

X-Ray Absorption Studies of the Restriction Enzyme from *Bacillus Species (BslI)*

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Restriction endonucleases are excellent model systems for studying protein-DNA interactions because of their high specificity towards their recognition sequence. They are part of the restriction-modification (R-M) systems of bacteria, and serve as a primitive immune system against bacteriophage infection. The phage DNA is cleaved with high specificity, while the host DNA is protected by a specific methylation within the recognition sequence. High specificity is vital in R-M systems; accidental cutting of the host DNA at non-specific sequences would result in self-destruction. This high specificity gives restriction endonucleases an important role in recombinant DNA technologies and in the diagnostics of certain genetic diseases.

BslI is unique among restriction endonucleases in that it is the first known endonuclease that contains Zn. It is also the first endonuclease in which the recognition and cleavage sites are on two separate subunits. Subunit α , which is believed to be the DNA recognition domain, has two to three Zn binding sites as revealed by atomic absorption studies. The α subunit is rich in cysteines and sequence comparison against the SWISSPROT database shows matches with several Zn finger motifs. Our preliminary X-ray absorption spectroscopic (XAS) data of the full enzyme are most consistent with the presence of Zn(Cys)₄ type Zn fingers. Since the sequence also includes several histidines, further XAS studies, in conjunction with mutagenesis studies, are necessary to investigate their possible involvement in Zn binding.

Surprisingly, the X-ray absorption spectroscopic (XAS) data of the full enzyme differs from that of the α subunit. According to our data the α subunit contains two Zn fingers and the full protein has an additional Zn site. Mutagenesis studies identified a possible site in the β subunit. An interesting possibility is that the third Zn site is shared between the two subunits and it is involved in protein-protein interactions. XAS studies are under way on the β subunit to see whether it has any Zn site.