Hypothesis

Structural parsimony in endonuclease active sites: should the number of homing endonuclease families be redefined?

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Abstract Homing endonucleases are classified into four families based on active site sequence motifs. Through structural comparisons we have found structural similarities between the endonuclease domain of colicin E9, an H-N-H motif-containing enzyme, and both the non-specific nuclease from *Serratia* and I-*Ppo*I, a His-Cys box-containing homing endonuclease. Our comparison identifies conservation at the heart of all three enzyme active sites and so argues for a re-classification of H-N-H and His-Cys box homing endonucleases as a single family. We suggest the 'ββα-Me family' of homing enzymes to reflect the three elements of secondary structure and the metal ion that define the motif.

Key words: Colicin E9; Nuclease active site motif; Homing endonuclease

1. Introduction

Homing endonucleases are found in all three biological kingdoms encoded by genes within mobile, self-splicing introns and inteins. Structural similarities have recently been discussed for the active site regions of the highly sequence specific homing endonuclease from *Physarum polycephalum* I-*Ppo*I and the non-specific endonuclease from *Serratia marcescens*, and a common catalytic mechanism has been proposed [1,2]. The structure of the T4 endonuclease VII probably also shares this active site motif [3]. Now the recently published structure of the colicin E9 DNase domain, in complex with its cognate immunity protein Im9 [4], identifies yet another endonuclease with the same fold at the active site. Colicin E9 and its homologue colicin E7 [5] were the first structures solved for the H-N-H motif found in homing endonucleases (Fig. 1a), enzymes previously classified into four families based on amino acid sequence motifs present in their active sites (H-N-H, His-Cys box, GIY-YIG and LAGLI-DADG) [6]. As with the His-Cys box enzymes, the H-N-H endonuclease family is also composed of highly sequence specific endonucleases such as I-TevIII as well as relatively non-specific enzymes such as the DNase colicins [6].

2. Structural comparison of the active sites

Although the overall fold of the three nucleases is different and there is no sequence homology, a stretch of 22 amino acids containing two β-strands and an α-helix that make up their active sites superimpose with an r.m.s. deviation of 1.2 Å for the main chain atoms of the E9 DNase domain with I-*PpoI* and 1.5 Å for the E9 DNase with the *Serratia* nuclease (Fig. 1b) (I-*Ppo*I and *Serratia* nuclease superimpose with an r.m.s.d. of 1.14 Å as reported by Friedhoff et al. [1]). This suggests that this conserved structural motif is highly effective for DNA cleavage since it has evolved in three otherwise very different nucleases.

In the centre of this ββα motif is a divalent metal ion located in the same position in all three nucleases. This is identified as Mg^{2+} in I-*Ppo*I and *Serratia* nuclease and Ni^{2+} in the colicin E9 DNase structure (although the physiologically relevant ion in the E9 DNase is more likely to be Zn^{2+} [5,7]). Interestingly, the identity of the metal ion is governed by conservative asparagine-for-histidine substitutions in the helix of the motif (Fig. 1c,d). Catalytically important residues seem to be conserved and displayed in a similar manner. For example, His-103 which co-ordinates a phosphate molecule in the E9 DNase is equivalent to His-98 and His-89 in I-*Ppo*I and *Serratia* nuclease, respectively, where it is postulated to stabilise the negative charge on the pentaco-ordinate phosphate in the transition state for phosphodiester cleavage. Another conserved feature is an arginine, Arg-61 in I-*Ppo*I, Arg-57 in *Serratia* nuclease and Arg-5 in the E9 DNase. This residue points into the active site and co-ordinates either the inorganic phosphate in the E9 DNase structure or the phosphate backbone of bound DNA (in I-*PpoI*). This arginine has already been identified by mutational studies to be catalytically important in *Serratia* nuclease [1] and is conserved throughout the DNase-type colicins. Lastly, Asn-118 in the E9 DNase, the conserved asparagine of the H-N-H motif itself which is involved in forming stabilising hydrogen bonds within the active site (Fig. 1a), is conserved in *Serratia* nuclease (Asn-110). In I-*Ppo*I it is substituted by a histidine residue (His-110) and is part of the His-Cys box co-ordinating a structural zinc atom.

