Public Abstract
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Graduation Term:SP 2011
Department:Biochemistry
Degree:MS
Title:DIVALENT ION-BINDING AND THERMAL STABILITY STUDIES ON RAT -PARVALBUMIN AND THE EVIDENCE OF INFLUENTIAL DISTANT AMINO ACID RESIDUES AFFECTING CD SITE ION AFFINITY

EF-hand proteins participate in a vast number of signaling pathways. This large family of well over 250 member proteins and 45 subfamilies is largely responsible for mediating the critical role of calcium ions and their concentrations in biological systems. These proteins contain a characteristic calcium ion-binding motif known as the EF-hand. This motif combines a central ion-binding loop, flanked by short helical elements. Certain EF-hand proteins, such as calmodulin and troponin C, perform regulatory roles, whereas others function as cytosolic calcium buffers, e.g., calbindin and parvalbumin (PV).
PVs are vertebrate-specific proteins, which harbor two EF-hand motifs known as the CD and EF sites. Although the CD and EF sites are typically high-affinity sites, the mammalian Î²-PV exhibits highly attenuated divalent ion-affinity. The physical basis for this attenuation remains unclear. A clarification of this behavior could advance our understanding of EF-hand protein structure-affinity relationships.
Rat Î²-PV and chicken parvalbumin 3 (CPV3) are identical at 74 of 108 residues. Interestingly however, they exhibit distinct divalent cation-binding properties. The overall free energy of binding values for calcium and magnesium ion binding are 2.03 and 3.92 kcal/mol, respectively. This is largely due to the fact that the CD site binds calcium and magneisum ions with significant less affinity than does teh EF site. This divergent metal ion-binding behavior in proteins exhibiting 69% sequence identity provides a good model system for investigating the influence of protein structure on divalent ion-affinity.
The question arises as to whether the difference in divalent ion-binding affinity in these proteins derives from local differences in and around the immediate binding site, or whether remote structural determinants play a role. To address this matter, site-directed mutagenesis was performed on rat Î²-PV at positions 49, 50, 57, 58, 59, and 60. Four variant proteins were produced, they are identified with Roman numerals I through IV. Variant I harbored the F49I and I50L substitutions; variant II had additional changes at Y57F and L58I. A mutation at D59E was added to make variant III. Variant IV housed a G60E mutation and all the previously mentioned substitutions making it identical to CPV3 at 27 of 30 residues at the CD site. Divalent ion affinity and thermal stability were evaluated for each of the variants using isothermal titration calorimetry and differential scanning calorimetry, respectively. All four variant proteins exhibited increased divalent ion affinity in relation to wild-type rat Î²-PV. All of the variants exhibited an increase in melting temperature. However, the increases in the calcium ion free state indicating heightened stability were small in comparison to CPV3. These findings suggest that structural determinants outside the metal ion-binding motif significantly affect the attenuated binding affinity observed at the CD site in rat Î²-PV.