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CHARACTERIZATION OF FOLDING INTERMEDIATES OF TIM BARREL PROTEINS BY HYDROGEN EXCHANGE AND MASS SPECTROMETRY

by

Hai Pan

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree Doctor of Philosophy

Major: Chemistry

Under the Supervision of Professor David L. Smith

Lincoln, Nebraska

December, 2002

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DISSERTATION TITLE

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CHARACTERIZATION OF FOLDING INTERMEDIATES OF TIM BARREL PROTEINS BY HYDROGEN EXCHANGE AND MASS SPECTROMETRY

Hai Pan, Ph.D.

University of Nebraska, 2002

Advisor: David L. Smith

How the amino acid sequence of a protein specify its three dimensional structure is referred as protein folding problem. It is the major unsolved problem in structural biology. TIM barrel is one of the most frequently encountered and the most versatile structures among the enzymes. Characterization of the folding intermediates of TIM barrel proteins is critical to solve the folding problem for TIM barrel proteins and it potentially offers insights to the folding of other protein architectures as well.

A new method, based on hydrogen/deuterium exchange, protein fragmentation and mass spectrometry was developed to identify and characterize the structures of folding intermediates at high resolution. In this method, the protein can be fragmented by an acid protease after the protein undergoes H/D exchange. The deuterium level and isotope pattern present in each fragment is determined by HPLC and mass spectrometry.

Equilibrium and kinetic folding intermediates of four TIM barrel proteins have been detected and characterized by this method. The results from rabbit muscle aldolase, a tetrameric TIM barrel protein, showed that folding to the native state involves three steps and two intermediates. Approximately 110 residues fold to highly compact forms in each step. These results also showed that each folding domain includes widely

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separated regions of the backbone. Interestingly, two intermediates are also populated in the equilibrium and kinetic folding experiments of monomeric *S. aureus* aldolase, which has high sequence homology with rabbit muscle aldolase. The structures of two intermediates are similar but not the same as those observed in the rabbit muscle aldolase, suggesting that quaternary structure affects folding/unfolding pathways. The sequential folding of two domains was observed in the folding of triosephosphate isomerase (TIM), the prototype in TIM barrel family. The C-terminal four ($\alpha\beta$) units folds much faster than the N-terminal four (α/β) units. Two intermediates are characterized in the equilibrium unfolding of α subunit of bacterial luciferase. The domain that unfolds at lower concentrations of denaturant is located at the C-terminus of the protein including the last two (α/β) units.

All these results suggest that several different models are required to describe the folding of TIM barrel proteins, despite their high level of structural and functional similarity.

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TO MY PARENTS AND BROTHER,

FOR THEIR SUPPORT,

TO MY WIFE, YUAN (VIVIAN) WANG,

FOR HER UNDERSTANDING AND SACRIFICE

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LIST OF ABBREVIATIONS

ACN	acetonitrile
CD	circular dichroism
CID	collision induced dissociation
Cyt C	cytochrome c
E/S	enzyme substrate ratio
ESI	electrospray ionization
GdHCl	guanidine hydrogen chloride
HisF	imidazoleglycerol phosphate synthase
HPLC	high performance liquid chromatography
HX	hydrogen exchange
HX MS	hydrogen exchange and mass spectrometry
LCMS	liquid chromatography and mass spectrometry
LEM	linear extrapolation method
MW	molecular weight
MS	mass spectrometry
M/z	mass to charge ratio
NMR	nuclear magnetic resonance
PDB	protein data bank
S/N	signal to noise ratio
TIM	triosephosphate isomerase
TFA	trifluroacetic acid
w/w	weight to weight ratio

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Chapter 1

Introduction and Background:

Protein folding & TIM barrel proteins

1.1 Protein Folding

1.1.1 Protein folding problem

Proteins are the molecules of life, responsible for a wide range of fundamental biological processes such as catalyzing the numerous chemical reactions, transport, signaling and energy conversion. Proteins are synthesized on the ribozymes as linear chains of amino acids. To become fully functional, all proteins must fold to unique three-dimensional structures. The question of how proteins efficiently and reliably achieve their native states following synthesis is referred as protein folding problem, which is one of the most intriguing questions in structural biology.

In 1973, Anfinsen established that the sequence of the protein contains all the information necessary to direct the unfolded protein to reach the native state (Anfinsen, 1973). However, the practice of this rule is not simple, which is illustrated by a very famous question known as the Levinthal paradox (Dill and Chan, 1997). One simple way to explain this problem follows. If it is assumed that each of the backbone ϕ and ψ angles in an polypeptide chain can adopt only three different values, there will be 3^{100} possible conformations for the protein with 100 residues. If only 10^{-11} s is required to change from one conformation to the other, a random search of all conformations will take about 10^{29} years, which is far more than the time since the earth was born. However, proteins are frequently observed to fold on the seconds to hours scale.

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The classical view to explain the Levinthal paradox is that the protein folding is not the random search. Instead, the protein passes through a limited number of intermediates (Fig 1.1). These intermediates guide the protein to reach the native state.

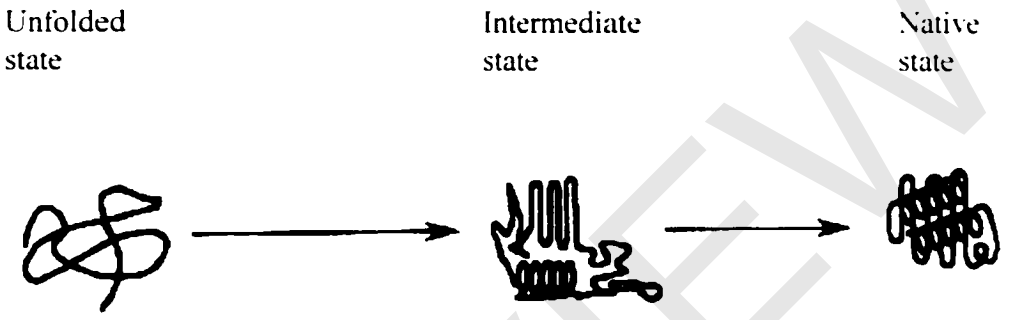

Figure 1.1 Schematic representation of a simple folding reaction with one intermediate

1.1.2 The significance of the protein folding

The protein folding is not just a fascinating challenge from an intellectual point of view, it has important practical implications. With the completion of the human genome project, solving protein folding problem is even more urgent. Tremendous efforts have been made to predict three dimensional structures from amino acid sequences. In addition, protein misfolding is found to relate to a number of diseases, such as Parkinson and Alzheimer's diseases (Dobson, 1999). For example, misfolded β-amyloid peptides form insoluble fibrils in the human brain, which is likely the cause of the Alzheimer's disease (Walsh, Lomakin et al., 1997; Walsh, Hartley et al., 1999). There is also

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