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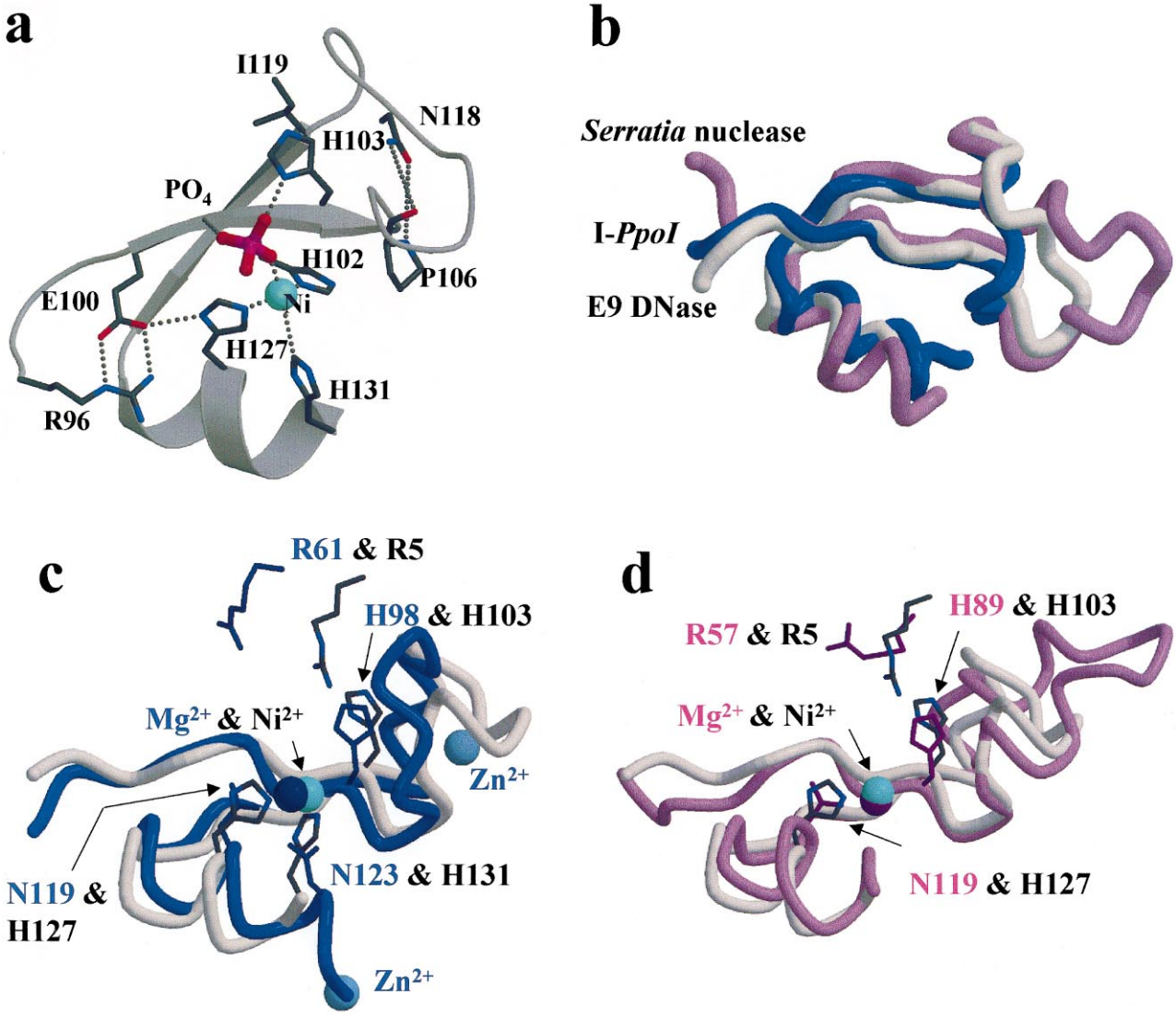

Fig. 1. a: Active site of the E9 DNase domain with the conserved residues of the H-N-H motif displayed along with the bound Mg^{2+} and phosphate molecule [4]. b: Superposition of the conserved motif of E9 DNase (grey), I-*Ppo*I (blue) and *Serratia* nuclease (magenta). Detailed views of the motif for E9 DNase (grey) overlaid with I-*Ppo*I and with *Serratia* nuclease are shown in panels c and d, respectively. Important residues are highlighted and coloured according to panel b. The active site Ni^{2+} ion (cyan) of the E9 DNase co-localises with the Mg^{2+} ion (magenta) of both I-*Ppo*I and *Serratia*. Light blue spheres in panel c show the positions of two structural zinc atoms in I-*Ppo*I.

3. Redefining homing endonuclease families

Since the H-N-H motif-containing E9 DNase and I-*Ppo*I, a His-Cys box enzyme, both display the same active site motif we suggest that the two families of homing endonucleases to which these enzymes belong should be redefined on a structural basis as being one and the same. Our structural comparison indicates that the H-N-H motif of the E9 DNase and the His-Cys box of I-*Ppo*I represent different ways of stabilising the same basic active site structure for a nuclease. We suggest the new name of the 'ββα-Me family' of homing enzymes to reflect the three elements of secondary structure and the metal ion that make up the core of their common active site fold.

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