Probing Stability and Unfolding of the N-terminal Domains 5-6 of Wilson Protein

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Abstract

The Wilson protein (ATP7B) is a copper transporting P-type ATPase found in the liver, brain, and other organs. The N-terminal end consists of six copper binding domains, which have a ferrodoxin [ββββ] fold with a CxxC motif. Despite similarities in copper binding affinities they interact differently with the HAH1 metallochaperone. Studying the stability of these domains will help understanding the differences in their functions. The stability of WLN5-6 was probed using several different methods: Dynamic light scattering (DLS), Circular dichroism (CD) and Fluorescence spectroscopy methods. Previous studies show that WLN5-6 has a spherical shape and CD chemical unfolding studies indicate that mutants prepared had the same stability. Variations in Two Photon Absorption (2PA) cross-section are attributed to changes in local electric fields of the protein as confirmed from femtosecond fluorescence anisotropy and fluorescence lifetime. Present results show that the 2PA cross-sections can be used as a tool to probe local environments and unfolding of proteins.

Introduction

Wilson disease is caused by a buildup of copper in the body.

Materials and Methods

Plasmid Engineering

5 original cysteines were mutated, one was produced at position 51

4 cysteines were mutated to serine, cysteine at position 6 remained

Protein expression and purification

The plasmid was transformed into E. Coli-Rosetta 2 cells and grown in LB media until the OD600 reaches 0.7 then the cells induced with 1mM IPTG for 4 hours at 28 °C. The cells harvested by centrifugation then the protein extracted by freeze thaw method. Purification of the protein was accomplished by anion exchange column (DEAE) and gel filtration technique.

Protein probing

Why 2PA cross sections for unfolding?

2PA cross sections (b) used to monitor protein unfolding: it is measured as the ratio between the intensities of the two-photon fluorescence (12P) and the one-photon fluorescence (1P) based on the following equation:

\[ \frac{I_{12P}}{I_{1P}} = \frac{I_{1P} \cdot \frac{1}{\sigma^2} \cdot \frac{1}{\varphi^2} \cdot \frac{1}{\varphi^3}}{I_{1P}} = \frac{1}{\sigma^2} \cdot \frac{1}{\varphi^2} \cdot \frac{1}{\varphi^3} \]

Why ultrafast anisotropy for unfolding?

Anisotropy measurements measure the average angular displacement of the fluorophore that occurs between absorption and subsequent emission of a photon. It is quite sensitive to micro environments.

Research Objective:

Probing stability of the last two metal binding domains WLN5-6

Results

Linear extrapolation method used to determine the free energy (ΔG).

\[ \Delta G = 3.33 \text{ Kcal/mol} \]

\[ C_m = 3.3 \text{ M} \]

Figure 1: Unfolding of WLN5-6 using Guanidine Hydrochloride, assuming a two-state unfolding mechanism.

Figure 2: Molar residue ellipticity at 222 nm. Unfolding of WLN5-6 and two different mutants that contain one cysteine residue.

Figure 3: 2PA cross-section of CPM dye bound to WLN5-6 at position 51 and 6. This method can be used to monitor unfolding of proteins at specific positions.

Figure 4: Ultrafast anisotropy at 10 ps time delay. Anisotropy changes are attributed to the changes in the orientation of the molecular dipole in the bound state, probably hydrogen bonding with amino acids adjacent to it.

The dye is bound to protein via hydrogen bonding and the unfolding of the protein alters it.

Conclusion

WLN5-6 unfolds in an apparent two-state pathway with a free energy of 3.33 Kcal/mol with a denaturation midpoint of 3.3 M. Alos, labeling WLN5-6 with CPM dye did not affect the chemical unfolding pathway.

The results of 2PA cross-sections of chromophores have shown a novel way to monitor the unfolding of proteins. The increase in 2PA cross-sections is ascribed to the differences in the local electric field around the chromophore during unfolding. This technique paves way for imaging folded and unfolded forms of proteins.

Femtosecond fluorescence anisotropy technique of the chromophores was successfully used to monitor the unfolding of WLN5-6 protein. This is one of the first reports where ultrafast anisotropy was used to study unfolding. It is important to note that the change in anisotropy is not due to HOMO-FRET but rather due to the hydrogen bonding of chromophore with protein.

References


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