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SHORT COMMUNICATION

Five-Stranded β-Sheet Sandwiched With Two α-Helices: A Structural Link Between Restriction Endonucleases *Eco*RI and *Eco*RV

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ABSTRACT **Examination of crystal structures of restriction endonucleases *Eco*RI and *Eco*RV complexes with their cognate DNA revealed a common structural element, which forms the core of both proteins. This element consists of a five-stranded β-sheet and two α-helices packed against it and could be described as α–β sandwich in which helices and β-strands lie in two stacked layers. While the spatial structure of this α–β sandwich is conserved in both enzymes, there are no detectable similarities between amino acid sequences except of a few residues involved in active site formation. Probably, other restriction endonucleases which have similar organization of the active site might possess similar structural element regardless of DNA sequence recognized and recognition elements in the enzyme used.**

Key words: protein tertiary structure, enzyme–DNA complex, cleavage pattern, active site, DNA recognition sequence

INTRODUCTION

Type II restriction endonucleases (for recent review see ref. 1) usually recognize a short (4–6 bp) palindromic sequence of double-stranded DNA and cleave phosphodiester bond at the defined position leaving "blunt" or "sticky" (with a 5′ or 3′ overhangs) ends. These enzymes show a remarkably high specificity in recognizing their DNA sites[1] and thus are of high interest both for studying protein–DNA interactions and for practical use. In general, comparison of protein sequences of type II restriction endonucleases revealed little or no sequence similarities (except a few pairs of isoschizomers).[2,3] This raises a reasonable question of whether the diversity of protein sequences of restriction endonucleases reflects the diversity of tertiary structures or these proteins still share similar structural elements despite the lack sequence similarities. To date there are only two restriction endonucleases—*Eco*RI (recognition sequence G/AATTC, cleavage site indicated by /)[4,5] and *Eco*RV (recognizes GAT/ATC),[6] for which crystal structures of their complexes with cognate DNA are solved. The comparative structural analysis of both enzymes led to the conclusion that their overall tertiary structures are quite different except the local structural homology in the active site region.[6,7] However, during close examination of crystal structures of *Eco*RI and *Eco*RV complexes with their cognate DNA we have found that similarity between tertiary structures of these proteins in the active site region could be extended to the larger structural element consisting of five-stranded β-sheet and two α-helices packed against it. Here we discuss the structural features of this element conserved between *Eco*RI and *Eco*RV.

ATOMIC COORDINATES AND SEQUENCES

Atomic coordinates of crystal structures for *Eco*RI–DNA and *Eco*RV–DNA cognate complexes as well as amino acid sequences of both enzymes were obtained from Brookhaven Protein Data Bank.[8] Their PDB entries are 1R1E and 4RVE, respectively.

RESULTS AND DISCUSSION

Structural comparison of *Eco*RI and *Eco*RV[7] revealed that both enzymes share a conserved structural motif of two acidic and one basic side chain residues (D91, E111, and K113 in *Eco*RI and correspondingly D74, D90, and K92 in *Eco*RV) which are similarly positioned in the vicinity of scissile phosphodiester bond. These residues were supposed to be involved in the formation of the catalytic/Mg^{2+} binding site.[7,9] Site-directed mutagenesis experi-

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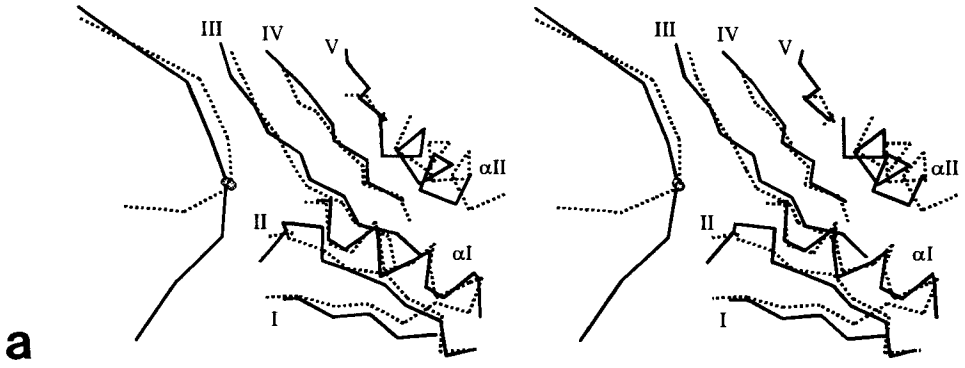

