Chapter 7

BIOLOGICAL IMPLICATIONS OF THE Zα STRUCTURE

Structural homologues of Zα

A structural similarity search of the protein structure database using the program DALI [206] uncovered numerous α+βHTH DNA binding proteins with highly significant Z-scores (table 11). Of the 14 similar α+βHTH domains, 11 bind to B-DNA indicating that structural and functional homology correlate well for this fold. Zα and its B-DNA binding homologues agree well in the arrangement of DNA contacting residues in α3, but Zα lacks DNA contacts at the N-terminus of α1 (fig. 47). Furthermore, Zα mediates three DNA contacts through its loop between β2 and β3, whereas the B-DNA binders form only one DNA contact. Another difference is that Zα forms only water mediated backbone/Z-DNA interactions through the N-terminus of α2, where B-DNA binding α+βHTH domains have direct side chain/DNA interactions. Overall, Zα and its B-DNA binding homologues agree well in the arrangement of DNA contacts in the recognition helix α3 and agree partially in those of the C-terminal β-sheet, but differ in those of α1 and α2.

table 11 **Structural homologues of Zα**

PDB ID	Z-score[1]	Rmsd [Å]	LALI[2]	Protein	α1 length
-	12.9	0.9	63	Crystal structure of Zα bound to Z-DNA	-
1smt-A	9.7	1.6	58	Transcriptional repressor SmtB	very short
1hst-A	8.7	1.9	61	Histone H5	very short
1lea	8.4	2.0	62	LexA repressor	short
1bia	7.5	2.2	57	BirA biotin operon repressor	short
1cf7-A	7.2	2.0	60	Transcription factor E2F4	short
2cgp-C	7.1	1.9	58	Catabolite gene activator protein (CAP)	very short
1bja-A	6.9	1.4	56	Transcription regulator MotA	short
1opc	6.7	2.5	60	Omp repressor	short
2fok-A	6.3	1.8	57	FokI restriction endonuclease [3]	no DNA
1xgs-A	5.9	1.7	51	Methionine aminopeptidase [3]	no DNA
1ecl	5.7	2.3	59	Topoisomerase I [3]	no DNA
1bm9-A	5.7	2.2	58	Replication terminator protein (RTP)	short
2tdx	5.6	1.8	59	Diphtheria tox repressor (DtxR)	very short
-	5.3	2.3	57	Hepatocyte nuclear factor 3γ (HNF3γ)	very short

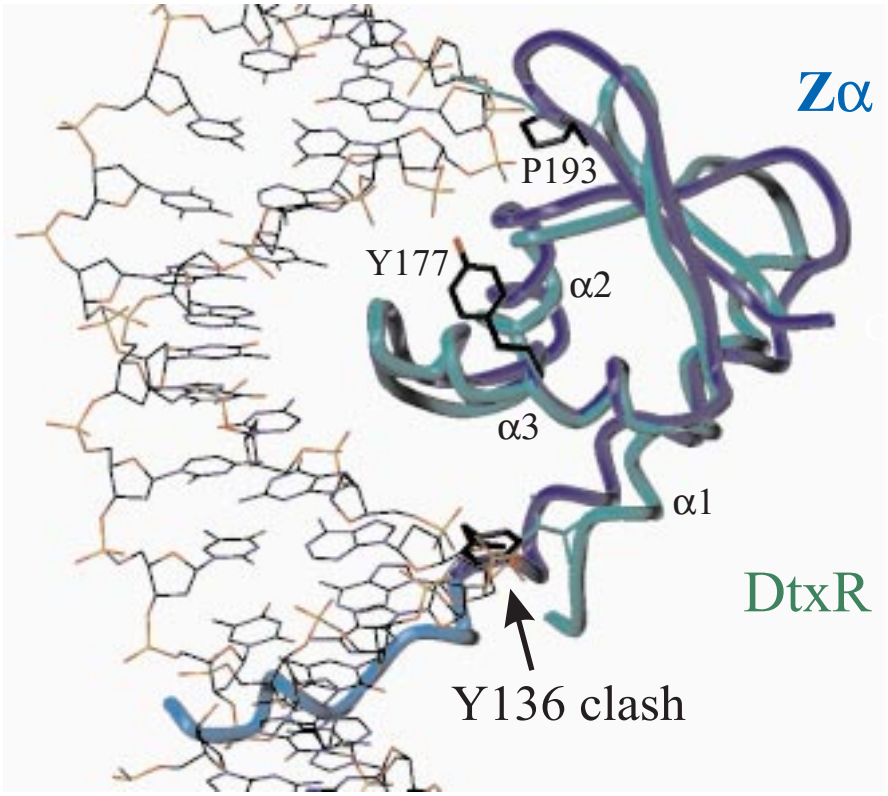
[1]The Z-score, calculated using the program DALI [206], describes the similarity between structures. Protein domains with Z-scores < 2.0 are structurally dissimilar.
[2]Length of equivalenced residues. The Zα core domain (63 residues) served as input.
[3]Function other than DNA binding.

