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Docking of Antizyme to Ornithine Decarboxylase and Antizyme Inhibitor using Experimental Mutant and Double-Mutant Cycle Data

Ori Cohavi[1], Dror Tobi[2] and Gideon Schreiber[1]*

[1]*Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel*

[2]*Department of Computer Science, Ariel University, Ariel, Israel*

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Antizyme (Az) is a highly conserved key regulatory protein bearing a major role in regulating polyamine levels in the cell. It has the ability to bind and inhibit ornithine decarboxylase (ODC), targeting it for degradation. Az inhibitor (AzI) impairs the activity of Az. In this study, we mapped the binding sites of ODC and AzI on Az using Ala scan mutagenesis and generated models of the two complexes by constrained computational docking. In order to scan a large number of mutants in a short time, we developed a workflow combining high-throughput mutagenesis, small-scale parallel partial purification of His-tagged proteins and their immobilization on a tris-nitrilotriacetic-acid-coated surface plasmon resonance chip. This combination of techniques resulted in a significant reduction in time for production and measurement of large numbers of mutant proteins. The data-driven docking results suggest that both proteins occupy the same binding site on Az, with Az binding within a large groove in AzI and ODC. However, single-mutant data provide information concerning the location of the binding sites only, not on their relative orientations. Therefore, we generated a large number of double-mutant cycles between residues on Az and ODC and used the resulting interaction energies to restrict docking. The model of the complex is well defined and accounts for the mutant data generated here, and previously determined biochemical data for this system. Insights on the structure and function of the complexes, as well as general aspects of the method, are discussed.
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Keywords: protein–protein interaction; docking; Ala scan mutagenesis; double-mutant cycles

Introduction

The pyridoxal 5′-phosphate-dependent enzyme ornithine decarboxylase (ODC) is a 106-kDa homodimer with molecular 2-fold symmetry. For activity, dimerization is essential as the two catalytic sites contain residues from both monomers.[1] ODC catalyzes the first and rate-limiting step in the biosynthesis of polyamines. It performs the decarboxylation of ornithine to putrescine, which is a precursor of the polyamines spermidine and spermine. These molecules have crucial roles in the cells: They are known to bind to RNA and chromosomal DNA and to participate in numerous processes, such as cell cycle, gene expression, apoptosis and embryonic development. They are essential for normal cell growth and proliferation.[2,3] Cells that are transformed by carcinogens,[4] oncogenes[5,6] and viruses[7,8] contain constitutively active ODC, and its overexpression can lead to transformation of cells.[9] These findings highlight the importance of polyamine regulation, which is executed by multiple pathways, such as biosynthesis, catabolism, uptake and excretion.[10] ODC activity is tightly controlled by its inhibitor—antizyme (Az), a 26-kDa intracellular protein that binds ODC as a monomer with high affinity.[11] Az–ODC interaction is possible due to the rapid equilibrium between ODC dimers and monomers.[12] This instability might be explained by the relatively small surface area (655 Å^2) that is buried upon dimer formation.[1] The interaction

*Corresponding author. E-mail address: gideon.schreiber@weizmann.ac.il.

Abbreviations used: Az, antizyme; ODC, ornithine decarboxylase; AzI, antizyme inhibitor; AZBE, Az-binding element; DMC, double-mutant cycle; NTA, nitrilotriacetic acid; SPR, surface plasmon resonance; PDP, protein docking potential; WT, wild type.

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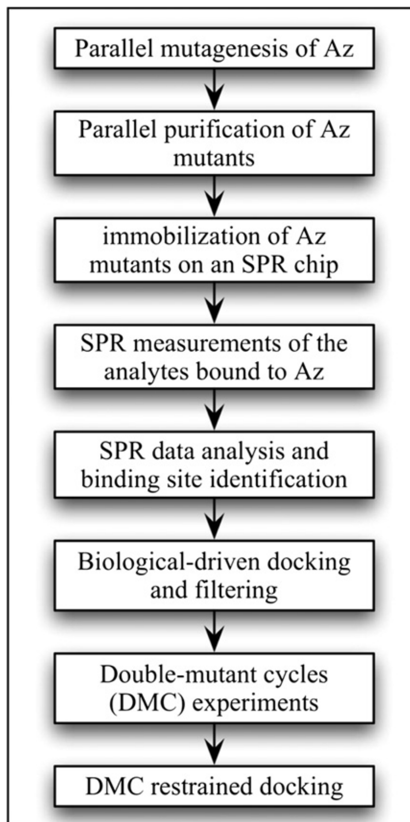