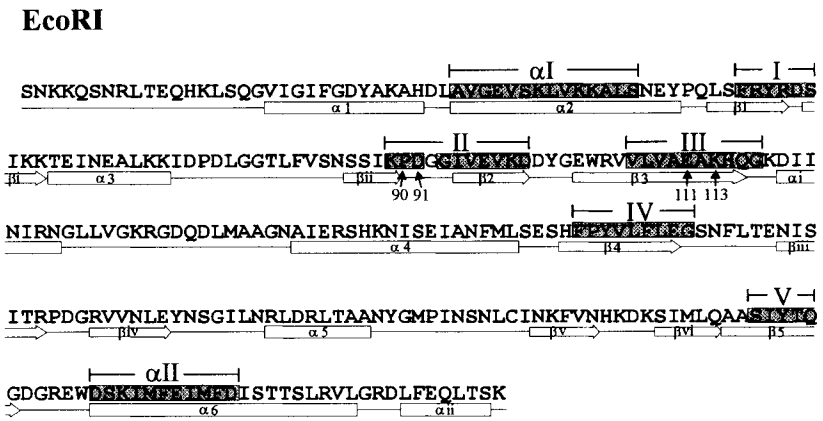

Fig. 1. Common structural element shared by *Eco*RI and *Eco*RV. (**a**) Stereo view of the *Eco*RI β-sheet sandwiched with α-helices and single DNA strand (solid lines) superimposed with an equivalent structure of *Eco*RV (dashed lines). Polypeptide chains are represented by their Cα atoms and DNA phosphodiester backbone by phosphorus atoms. Phosphorus atom at scissile bond is indicated by the circle. (**b**) Amino acid sequences of *Eco*RI and *Eco*RV. Secondary structure elements for *Eco*RI[4] and *Eco*RV[6] are indicated below the sequences. Sequences of the conserved structural elements are shadowed and designated as in (**a**). Putative catalytic site residues of *Eco*RI and *Eco*RV[7] are indicated by arabic numerals.

