Differential effects of ligand binding on protein dynamics

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Summary

The enzyme aminoglycoside phosphotransferase (3')-IIIa (APH) confers resistance against a broad range of aminoglycoside antibiotics. We use the Gaussian Network Model, a coarse-graining method, to investigate how the binding of nucleotide and antibiotic influences the dynamics and thereby the ligand binding properties of APH.

Introduction

APH catalyzes ATP-dependent phosphorylation of more than ten different aminoglycoside antibiotics, whereby the antibiotic's affinity for the ribosome is decreased. The crystal structure of APH is solved in its apo form, in complex with nucleotide,[1] and in complex with nucleotide and kanamycin A or neomycin B.[2] Interestingly, in NMR experiments, the dynamics differs significantly in the various APH complexes,[3] although the crystallographic studies indicate that no larger conformational changes occur upon ligand binding. A complete exchange of backbone amides is observed within 15h of exposure to D_2O in the apo form. While antibiotic binding leads to significant stabilization, nucleotide binding to the APH-aminoglycoside complex decreases the protection and renders several amides of β-sheet residues exchangeable.

Materials and Methods

A Gaussian Network Model[4] was applied. The nodes of the elastic network are grouped into dynamic domains based on the correlations of fluctuations. Details are described elsewhere.[5]

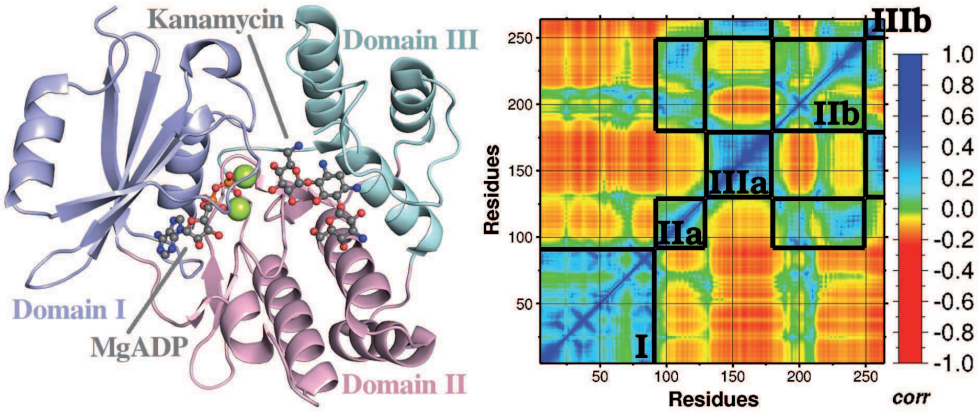

Figure 1: Definition of three dynamic domains of APH based on correlations of residue fluctuations. The residues of one domain are positively correlated to each other, while they are anticorrelated to residues of the other domains. MgATP binds between domains I and II, the antibiotic binds between domains II and III.

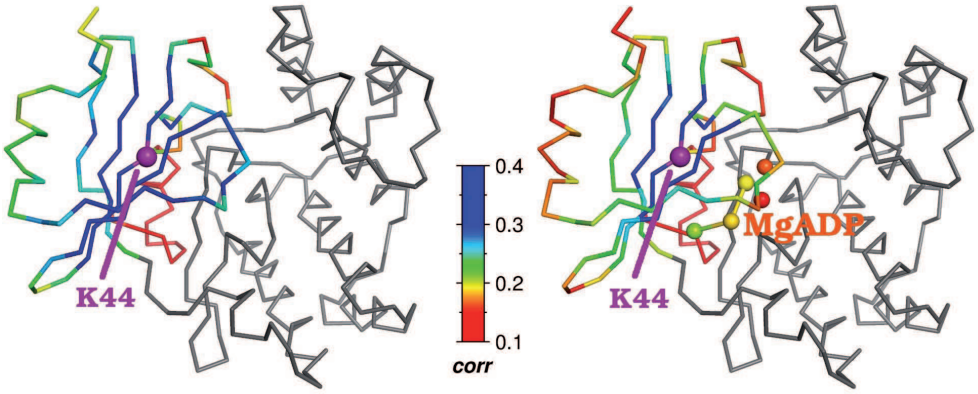

Figure 2: Correlations of nodes of domain I to the node representing K44. The correlations are higher in the absence of nucleotide (left side).

Results

Based on the correlations of fluctuations of apo- APH, three dynamic protein domains are identified (Figure 1). Ligand binding between the dynamic domains reduces the correlation within the domains (Figure 2) and reduces the flexibilities of residue nodes which are located near the ligand.

Conclusions

Stabilization of APH due to antibiotic binding can be explained by reduced residue

flexibilities upon ligand binding. But the surprising destabilization of β-sheet residues upon nucleotide binding shows that not only the number of closest neighbors, but the overall protein architecture is important for the dynamical properties. Nucleotide binding disturbs the rigid-body movement of domain I, manifested by reduced correlations, and weakens hydrogen bonds between the strands of the β-sheet. The differential effects of antibiotic and nucleotide binding on dynamics arise from structural differences between the binding domains I and III. While the antibiotic binding loop of domain III is very flexible and can be stabilized by additional connections to the ligand, the β-sheet of domain I underlies many constraints even in the absence of ligand. Nucleotide binding leads to frustration. The arrangement of the protein into three anticorrelated dynamic domains with different rigidities allows for the tuning of APH dynamics upon ligand binding, which is a key factor in explaining the substrate promiscuity of the enzyme.

Acknowledgements

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