Lanthanide Spectroscopic Studies of Mg(II)-Dependent *PvuII* Restriction Endonuclease

Lori M. Bowen, Gilles Muller, James P. Riehl, and Cynthia M. Dupureur

Department of Chemistry and Biochemistry, University of Missouri St. Louis, and Department of Chemistry, University of Minnesota Duluth.

Type II restriction enzymes are homodimeric systems which bind 4-8 base pair palindromic recognition sequences of DNA and catalyze metal ion-dependent phosphodiester cleavage. While Mg(II) is required for cleavage in these enzymes, in some systems Ca(II) promotes avid substrate binding and sequence discrimination. These properties make them useful model systems for understanding the roles of alkaline earth metal ions in nucleic acid processing. We have previously shown that two Ca(II) ions stimulate DNA binding by *Pvu*II endonuclease, and that the trivalent lanthanide ions Tb(III) and Eu(III) support subnanomolar DNA binding in this system. Here we capitalize on this behavior, employing a unique combination of luminescence spectroscopy and DNA binding assays to characterize Ln(III) binding behavior by this enzyme. Upon excitation of Tyr residues, the emissions of both Tb(III) and Eu(III) are enhanced several-fold. This enhancement is reduced by the addition of a large excess of Ca(II), indicating that these ions bind in the active site. Poor enhancements and affinities in the presence of the active site variant E68A indicate that Glu68 is an important Ln(III) ligand, similar to that observed with Ca(II), Mg(II), and Mn(II). At low micromolar Eu(III) concentrations in the presence of enzyme (10-20 uM), Eu(III) excitation $^7F_o \rightarrow {}^5D_o$ spectra yield one dominant peak at 579.2 nm. A second, smaller peak at 579.4 nm is apparent at high Eu(III) concentrations (150 uM). Titration data for both Tb(III) and Eu(III) fit well to a two-site model featuring a strong site (K_d 1-3 uM) and a much weaker site ($K_d \approx$ 100-200 uM). Experiments with the E68A variant indicate that the Glu68 sidechain is not required for the binding of this second Ln(III) equivalent; however, the dramatic increase in DNA binding affinity around 100 uM Ln(III) for the wildtype enzyme and metal-enhanced substrate affinity for E68A are consistent with functional relevance for this weaker site. This discrimination of sites should make it possible to use lanthanide substitution and lanthanide spectroscopy to probe individual metal ion binding sites, thus adding an important tool to the study of restriction enzyme structure and function.