Crystal Structure and Catalytic Mechanism of Proline Racemase

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Amino acid racemases catalyze an otherwise extremely unfavorable reaction: the stereoinversion of the chiral α-carbon. Amino acid racemization allows cells to produce the D-enantiomers that participate in biological processes such as bacterial cell wall construction or neuro/endocrine signaling in mammals.

Proline racemase has been extensively studied as a model of pyridoxal-phosphate-independent amino acid racemases. We report the crystal structure of the proline racemase from *Trypanosoma cruzi*, which is also known to be a powerful B-lymphocyte mitogen [1].

The enzyme is a homo-dimer, with each monomer folded in two α/β domains separated by a deep crevice. In contrast with the accepted model of one symmetric reaction center per dimer [2], the crystal complex with a transition-state analog (pyrrole-2-carboxylic acid) reveals one competent catalytic site per monomer, buried in the inter-subunit crevices. Two cysteine residues are optimally located to perform acid/base catalysis through a carbanion stabilization mechanism. Crystallographic and calorimetric evidence prove that proline racemase undergoes a substrate-triggered closure of the inter-domain crevice, which might regulate the protein's mitogenic activity.

[1] Reina-San-Martin B., Degrave W., Rougeot C., Cosson A., Chamond N., Cordeiro-da-Silva A., Arala-Chaves M., Minoprio P., *Nature Med.*, 2000, **6**, 890. [2] Rudnick, G., Abeles, R.H., *Biochemistry*, 1975, **14**, 4515.

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