Steric hindrance disfavors B-DNA binding by Zα

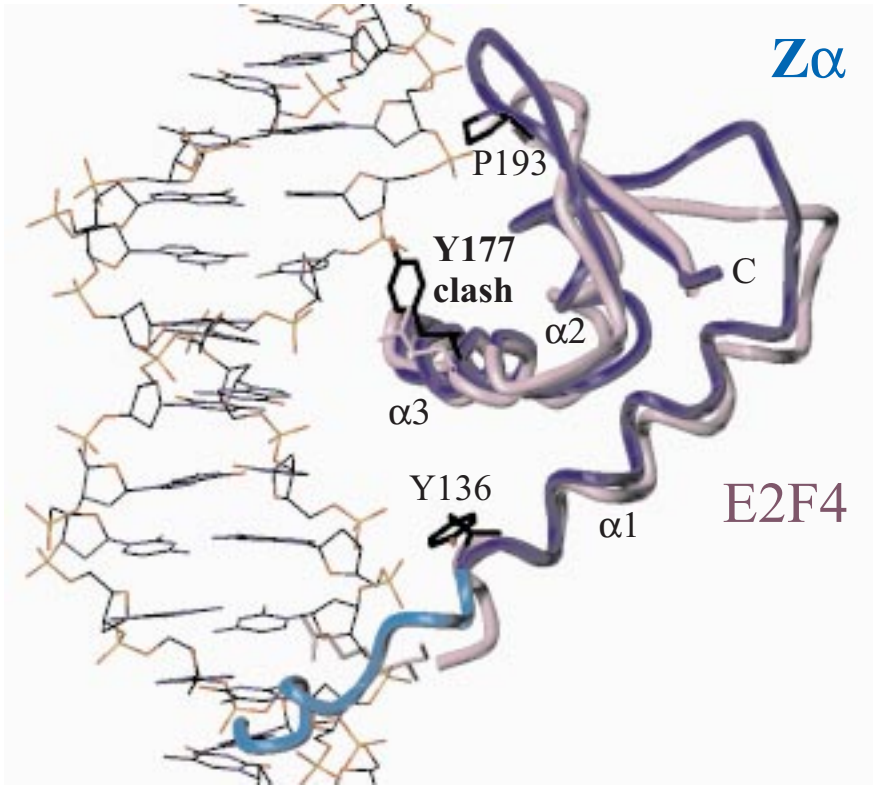
Searching for structural discriminants that distinguish between B- and Z-DNA recognition by α+βHTH domains, the 11 B-DNA binding homologues of table 11 were superimposed with Zα based on the residue matches in α3, β2 and β3 suggested by DALI. Five of them show a helix 1 shorter by one turn or more than Zα (designated 'very short' in the final column of table 11), and six show a helix 1 shorter by less than one turn in the superposition (designated 'short'). Diphtheria toxin repressor (DtxR) is a suitable example for the 'very short α1' class because it uses nine residues to form contacts to the B-DNA backbone and only one residue for base-specific contacts [39], very similar to Zα. The superposition of the lowest energy NMR structure of Zα with the crystal structure of the DtxR/DNA complex shows that DtxR differs from Zα in having a helix α1 shorter by ~1 turn (fig. 53a). The residues of DtxR preceding α1 are bent out of the way, whereas the N-terminal residues of Zα, including Y136, clash with B-DNA in the minor groove (fig. 53a). Y136 shows long-range NOE and is defined in all of the three complexes in the asymmetric unit of the crystal structure [6]. Therefore Y136 can only bend out of the way at the expense of free energy of binding to accommodate B-DNA binding. In contrast, Y136 and the prehelix have ample space in the distinct binding geometry of the $(Z\alpha)_2$/Z-DNA complex. The superposition of Zα with the crystal structures of the HNF3γ/DNA [36] and the CAP/DNA complex [37], further members of the 'very short α1' category, also show steric hindrance between Y136 of Zα and the minor groove of B-DNA. Consequently, these comparisons suggest that steric hindrance through the extended helix 1 of Zα may disfavor B-DNA binding by Zα in the binding mode of the 'very short α1' class of α+βHTHs.

