Linker structure in a rhomboid protease domain-swapped dimer monitored by solvent deuterium exchange

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Abstract

Rhomboid proteases are a highly-conserved family of membrane-spanning proteases initially discovered in *Drosophila*. These serine proteases are involved in multiple cell-signaling pathways and are increasingly linked to a variety of human diseases including diabetes and Parkinson's disease. The dimeric structure of a cytoplasmic domain from the *Escherichia coli* rhomboid protease GlpG has recently been characterized. This dimer undergoes an unusual domain-swapping event that may play a role in the regulation of protease function. However, one part of the structure that is less precisely defined is the linker that separates the two protomers, making it difficult to determine their relative arrangement. Measurement of backbone amide solvent deuterium exchange rates by solution nuclear magnetic resonance (NMR) can help identify hydrogen bonding partners in this region and refine this structure. Therefore, the primary objective of this project is to measure these exchange rates for the rhomboid cytoplasmic domain in order to gain insight into the structural aspects of this dimer. This information may help future investigations of rhomboid structure-activity relationships which could lead to the discovery of novel drug targets.

Project goal

Characterize hydrogen bonding interactions in and around the hinge region of *ec*GlpG CytD in the monomer and dimer.

Introduction

Rhomboid proteases

- Intramembrane serine petidases present in nearly all life forms.
- Regulate various pathways ranging from mitochondrial remodeling to parasitic invasion.
- Catalytic site located in a hydrophilic cavity formed by 6 transmembrane helices.
- Domains outside catalytic core may modulate function, E. *coli* GlpG rhomboid has one such region, called the cytoplasmic domain (CytD) (1,2).

Domain Swapping in the *Escherichia coli* GlpG cytoplasmic domain (*ec*GlpG CytD)

- *ec*GlpG CytD has been previously shown to exchange domains between its two identical monomers using a flexible hinge region between sheets β2 and β3 (figure 1).
- Previous NMR experiments have identified residues H32, Q34, S35 and D36 as the ones which experience the greatest peak shift after dimerization; suggesting they are the principal residues characterizing the hinge region (figures 2 &3) (4).

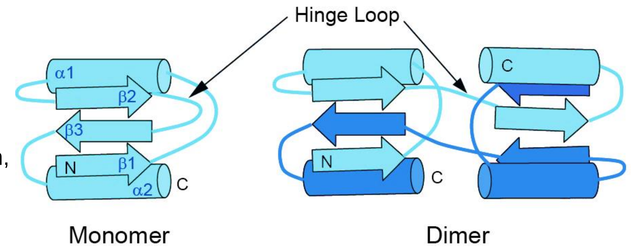


Figure 1. Domain swapping model for *ec*GlpG CytD (4)

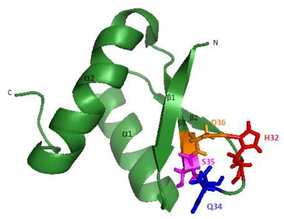


Figure 2. *ec*GlpG CytD monomer with hinge residues shown ((3) PDB ID:2LEP)

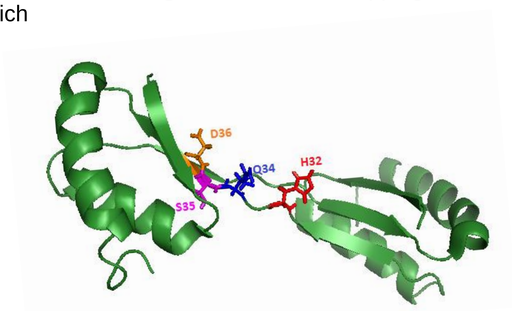


Figure 3. *ec*GlpG CytD dimer with hinge residues shown (PDB ID:2MJA)

Experimental design

Expression and purification of *ec*GlpG CytD

- Nitrogen-15 labelled *ec*GlpG CytD was expressed in BL21(DE3) cells.
- Monomer and dimer were purified using Nickel affinity chromatography and separated by Fast-Protein Liquid Chromatography (FPLC).
- The samples were prepared for NMR by buffer exchange into D_2O using a NAP-5 purification column.

