Probing the Reaction Mechanism of Co(II)-substituted VanX

Michael W. Crowder[1], Megan L. Matthews[1], Robert M. Breece[1], Alison Costello[2], Brian Bennett[3], Tara K. Sigdel[1], and David L. Tierney[2]

[1]Department of Chemistry and Biochemistry, Miami University, [2]Department of Chemistry, University of New Mexico, and [3]National Biomedical EPR Center, Department of Biophysics, Medical College of Wisconsin

VanX is a Zn(II)-dependent, *D*-ala-*D*-ala dipeptidase that is required for high-level vancomycin resistance in bacteria. In an effort to structurally probe the metal binding site in VanX, electronic absorption, EPR, and EXAFS spectroscopic studies were conducted on Co(II)-substituted VanX. Electronic spectroscopy revealed the presence of Co(II) ligand field transitions that had molar absorptivies of *ca.* 100 $M^{-1}cm^{-1}$, which suggests that Co(II) is five-coordinate in Co(II)-substituted VanX. Low temperature EPR spectra of Co(II)-substituted VanX were simulated using spin Hamiltonian parameters of $M_S = |\pm{}^1/_2\rangle$, $E/D = 0.14$, $g_{\mathrm{real(x,y)}} = 2.37$, and $g_{\mathrm{real(z)}} = 2.03$. These parameters lead to the prediction that Co(II) in the enzyme is five-coordinate and that there may be at least one solvent-derived ligand. Single-scattering fits of EXAFS data indicate that the metal ions in both native Zn(II)-containing and Co(II)-substituted VanX have the same coordination number and that the metal ions are coordinated by 5 N/O ligands at ~ 2.0 Å. These data demonstrate that Co(II) (and Zn(II) from EXAFS studies) is five-coordinate in VanX. Stopped-flow UV-Vis and rapid-freeze quench EPR spectroscopic studies have been used to probe the reaction of Co(II)-substituted VanX with *D*-ala-*D*-ala.