The superposition of Zα with the crystal structure of the E2F4/B-DNA complex [38] (fig. 53b) shows that E2F4 belongs to the 'short α1' class of α+βHTHs. Here the N-terminus of α1 of Zα does not collide with the minor groove. Steric hindrance with the prehelix of Zα may be circumvented by rearranging the loosely folded prehelix. However, the aromatic ring of Y177 of Zα clashes with a phosphate in the major groove. Y177 is the only residue in the crystal structure of the $(Z\alpha)_2$/Z-DNA complex mediating a van der Waals contact with a base in the *syn* conformation specific for Z-DNA. Moreover, in CD spectroscopy experiments, the Y177A mutant showed a significantly reduced ability to bind specifically to Z-DNA and stabilize this left-handed DNA conformation [5]. Taken together, these data suggest that B-DNA binding by Zα may be also disfavored in some cases due to steric hindrance through the phenolic ring of Y177. Indeed, it may be possible to replace Y177 with a more flexible residue to produces a protein that can bind both B- and Z-DNA. The lack of conservation of Y177 in other Zα family members is thus of great interest. Domains capable of recognizing both B- and

fig. 53 **Steric hindrance disfavors B-DNA binding by Zα (see next page).** ***a***, The superposition of the lowest energy structure of Zα (blue) with the crystal structure of diphtheria toxin repressor (DtxR in green) complexed with B-DNA shows that the N-terminus of Zα (residues Y136) and possibly also the prehelix of Zα (light blue) cause steric hindrance with B-DNA in the minor groove. For reference, the B-DNA contacting residues of DtxR close to Y136 and P193 of Zα are shown in green. ***b***, The superposition of Zα with the E2F4/DNA complex (pink) shows that Y177 of Zα may clash with the B-DNA backbone in the major groove. The B-DNA contacting residues of E2F4 corresponding to Y136, Y177 and P193 of Zα are represented in pink. P193 is within van der Waals distance to the B-DNA in both superpositions.


Zα
P193
Y177
α2
α3
α1
DtxR
Y136 clash

(A)


Zα
P193
Y177
clash
C
α2
α3
Y136
α1
E2F4

(B)

Z-DNA may bind initially in a B-DNA sequence-specific fashion, e.g. to initiate transcription. The ability to bind Z-DNA allows the interaction with DNA to persist even when negative supercoils arising from the action of enzymes, such as RNA polymerase, disrupt the B-DNA specific interaction. In this manner targeting can be maintained.

In conclusion, the specificity of Zα for left-handed Z-DNA probably results from two different structural mechanisms: B-DNA binding by Zα is disfavored by steric hindrance. Second, Z-DNA binding by Zα is favored because seven of the nine Z-DNA contacting residues are prepositioned to bind the distinct backbone of Z-DNA. These structural modifications of the α+βHTH fold may enable Zα to recognize Z-DNA in the presence of excess B-DNA in the nucleus of a living cell.

Summary

The Zα domain shows close structural homology to several (α+β)HTH DNA binding protein domains which all bind to right-handed B-DNA. Superposition of Zα with the crystal structures of four homologous (α+β)HTH/B-DNA complexes suggests that binding of Zα to B-DNA is disfavored by steric hindrance through the extended helix α1 of Zα and in some cases through the rigid aromatic ring of Y177. Since Zα contains a prepositioned binding surface for Z-DNA, it prefers to bind to Z-DNA rather than B-DNA. However, the minute structural differences between Zα and its B-DNA binding homologues raise the possibility that Zα-related domains may bind to both B- and Z-DNA.