Validating a Proposed Mechanism for Cyclophilin-A

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Introduction

Cyclophilin-A (CypA) is the prototypical cyclophilin family member responsible for peptidyl-prolyl isomerization inside most human cells. However, despite the roles of this enzyme in a range of functions from protein folding to signal transduction, the underlying mechanism of catalytic turnover has been elusive for decades. Together with the Vendruscolo laboratory, chemical shift based approaches were used to probe the conformational changes that occur within active CypA during catalysis of a model peptide with the discovery of a potential "electrostatic lever" mechanism to facilitate isomerization. In order to validate such a possibility, we specifically altered the dipole of the glycine residue preceding the proline in our model substrate from CO to CS (Sulfer replacing Oxygen in the carbonyl group). This ligand is NOT isomerized by CypA, thereby lending creedence to the proposed mechanism.

Experimental

Spectroscopy was performed on a solutions 600 MHz instrument at the NHMFL. Standard VNMR BioPack sequences were used to assign both active CypA during turnover as well as a catalyzed substrate for subsequent use in chemical shift based ensemble calculations. ZZ-exchange experiments were conducted with either 1 mM of the model peptide substrate, GSFGPDLRAGD, or with 1 mM of the thioamide substituted peptide that comprised a CS. The unmodified peptide was recombinantly made and purified as previously described[1] and the thioamide counterpart was synthesized with residue L7 15N labeled. Catalytic concentrations of CypA were used as indicated.

Results and Discussion

ZZ-exchange spectroscopy affords a relatively simple and quantitative means to probe CypA-mediated catalysis and was therefore used here. In the absence of CypA, only the respective Cis and Trans peaks are observed as can be seen for L7 of both the unmodified and CS modified peptide (Fig. 1, top). However, in the presence of catalytic amounts of CypA, exchange peaks arise that indicate catalysis and are only observed using the unmodified peptide.

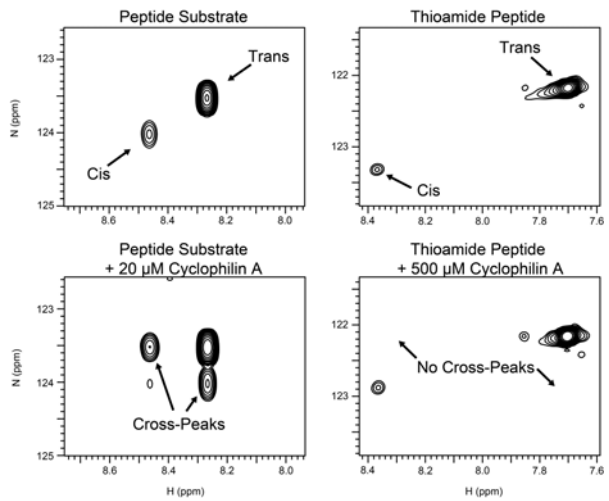

Figure 1. CypA does not catalyze the thioamide peptide. ZZ-exchange experiments of L7 amide of 1 mM 15N-labeled GSFGPDLRAGD (left) and thioamide counterpart labeled at L7 (right). The top panels are in the absence of CypA and the bottom panels are with CypA present at the indicated concentrations. The respective Cis and Trans conformations are indicated (top) and exchange peaks (bottom).

Conclusions

Altering the dipole on the glycine carbonyl preceding the catalyzed proline results in no measurable CypA-mediated peptidyl-prolyl isomerization, as we have recently published[2].

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References

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[2] Camilloni, C., *et al.,* Proceedings of the National Academy of Sciences of the United States of America, **111**, 10203-10208 (2014).