Exploring Protein Folding Using Gromacs Simulation Software

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Introduction

Chymotrypsin inhibitor 2 (2ci2) is a serine proteinase found in barley seed, that is of interest in many protein transition state studies due to its two-state model. (Mcphalen & James, 1987; Jackson & Fersht, 1991).

What is a two-state model?

A two-state model is when the protein does not have an intermediate state when flipping between the folded and unfolded state. The transition state is found in between the folded and unfolded state.

The transition state is of interest in many studies to analyze what bonds are created as well as how those bonds are affected in terms of a mutation.

Objective

This study uses the Gromacs software to explore the parameters that enabled chymotrypsin inhibitor 2 to be found in between the folded state and the unfolded state. This further allowed for the analysis of the protein's structure and phi value when found within the transition state. The results of this study are then compared to previous experiments done.

Big Question

How are bonds affected within chymotrypsin inhibitor 2 during the transition state vs. the folded and unfolded state; and how does this compare to experiment data?

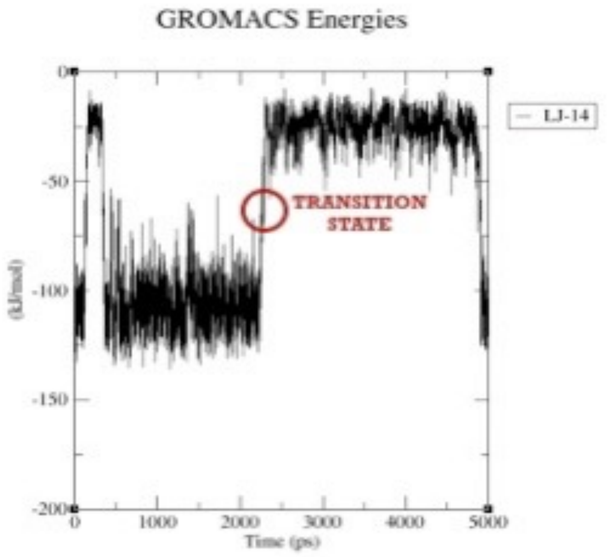
METHODS AND MATERIALS

1. Using Gromacs Software, the protein was simulated with different temperature to attempt to find the temperature at which the protein was in both the folded and unfolded state. This was done looking at the energy graphed.

115K – the protein was in the folded state
150K – the protein was in the unfolded state

METHODS AND MATERIALS

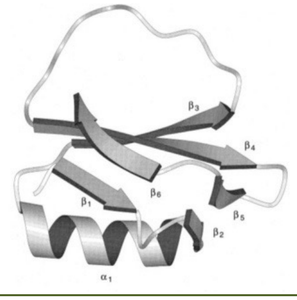
The temperature at which the protein was found to be flipping between both states is 138K. Look at graph below for energy diagram.



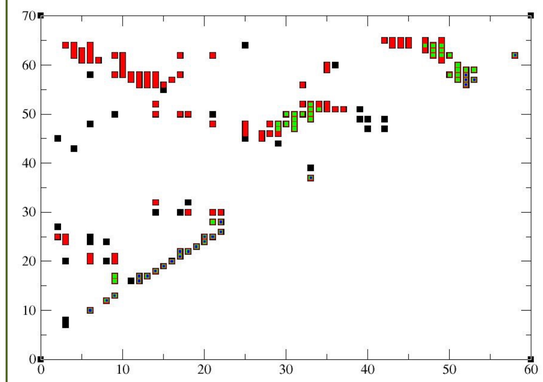
2. The program "xmgrace" was then used to analyze and compare data in the transition state

- Contacts graph was made to compare the contacts made in the crystal structure, folded state, transition state, and unfolded state
- The absolute probability of the contacts in the protein were graphed
- The phi values from this study was then compared and graphed to the experimental values

Crystal structure of the protein:

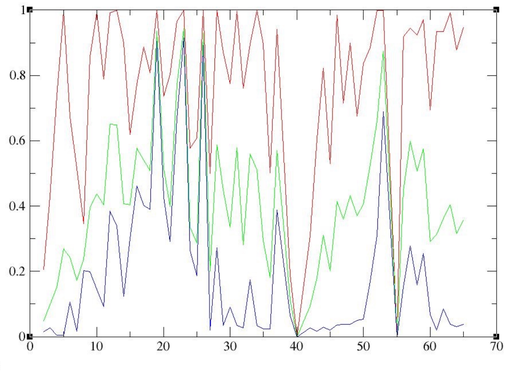


RESULTS



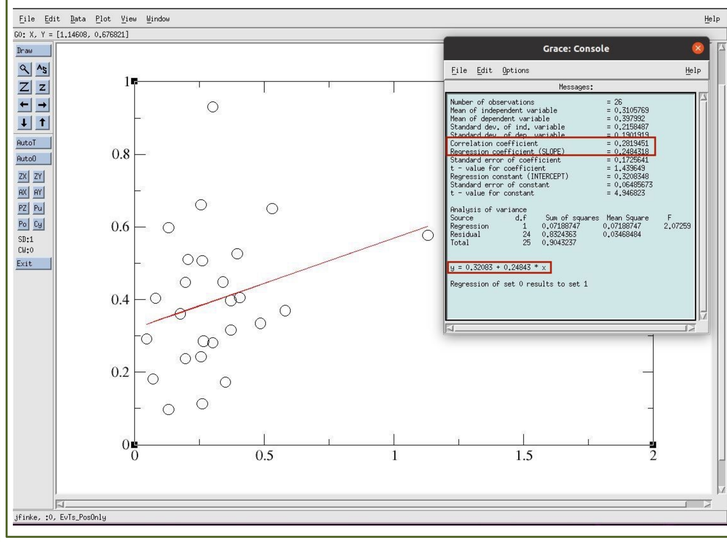
Black: Crystal structure of the protein
Green: Transition State of the protein

Left Graph:
This graph shows the contacts made in each state of the protein. The protein polypeptide is made up of 65 amino acids. The X and Y axis show the contacts made within each amino acid. Squares in the top left corner are long distance contacts made within the protein. As seen in the graph above there are much more contacts being made in the folded vs. transition vs. unfolded state which is expected.



Red: Folded State of the protein
Blue: Unfolded State of the protein

Right Graph:
This graph shows the absolute probability of each residue. Amino acid residue numbers are in the X-axis (1-65), and the probability is on the Y-axis. For example, in residue 20 you have about an 80% average contact forming in the folded state, 50% in the transition state, and about 40% in the unfolded state.



This graph shows the transition state phi values from the simulated protein using Gromacs software (X-axis), compared to the experiment transition state phi values (Y-axis). Phi values normally range between 0-1. Each circle in the graph represents a different amino acid. What's notable is the correlation coefficient and the slope as shown with the red square. The correlation coefficient is at 0.28 while the slope is at 0.25 showing that the experiment phi values and the phi values in the simulation do not match well. This shows that the simulated protein is not a perfect match to the experimental structure of chymotrypsin inhibitor 2.

REFERENCES

Mcphalen & James. 1987. Crystal and molecular structure of serine proteinase inhibitor CI-2 from barley seeds. 26(1):261-9. doi: 10.1021/bi00375a036. Retrieved from: https://pubmed.ncbi.nlm.nih.gov/3828302/

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