OVERVIEW

Overall Summary of this Ph.D. thesis

The discovery of the first naturally occurring Z-DNA binding protein domain, Zα, at the N-terminus of the mammalian RNA editing enzyme ADAR1 raised questions concerning the structural and functional characteristics that allow Zα to specifically interact with Z-DNA. In this Ph.D. thesis, the solution structure of human Zα was determined with high coordinate precision by multi-dimensional NMR spectroscopy. The structure was set in a functional context by scanning mutagenesis, interaction mapping and biochemical binding studies. By using site-directed mutagenesis, a number of residues were identified that diminished binding to Z-DNA when mutated to alanine. In conjunction with the complementary data from interaction mapping, these results allowed one to determine the interaction surface between Zα and Z-DNA in solution. Residues from helix α3 and the C-terminal β-sheet form a contiguous, positively charged surface on Zα that is suitably shaped to bind to the negatively charged backbone of Z-DNA. Binding studies by analytical ultracentrifugation and surface plasmon resonance spectroscopy showed that two Zα domains bind to one $d(CG)_3T_4(CG)_3$ hairpin in the Z-DNA conformation with a K_d of 30 nM.

Comparison with the crystal structure of Zα complexed with Z-DNA [6] showed that seven of a total of nine Z-DNA contacting residues are prepositioned in the solution structure of unbound Zα, though they are exposed on the protein surface. The prepositioned residues showed a large decrease in the free energy of binding ($\Delta\Delta G$) when mutated to alanine, while the flexible Z-DNA contacting residues showed no effect. This suggests that Zα uses prepositioned residues to minimize the entropic cost of binding.

Searching the structural database for similar proteins with the program DALI [206] revealed a number of structurally homologous (α+β)HTH DNA binding proteins, such as histone H5, CAP, DtxR, E2F-4 and others. Comparison of the solution structure of Zα with the crystal structure of these homologues complexed with cognate B-DNA suggests that Zα is disfavored from binding to B-DNA by steric hindrance at two sites. Firstly, Zα causes steric hindrance with the minor groove of B-DNA because it contains an extended helix α1 that is further elongated by a prehelix. Secondly, the aromatic ring of Y177 of Zα collides with the major groove of B-DNA in the superposition with some homologous protein/B-DNA complexes. Consequently, Zα may bind to Z-DNA rather than B-DNA firstly because it possesses a suitably shaped binding surface for Z-DNA, and secondly because binding to B-DNA is disfavored by steric hindrance.