STRUCTURAL INVESTIGATIONS OF THE ENZYME CYCLOPHILIN-A DURING CATALYSIS

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Introduction

CypA is a prolyl-isomerase that was initially found as the target of the immunosuppressive drug cyclosporine (CSA). Though multiple X-ray crystal structures have been determined both free and in complex with various targets, including the HIV Capsid protein, the mechanism has still remained elusive. The N-terminal residues to the catalyzed proline have been suggested to rotate based on several X-ray crystal structures (Sundquist et al., 2003) yet this is in sharp contrast to previous dynamics data (Eisenmesser et al., 2002). In that study residues exhibiting chemical exchange could be explained if the C-terminal substrate residues were rotated. Here we have employed structural investigations of CypA in the presence of several peptide substrates that support C-terminal rotation though N-terminal rotation cannot be completely ruled out.

Experimental

Recent NMR experiments that use isotope-edited techniques to assess both intermolecular and intramolecular interactions (Eichmuller et al, 2001) were applied to CypA in the presence of several of three peptide substrates that included AAPF, AGPF, and AFPF all with an N-terminal succinyl group and C-terminal pNA group. These experiments separate proton/proton NOEs based on the attached nuclei. Thus, 13C,15N labeled enzyme was used in the presence of the unlabelled substrate.

Results and Discussion

All three substrates gave similar intermolecular NOE patters suggesting similar modes of binding. Additionally the C-terminal substrate residues had multiple NOE contacts exceeding 10 angstroms relative to the static X-ray crystal structure (Figure 1). These could not all be fit simultaneously indicating multiple binding modes of the C-terminal residues.

Conclusions

Collectively, the results presented here lend structural evidence to at least a C-terminal rotation around the prolyl-peptide bond. Investigations are ongoing with other substrates such as the HIV-Capsid protein to aid in our understanding of the CypA mechanism.

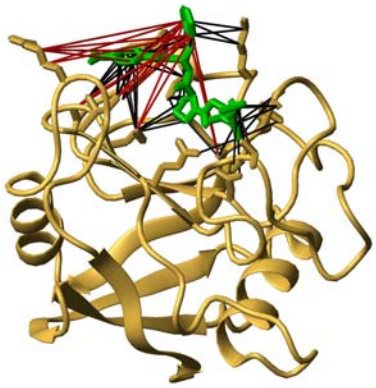

Figure 1: Intermolecular NOEs obtained between CypA and the substrate succinyl-A-G-F-P-pNA during turnover. NOEs are color coded based on their distance from the X-ray crystal structure recently determined (data not published) as more than 10 angstroms (red) and less than 10 angstroms (black).

References

Eichmuller, C. et al. (2001) J.Biolmol. NMR 21, 107-116.
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Howard, B.R. et al., Nature Structure Biology, (2003) **10**, 475-481.