LA-UR-97-3507

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Author(s): W. H. Woodruff, CST-4
R. B. Dyer, CST-4
R. H. Callender, Dept. Phys., City Univ. of New York

Submitted to: DOE Office of Scientific and Technical Information (OSTI)

Los Alamos
NATIONAL LABORATORY

Form 836 (10/96)
ST 2629

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Ultrafast Studies of Solution Dynamics

William H. Woodruff* and R. Brian Dyer
Chemical Sciences and Technology Division
Los Alamos National Laboratory

Robert H. Callender
Department of Physics
City University of New York

Abstract

This is the final report of a one-year, Laboratory Directed Research and Development (LDRD) project at Los Alamos National Laboratory (LANL). Fast chemical dynamics generally must be initiated photochemically. This limits the applicability of modern laser methods for following the structural changes that occur during chemical and biological reactions to those systems that have an electronic chromophore that has a significant yield of photoproduct when excited. This project has developed a new and entirely general approach to ultrafast initiation of reactions in solution: laser-induced temperature jump (T-jump). The results open entire new fields of study of ultrafast molecular dynamics in solution. We have demonstrated the T-jump technique on time scales of 50 ps and longer, and have applied it to study of the fast events in protein folding. We find that a general lifetime of alpha-helix formation is ca 100 ns, and that tertiary folds (in apomyoglobin) form in ca 100 μs.

Background and Research Objectives

Proteins consist of a chain of amino acid residues covalently linked together by the peptide bond. The sequence of amino acid residues defines the exact nature of the protein, its structure, and function. In solution, proteins are found in compact dense structures. It is generally believed that most if not all proteins fold into their native compact structures in ways that are determined by primary sequence and the interactions of the component residues with themselves and with their surroundings. The molecular details of the folding process are under intense investigation, both theoretically and experimentally.

At present, kinetic measurements of protein folding, or the reverse situation of protein unfolding, are performed by using stop flow measurements techniques. The reversibly "denatured" unfolded protein is rapidly mixed together with solution to reverse the denaturing conditions. For example, high concentrations of urea or guanidine hydrochloride will unfold a protein and their rapid dilution will often initiate refolding. Of

* Principal Investigator, E-mail: woody@lanl.gov

more interest to the present work, extremes of pH (either high or low) will also unfold proteins, and neutralization will refold them reversibly in certain cases. In addition, proteins fold and unfold as a function of temperature. The time constant of these kinetic studies is the mixing time of the stop flow apparatus, generally around one millisecond. Some intermediates are observed in the folding process with time constants on the millisecond to minute time scale. However, considerable changes in structure during the folding process occur in shorter times. Obviously these events have not been monitored as yet, and a central goal of this work is to investigate and establish methods to observe early intermediates.

Our approach is to employ laser-induced temperature-jump (T-jump) techniques to initiate protein unfolding/folding and time-resolved vibrational spectroscopy to follow the time course of these reactions. The advantages of time-resolved vibrational measurements as structure-specific probes of chemical dynamics are well established. The structural specificity of vibrational spectroscopy derives from the connection of molecular vibrations to specific structures which determine the frequency, intensity and linewidths of the absorptions. In principle, infrared spectroscopy yields information on every discrete structure and interaction in the protein, because virtually all of the normal vibrations of the protein are infrared active. The amide vibrations are sensitive to secondary structure, while higher order structures can in some cases be probed via the vibrations of amino acid side chains.

Importance to LANL's Science and Technology Base and National R&D Needs

OBER structural biology initiative: The DOE Office of Biological and Environmental Research (OBER) has instituted a major new initiative in structural biology. There are two interrelated components of this initiative that will be developed in parallel: i) to build up the unique facilities of the national laboratories as structural biology centers for external and internal users and ii) to strengthen the in-house structural biology research programs to address the missions of the DOE. Stated missions are the structural problems associated with the Human Genome Project (HGP), with DNA damage and repair and the application of unique capabilities to important biological problems. Our work will greatly enhance our ability to respond to this new initiative. First, the work will apply the Laboratory's unique laser/protein dynamics facilities to an important problem in structural biology, in collaboration with an external (academic) user. Second, it directly addresses one of the stated DOE missions since one of the major structural problems associated with

the HGP is the development of algorithms for the prediction, from primary structure (amino acid sequence), of the folding pathways of proteins to their native three-dimensional structures.

This work, which complements ongoing Los Alamos research in protein dynamics within the Chemical Science and Technology Division, will further enhance the visibility and reputation of LANL in the areas of structural biology and protein dynamics. In addition, other LANL programs in biosciences benefit from both the molecular understanding of protein folding and the technology developed by this work.

Scientific Approach and Accomplishments

General Methodology

A number of small proteins show early transients in their folding/unfolding kinetics. We began our investigations by studying the kinetics of apomyoglobin and synthetic peptide fragments which serve as models for the folding of small protein regions. This protein has served as an archetypal model for the folding kinetics in small proteins. Our main approach to the problems at issue is to employ laser pump probe techniques on all relevant time scales (picoseconds to seconds). Protein folding/unfolding reactions were initiated using laser T-jump. Time-resolved infrared techniques, described below, were used to identify and characterize the protein folding intermediates and their dynamics.

Protein and Model Systems

Apomyoglobin was chosen as the protein system to study. This small, mostly helical protein has been used as an archetype for the folding of single-domain proteins. A synthetic helical model peptide (suc-21) was chosen as the complement for the protein studies.

