

Crystal Structure of the Complex of the DNA-Binding Domain of the Intron-Encoded Endonuclease I-TevI with its Homing Site

P. Van Roey, C.A. Waddling, K.M. Fox, M. Belfort, and V.Derbyshire (Wadsworth Center)

Abstract No. Van2738

Beamline(s): X12C

Introduction: I-TevI is a 28-kDa homing endonuclease encoded by the *td* intron of bacteriophage T4. The enzyme binds a long (37 base pairs) DNA target, exhibiting high sequence tolerance. I-TevI consists of two functionally distinct domains, an N-terminal catalytic domain and a C-terminal DNA-binding domain, connected by a long flexible linker. The isolated DNA-binding domain, residues 130 to 245, contacts a 20 base pair region of the homing site and binds with the same affinity as the full-length protein.

Methods and Materials: The crystal structure of the DNA-binding domain of the intron encoded endonuclease I-TevI in complex with a 21-base pair DNA duplex, representing its homing site was has been determined by SIRAS methods. DNA modified by replacing four thymidines by 5-iodouridine was used as the heavy atom derivative. The derivative data were measured at a wavelength of 1.5 Å to increase the anomalous scattering of the iodine derivative while the native data were measured at 1.1 Å. The native data were measured to 2.2 Å resolution using the Brandeis B1 detector, R_{mer} 0.059, 99.4% completeness, $\langle I/\sigma(I) \rangle = 3.8$ in the high-resolution shell. The structure was refined (CNS) to an R of 0.215 and an R_{free} of 0.248.

Results: The protein interacts with the DNA along the full length of the duplex, wrapping around it along the minor groove. It includes three different DNA-recognition subdomains connected by long linkers that lack secondary structure. The three subdomains are a zinc-finger, an α -helix that binds in the minor groove and a helix-turn-helix domain that contacts the major groove.