Comparison of the Native Zn(II) and Co(II)-substituted Active Sites For Protein Farnesyltransferase and Cobalamin-Dependent Methionine Synthase.

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The enzymes protein farnesyltransferase (FTase) and cobalamin-dependent methionine synthase (MetH) are two examples of a growing class of alkyl-transfer enzymes that have an active site zinc ion necessary for catalysis. Because Zn(II) has a d^{10} electron configuration, it is often replaced with Co(II) for spectroscopic studies. The electronic properties of Co(II) make it ideally suited for further spectroscopic investigation of the active site of these enzymes. For both enzymes, optical absorption spectroscopy of the Co(II)-substituted enzyme has previously been used to characterize the reaction. Although the Co(II)-substituted active site is generally assumed to be isostructural with the native Zn(II) active site, this assumption has not been tested. We have used extended x-ray absorption fine structure (EXAFS) to compare directly the structures of the Zn(II) and Co(II) active sites in these enzymes in the resting state, in the substrate-bound structure, and in the product bound form. Cobalt EXAFS data reveal structural differences between the Co(II) and Zn(II) forms, but confirm earlier Zn-EXAFS data showing that the substrate thiolate, but not the product thioether, bind directly to the metal. Electron nuclear double resonance (ENDOR) spectroscopy of Co(II)-substituted FTase confirm that there are dramatic structural changes when substrate binds, and suggest that a water is coordinated to the Co(II) in the resting state of the enzyme.