bounds were used was it possible to define the few remaining loci of conformational isomerism in this structure. The differences in the convergence and structural precision obtained are illustrated in the **Research Highlight** accompanying this report.

SIGNIFICANCE: Until our most recent studies, regions of proteins which were ill-defined in the ensemble of NMR-refinement structures were dismissed as "disordered" and the standard NOESY methodology did not work for smaller peptides that display conformational isomerism. The methods that we are developing should extend the range of systems that can be structurally characterized by NMR to include peptides displaying multi-conformer equilibria and the loop dynamics in proteins.

MEETING PRESENTATIONS: FOUR (4) posters based on ONR-supported research were presented by my group at the Apr.'92 E.N.C. Meeting.

PUBLICATIONS (last 12 months, plus those in press or submitted): [* Will be submitted when reprints become available, § Utilize methods developed under ONR support but acknowledge primary support from other sources.]

1) Andersen, N.H., Lai, X., and Marschner, T.M. (1991), NOESYSIM/DISCON Docu-mentation, copyrighted by Univ. of Washington. (80 pages, copy available upon request)

§ 2) Krystek, S.R., Bassolino, D.A., Novotny, J., Chen, C., Marschner, T.M., and Andersen, N.H. (1991), Conformation of Endothelin in Aqueous Glycol Determined by 1H-NMR and Molecular Dynamics Simulations, *FEBS Lett.* ***281***, 212-218. (previously submitted to the program officer)

3) Rovnyak, G., Andersen, N., Gougoutas, J., Hedberg, A., Kimball, S.D., Malley, M., Moreland, S., Porubcan, M., and Pudzianowski, A., Active Conformation of 1,4-Dihydropyridine Calcium Entry Blockers: Effect of Size of 2-Aryl Substituent on Aryl Rotamer Preference, *J. Med. Chem.* **34**, 2521-2524. (previously submitted to the program officer)

§ 4) Andersen, N. H., & Hammen, P. K. (1991) A Conformation-Preference/ Potency Correlation for GnRH Analogs: NMR Evidence, Biorganic & Med. Chem. Lett. **1**, 263-266. (reprint enclosed)

5) Andersen, N.H., Chen, C., Marschner, T.M., Krystek, S.R., & Bassolino, D.A. (1992) Endothelin Conformational Dynamics in Acidic Aqueous Media from Quantitative NOESY Analysis, *Biochemistry* **31**, 1280-1295. (reprint enclosed)

* 6) Lai, X., Chen, C., and Andersen, N.H. (1992) Extracting Experimental Distances from NOESY Data: The DISCON Algorithm, an Accurate and Robust Alternative to an Eigenvalue Solution, *J. Magn. Reson.*, in press.

* 7) Lai, X., Reid, B., & Andersen, N. H. (1992) A Comparison of BKCALC-Adjusted Constraints and those from DISCON using Simulated Data for a B-DNA Structure, *J. Magn. Reson.*, in press.

* 8) Andersen, N.H., Cao, B., Rodriguez, A., & Arreguin, B. (1992) Hevein: the NMR Asignment and an Initial Assessment of Solution-state Folding for the Agglutinin-Toxin Motif, *Biochemistry*, submitted.

A-1

ANNUAL REPORT QUESTIONNAIRE
(for ONR use only)

Principal Investigator Name: Niels H. Andersen, Professor of Chemistry

Institution: University of Washington, Seattle, WA 98195

Project Title: Magnetic Resonance Elucidation of Molecule/Macromolecule Interaction Stereochemistry

Number of ONR supported

Papers published in refereed journals: 4 (+ 2 in press, + 2 submitted)

Papers or reports in non-refereed publications: 5

Books or book chapters published: 1

Number of ONR supported patents/inventions
Filed: none

Granted: none **Patent name and number:** _____

Number of presentations:	Total	ONR Project
Invited:	3	2
Contributed:	5	4

Trainee Data (only for those receiving full or partial ONR support):

	TOTAL	FEMALE	MINORITY	NON-US CITIZEN
No. Grad. Students:	5			2
No. Postdoctorals:	1			1
No. Undergraduates:				

AWARDS/HONORS TO PI AND/OR TO MEMBERS OF PI'S RESEARCH GROUP (please describe):

None.