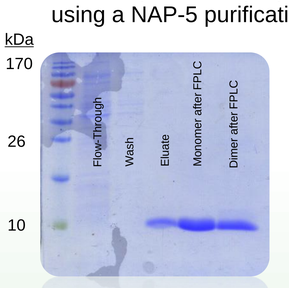


Figure 4. SDS PAGE gel of the *ec*GlpG CytD purification samples.

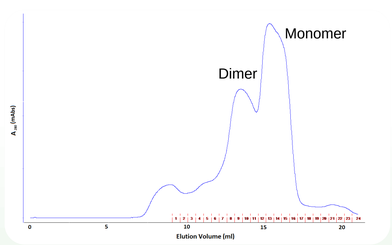


Figure 5. Size Exclusion Chromatography (SEC) profile of the *ec*GlpG CytD sample showing dimer and monomer peaks.

Nuclear Magnetic Resonance Spectroscopy (NMR)

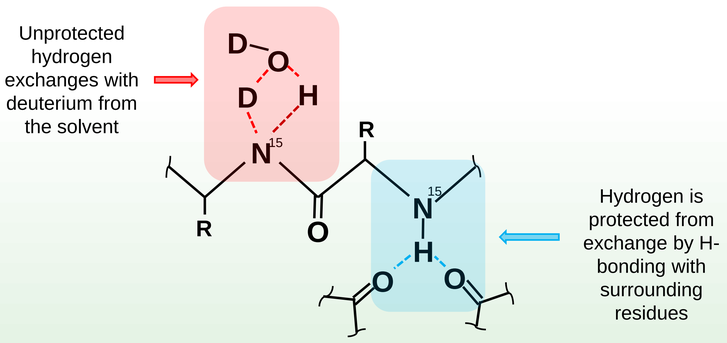
Peak data was acquired on the D_2O protein samples using the Varian Inova 500 spectrometer.

^1H-^{15}N Heteronuclear Single Quantum Coherence (HSQC) spectra were processed using NMRPipe software and peaks were mapped using NMRView software. Hydrogen exchange rates were calculated using the server program Sphere.

Data analysis and results

Each peak is assigned to a unique backbone amide hydrogen attached its corresponding nitrogen.

As the molecule interacts with the D_2O media, hydrogens which are not involved in hydrogen bonding interactions (less protected) can undergo exchange with a deuterium atom and their peak disappears from the spectra.

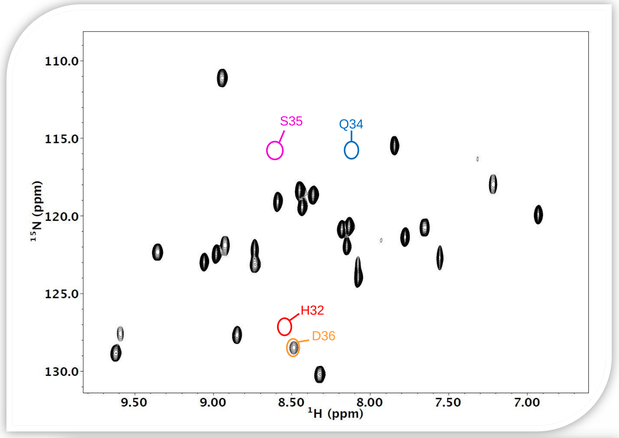


Exchange rates (k_{ex}) can be calculated from the peak decay curves.

A protection factor (P.F.) for each residue is determined from the exchange rates.

$$P.F. = -\log\left(\frac{\textit{Observed } k_{ex}}{\textit{Intrinsic } k_{ex} \textit{ for a completely exposed amide proton}}\right)$$

A.



