DIVALENT ION-BINDING AND THERMAL STABILITY STUDIES ON RAT β-PARVALBUMIN: EVIDENCE THAT REMOTE AMINO ACID RESIDUES INFLUENCE CD SITE ION AFFINITY

Kelly Ndubuka

Dr. Michael T. Henzl, Thesis Supervisor

ABSTRACT

Parvalbumins (PVs) are vertebrate-specific proteins (M_r 12,000), 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 β-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.

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 β-PV at positions 49, 50, 57, 58, 59, and 60, 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. The mutations resulted in an increase in melting temperature. However, the increases in the Ca^{2+}-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 β-PV.