Fig. 1. Flowchart describing the methods to obtain docked structures between Az and ODC or AzI.

between Az and ODC inactivates the latter by trapping the inactive monomers[13] and by inducing degradation via the 26S proteasome in an ATP-dependent but ubiquitin-independent manner.[13,14] As a result, ODC is rapidly degraded, with a half-life of a few minutes.[15] Az itself is induced by high levels of polyamines in a rare mechanism involving +1 ribosomal frameshifting.[16] In this manner, polyamines limit their own concentration in the cell. The third component in this system is the Az inhibitor (AzI). It is a close homologue of ODC that lacks enzymatic activity but has the ability to bind Az with higher affinity than ODC.[10] In this manner, it prevents Az from binding and inhibiting ODC. Indeed, elevated levels of AzI were found in gastric tumors.[17] AzI is also rapidly degraded by the proteasome, but the degradation is ubiquitin dependent. Az stabilizes AzI upon interaction by inhibiting its ubiquitination.[10] Moreover, Az is known to bind the cell-cycle protein cyclin D1[18] and to inhibit the transporter for the uptake of extracellular polyamines.[19]

The high-resolution crystal structures of ODC[1] and AzI[20] and an NMR solution structure of Az (the conserved domain consisting of amino acids 87–227)[21] have been determined. Yet the location of the binding sites of ODC and AzI on Az and the structures of the two complexes remain unknown. Nevertheless, the available structures, combined with mutation and deletion studies, provided some clues to that matter. A proposed Az-binding element (AZBE) on ODC (residues 117–140) was shown to contain an electropositive patch that is partially buried in the ODC dimer (as seen in the crystal structure) but is proposed to be solvent accessible in the monomeric form (which binds Az).[22,23] The NMR solution structure of Az, on the other hand, reveals a well-conserved patch of negatively charged residues that was suggested to be important for Az binding. It should be noted, however, that other well-conserved surface-exposed residues are positively charged. On the opposite side of the protein, there are a few conserved surface-exposed hydrophobic residues. These residues form an accessible hydrophobic patch that was suggested as a possible site for interaction with the Az partners.[21] It is also known that only the C-terminal half of Az is important for binding.[24]

In this work, we implemented high-throughput Ala scan to produce biological data for precise docking of Az to AzI and ODC. First, we created a nonbiased panel of 41 single alanine mutants of Az and determined their binding kinetics and affinities toward ODC and AzI. Nine residues that participate in binding to both counterparts were found. The experimental data were used to dock Az to ODC and AzI. To refine the docking results, we measured double-mutant cycles (DMCs)[25] between five highly conserved surface residues on ODC and Az. It was previously shown that DMC information can be used for high-resolution docking as they provide distance constraints between residues, much like NMR data.[26] Indeed, implementing the residue–residue interaction energies as distance restraints

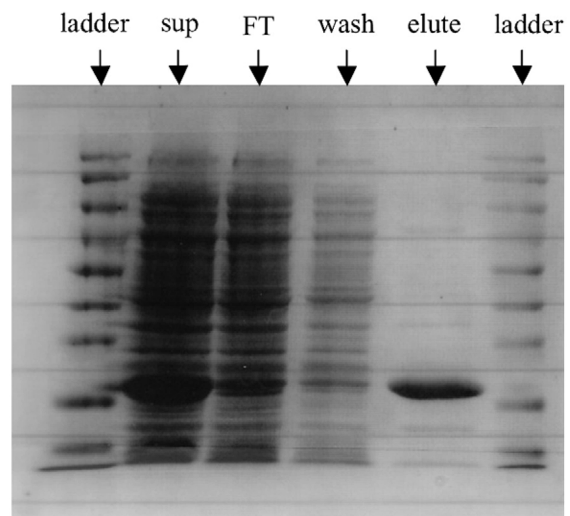

Fig. 2. Single-step purification of Az-V198A on a microcolumn loaded with Ni-charged resin. The Az-V198A gene was expressed in 15-ml media. The cells were centrifuged and resuspended in lysis buffer and then subjected to sonication and centrifugation. *sup*, supernatant; *FT*, flow-through; *wash*, proteins that were washed with binding buffer containing 15 mM imidazol; *elute*, proteins that were eluted with buffer containing 500 mM imidazol. All other Az mutants were purified in parallel (~12 mutants in each round) in the same manner.

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