Several questions concerning the fast events in folding were obvious. Just how fast are the transitions? Does the reaction involve a simple single step (quite unlikely) or are there several steps? Static thermal denaturation experiments suggest several steps. Is the fast unfolding reaction the same as the fast folding reaction? This issue is key to whether the reactions are thermodynamically controlled and whether microreversibility holds.

The helix-coil transition in peptides and proteins is a basic structural motif. It is of importance by itself and as a component in the folding/unfolding of larger scale motions. Dynamics simulations have been performed on simplified models of small peptides, and

these calculations suggest that the unwinding of a helix takes place in stages, with localized motions occurring on the 10^{-9} s time scale and with full unwinding happening in about 10^{-7} s. Using such substructures as components to a diffusional-collisional model of the folding of a protein, one calculation suggests that folding is completed in several microseconds. This calculation again emphasized that the folding pathway proceeded in stages over rather large time scales. We will study this transition in model oligopeptides that have very stable helical structures, for example the suc-21 peptide that we have obtained through collaboration with Peter Kim.

Laser Pump/Probe Methods

The Laser-Induced T-jump. Our laser systems produce millijoule energy pulses throughout the IR range. In order to rapidly heat a protein in solution, a laser pulse at a wavelength where water has absorption is the "pump" pulse in the T-jump experiments. The laser energy is absorbed by the water and the temperature of the volume of water in the laser beam is rapidly (on the subpicosecond time scale) increased. Studies have shown that the temperature is transmitted across proteins on the 1-5 picosecond time scale. The D_2O bands at ca 2 μ were used for this purpose, partly because they are strong absorption bands and partly because proteins do not absorb at this wavelength. The laser "pump" pulse is tuned to this band at a point where the absorption coefficient is such that about 10% of the IR light is absorbed to insure uniform heating of the sample. We have recorded T-jumps of 10°C with rise times of 45 ps, and 30°C or greater with rise times of 10 ns.

Time Resolved Infrared Spectroscopy (TRIS). Two TRIS approaches were employed. The first is real-time TRIS, a single-wavelength technique developed in our laboratories for obtaining infrared transients on time scales from tens of nanoseconds to seconds. For survey TRIS spectra on time scales of 1 μs and longer, we have developed another less time-consuming technique, time-resolved step-scan FTIR (TRS^2-FTIR) in collaboration with Richard Palmer at Duke University. The general method requires initiating a transient event (e.g., photolytically) and recording the temporal evolution of the IR signal at each point in the interferogram. The mirror is held in a fixed position while the event (photolysis) is repeated a sufficient number of times to obtain adequate signal-to-noise ratio, then stepped to the next position (the number of positions determined by the resolution and free spectral range required). Processing of the data involves sorting by time in order to produce interferograms for each time, which are subsequently Fourier transformed to yield a set of time-resolved spectra.

Protein Folding Results

We have measured the fast relaxation dynamics of "native" apomyoglobin following a 10-nanosecond, laser-induced temperature jump. The structural dynamics were followed using time-resolved infrared spectroscopy. The infrared kinetics monitored within the amide I absorbance of the polypeptide backbone exhibit two distinct relaxation phases that have different spectral signatures and occur on very different time scales (ν=1633 cm^{-1}, τ = 50 ns; ν=1650 cm^{-1}, τ = 120 μs). We assign these two spectral components to discrete substructures in the protein: helical structure that is solvated (1633 cm^{-1}) and native helix that is protected from solvation by interhelix tertiary interactions (1650 cm^{-1}). Folding rate coefficients inferred from the observed relaxations at 60°C are $k_{f(solvated)}$ = (7 to 20) $\times$ 10^6 s^{-1} and $k_{f(native)}$ = 3.6 $\times$ 10^3 s^{-1}, respectively. The faster rate is interpreted as the intrinsic rate of solvated helix formation, whereas the slower rate is interpreted as the rate of formation of tertiary contacts that determine a native helix. Thus, helix formation precedes the formation of tertiary structure by over three orders of magnitude in this protein.

The distinct thermodynamic and kinetic properties observed for the apoMb substructures suggest that they fold independently, or quasi-independently. Remarkably, apoMb does not fold homogeneously despite the relatively small size and structural simplicity of this protein. Results on the "molten globule" form of apomyoglobin show only the solvated helix relaxation at ca 100 ns, and in addition show that a substantial fraction of the protein structure is in the native helical form and does not melt under our conditions. This structure is assigned as the hydrophobic core of the protein comprising the A, G, and H helices. Results on the suc-21 peptide show only the solvated helix relaxation at 160 ns. The folding lifetime associated with the formation of solvated helix is ca 100 ns in all systems observed, which we take to be a characteristic lifetime for the formation of this structure.

Publications

1. Williams, S. ; Causgrove, T.; Gilmanshin, R. ; Fang, K.; Callender, R.; Woodruff, W.; Dyer, R., "Fast Events in Protein Folding: Helix Melting and Formation in a Small Peptide." Biochemistry **35(3)**, 691-7 (1996).

2. Gilmanshin, R. ; Williams, S. ; Callender, R.; Woodruff, W.; Dyer, R., "Fast Events in Protein Folding: Relaxation Dynamics of Secondary and Tertiary Structure in Native Apomyoglobin." Proc. Nat. Acad. Sci, in press (1997).