Equipment purchased on grant (number and description of items costing >$1,500):

$2759 ; BIOSYM, software maintenance

$6378 ; Silicon Graphics, Refurbished IRIS 4D-25TG + maintenance

RESEARCH HIGHLIGHTS (GRANT # N00014-91-J-1393)

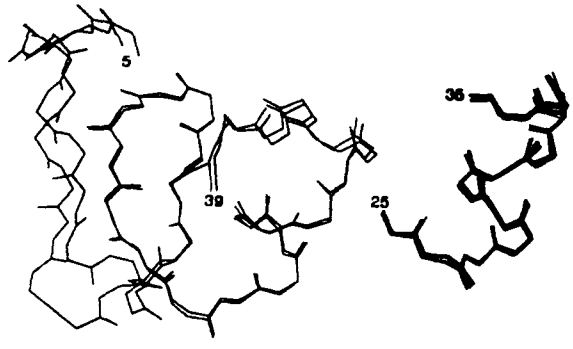
Objectives

- Elucidate the structures of biomolecular systems using novel quantitative NOESY analysis software
- Develop NOESY analysis methods that distinguish motional disorder (and its nature) from structural imprecision due to insufficient experimental constraints

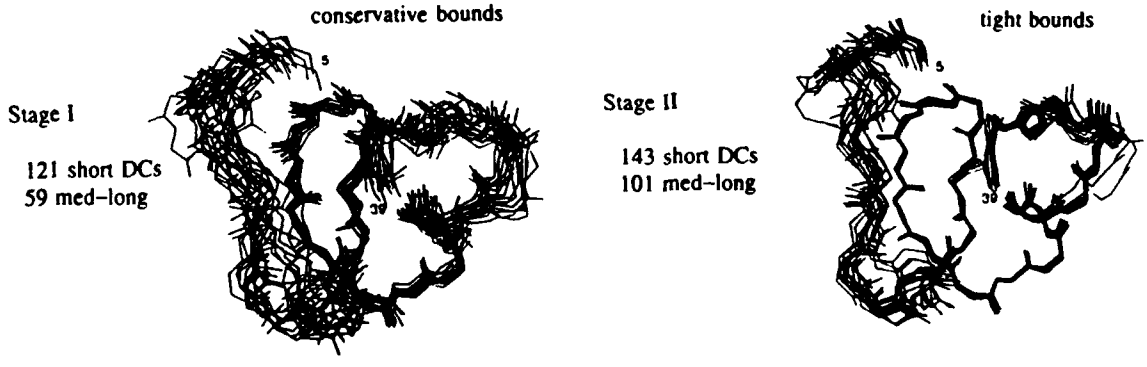
Accomplishments

- The incorporation of model-free order parameters into NOESYSIM improves the accuracy of the resulting spectral simulations.
- Program DISCON provides cross-rates and their precision directly from the analysis of a single NOESY spectrum. These can be converted to high precision constraints for structure/conformation refinement. Tight distance constraints improve structural precision and facilitate the diagnosis of equilibria between conformers with μsec lifetimes occurring in the solution state for peptides and within external loops of protein structures.
- The structure elucidation of ET-1 (an endogenous 21 residue bisdisulfide vasoconstrictor in which a persistent α-helical from residues 9→15 occurs but conformational isomers occur in other regions) and its analogs has revealed a method for analyzing $\Delta\delta/\Delta T$ data for backbone NH's which locates the sites of change and defines the time scale of those changes.
- Tight distance constraints have been used to elucidate the conformation of hevein, a 43 residue allergen with 4 disulfide crosslinks — 3/18, 12/24, 17/31, 37/41.

The structural features of (and distinctions between) the two major conformers are shown. These include, besides a short stretch of 3 stranded anti-β sheet, what may be the shortest helix (residues 28-32) known.



- The hevein structure elucidation also provided an example of the value of tight constraints. The two conformers in the N-terminal loop could not be discerned with conservative bounds. The structure ensembles that result with tight versus conservative bounds are compared below.



Significance. Discreet **Conformer structures** and loop dynamics are discernible in the regions that are "disordered" using standard protein NMR refinement methods.

N. H. Andersen, UW, 1992