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Crystallographic and Computational Studies of the Metal Ion Binding Properties of Parvalbumin

by

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Abstract

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An astonishing number of important physiological processes are regulated by the small alkaline earth metal, calcium. Regulatory Ca^{2+}-binding proteins must be able to distinguish Ca^{2+} ions in the presence of greater concentrations of other metal cations, such as Mg^{2+}, Na^{+} and K^{+}. The EF-hand family is a large class of Ca^{2+}-binding proteins that displays this sort of preferential Ca^{2+}-binding. The secondary and tertiary structure of the EF-hand metal ion binding site is highly conserved from one member of the family to the next. Because of this conservation, we can use the small, amenable, EF-hand protein, parvalbumin, as a model system to study the mechanisms that define the metal ion affinities and specificities of EF-hand Ca^{2+}-binding sites in general.

Our collaborator, Dr. James Potter, has designed a mutant to test directly the role of the last coordinating residue in the EF-hand binding site, the PVEF-E101D parvalbumin mutant. The crystal structures of both the Ca^{2+}- and Mg^{2+}-bound complexes of PVEF-E101D have been determined. The PVEF-E101D mutant displayed a 100-fold decrease in the binding affinity for Ca^{2+}, and the Mg^{2+}-binding affinity was increased 10-fold. Moreover, the Ca^{2+} off-rate escalated from 1 s^{-1} in wild-type parvalbumin to 600 s^{-1} in the PVEF-E101D mutant. The conformation of the

mutated EF-hand in the PVEF-E101D/Mg^{2+} structure was typical of a Mg^{2+}-bound EF-hand, with the exception of an F helix movement of ~1 Å toward the bound cation that allowed the shorter aspartate residue to coordinate the Mg^{2+} ion. The PVEF-E101D/Ca^{2+} structure showed that the aspartate residue is unable to bind Ca^{2+} in the bidentate mode normally adopted by the wild type glutamate. The resulting sixfold Ca^{2+} coordination in the mutant is usually characteristic of Mg^{2+}-bound EF-hands, and this finding indicates that the binding loop is not sufficiently flexible to allow the aspartate residue to move in far enough to offer bidentate ligation of the Ca^{2+} ion.

Two MD simulations were used to further investigate the relationship between the last coordinating residue of the EF-hand binding loop and the overall plasticity and flexibility of the loop region. The first simulation, called Alchemy, simulated the transition from Ca^{2+} to Mg^{2+} coordination through varying the van der Waals parameters for the bound metal ions. The glutamate at position 12 was accurately and reversibly predicted to be the source of bidentate ligation of Ca^{2+} in our simulations. A second simulation, the Aspartate simulation, produced results that correlated well with the experimental result that an E101D substitution at EF loop position 12 resulted in monodentate Ca^{2+} coordination. The F helix was able to move in to the binding cavity during the simulation to allow one aspartate oxygen to bind the Ca^{2+} ion, but the aspartate was unable to achieve a favorable orientation for bidentate Ca^{2+} coordination. The findings indicate that the interplay between the last coordinating residue of the loop, and the plasticity, or flexibility, of the binding loop, to a great extent determines the species of cations that are allowed to bind in a particular EF-hand site.

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properties. Finally, Mike Berry and Emai Ho, who worked on this project before I joined the lab, determined the structure of the F102W/Ca^{2+} mutant complex, and performed all but the final few rounds of refinement on the PVEF-E101D/Mg^{2+} complex.

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