B.

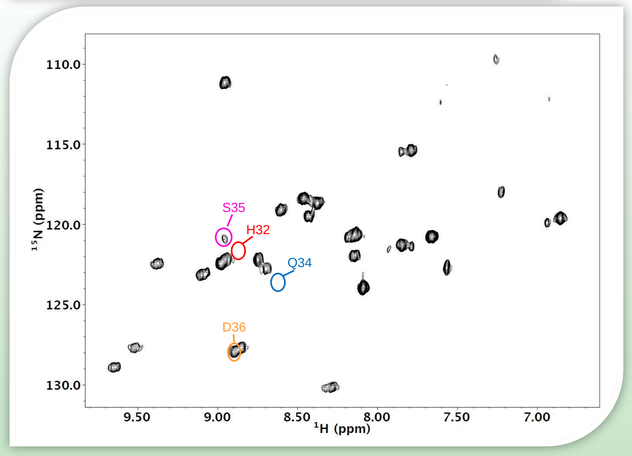


Figure 6. ^1H-^{15}N HSQC spectra of A. the monomer and B. the dimer structures at the beginning of the hydrogen-deuterium exchange process. Each amino acid in the protein that has not undergone exchange with solvent D_2O will give rise to a peak in this spectrum. Peak positions expected for residues in the hinge region are labeled on each spectrum.

P.F. legend

- >7 (More protected)
- >6
- >5
- >4
- <4 (Less protected)
- Absent from spectrum

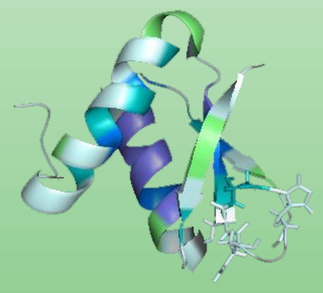


Figure 7. *ec*GlpG CytD monomer with hinge residues and protection factors shown (PDB ID:2LEP)

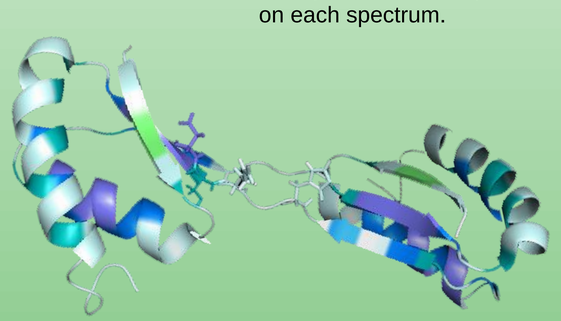


Figure 8. *ec*GlpG CytD dimer with hinge residues and protection factors shown (PDB ID:2MJA)

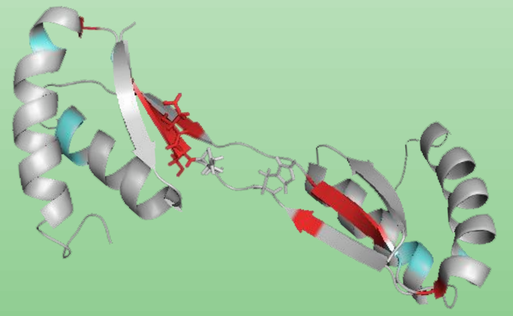


Figure 9. *ec*GlpG CytD dimer with hinge residues shown. Red residues are more protected in the dimer and those in blue are less protected. (PDB ID:2MJA)

Conclusions

- Protection factors in the monomer and dimer are highly similar.
- The dimer appears to have a more stable H-bonding network in the central beta-sheet and hinge residues D36 and S35 are more protected in the dimer.
-Overall, the hinge residues did not show evidence of high protection in either monomer or dimer, suggesting that hydrogen bonding is weak or not occurring.
- Therefore hydrogen bonds between hinge residues can not be added to structure data.

Acknowledgments

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References

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