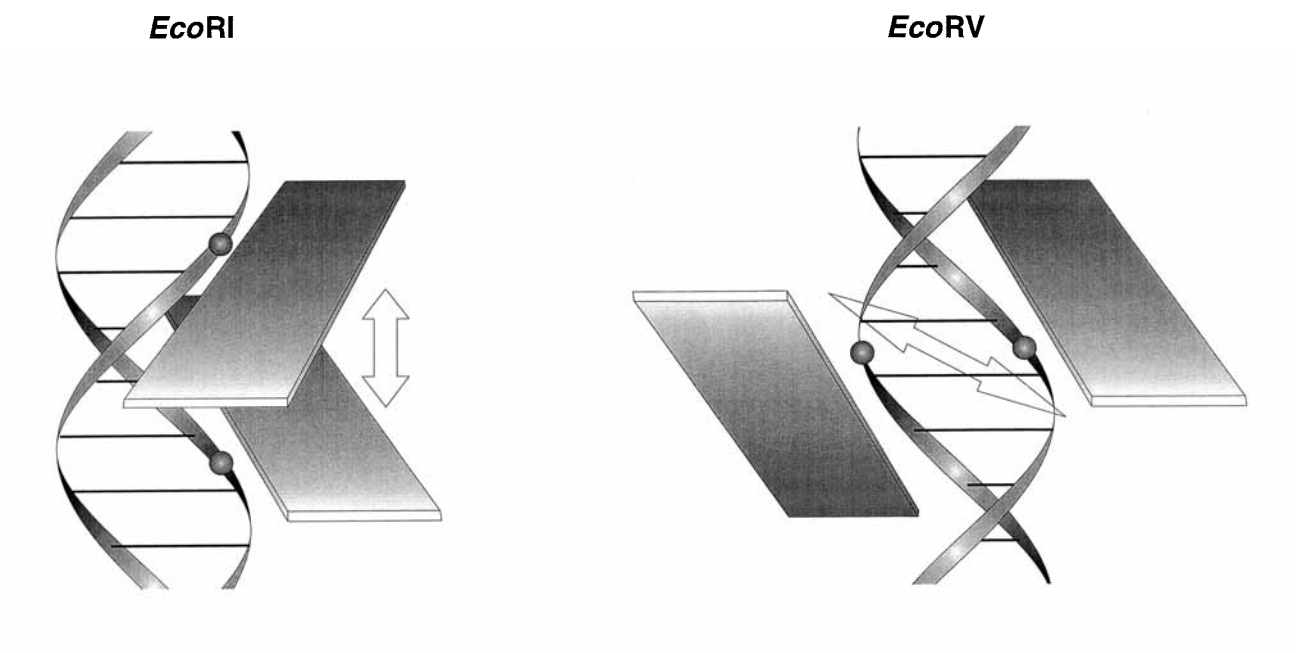

Fig. 2. Representation of the spatial arrangement of β-sheets in homodimeric complexes of *Eco*RI and *Eco*RV with DNA (spheres denote phosphorus atoms at scissile bond). Arrows indicate the dimerization mode of *Eco*RI and *Eco*RV.

ments of these residues in both restriction endonucleases confirmed their important role in catalysis.[9–13] Analyzing crystal structures of *Eco*RI and *Eco*RV complexes with cognate oligonucleotides we have found that similarity between the tertiary structures of these enzymes is not restricted to the structural motif in the active site region. This active site motif actually seems to be a part of larger structural element common for both enzymes. The latter includes a mixed parallel/antiparallel five-stranded β-sheet and two α-helices packed against it (Fig. 1a). This element could be described as an α–β sandwich in which β-strands and α-helices occupy two separate and distinct layers. The most structurally conserved part of superimposed structures are four β-strands (I–IV) and one α-helix (αI). They are arranged in the same order along protein chain and have the same topology in both enzymes as well (Fig. 1). Superposition of 49 Cα atoms [four β-strands (I–IV) and one α-helix (αI)] of *Eco*RI with equivalent atoms of *Eco*RV gives root-mean-square (rms) deviation of 1.9 Å. The αII helices, though similarly arranged in both enzymes, have an opposite orientation and are slightly shifted with respect to each other thus introducing an uncertainty in determining spatially equivalent positions, and for that reason they were not included in rms calculations. It seems that the differences observed in the location of α-helices (αII) are mostly due to the different twist angle of corresponding β-sheets in *Eco*RI and *Eco*RV.

The positions of αII helices (Fig. 1) also differ regarding protein sequences. In *Eco*RV the αII helix corresponds to the N-terminal α-helix; in *Eco*RI it corresponds to the part of the helix at the C-termini. The fifth strands (V) of corresponding β-sheets as αII helices also have an opposite orientation in the superimposed structures and possess larger displacement values than the rest of the β-strands. The spatial and topological conservation of common structural elements between *Eco*RI and *Eco*RV is striking since these restriction endonucleases recognize different hexanucleotide sequences and cleave them at different positions. Active forms of both *Eco*RI and *Eco*RV, as it is usual for type II restriction enzymes,[1] are dimers. Figure 2 schematically illustrates how the conserved β-sheets from both subunits of restriction endonucleases *Eco*RI and *Eco*RV are arranged in each enzyme–DNA complex. While corresponding β-sheets of both enzymes are similarly positioned with respect to the scissile DNA strand (Fig. 1a), different cleavage patterns in each case determine both the different arrangement of β-sheets regarding the DNA double helix and their mutual orientation (Fig. 2). The different mutual orientation of central β-sheets in each case reflects the differences in protein regions and structural elements involved in dimer formation (Fig. 2). For instance, in *Eco*RV tertiary structure α-helix B is bent and two turns at its N-termini contribute directly to the dimer interface.[6] The C-terminal part of this helix which is packed against the central β-sheet in *Eco*RV has its counterpart in the tertiary structure of *Eco*RI (compare αI helices in Fig. 1). However, the

corresponding helix in *Eco*RI lacks two N-terminal turns. It is not involved in dimer formation in *Eco*RI and possibly that might be the reason for differences between these helices. On the other hand, there are structural elements contributing to dimer formation in *Eco*RI which have no counterparts in *Eco*RV. Namely, β1 and β2 strands of *Eco*RI central β-sheet are connected through the loop, which includes the β-meander with subsequent α-helix (Fig. 1b). It participates in dimerization[4,5] on the DNA major groove side. In contrast, corresponding β-strands of *Eco*RV (βc and βd in Fig. 1b) are connected only through the short loop which faces the DNA minor groove.

It is provocative to suppose that other restriction endonucleases whose active site organization is similar to that of *Eco*RI and *Eco*RV, might share a similar structural element consisting of a β-sheet sandwiched with two α-helixes as a core part of their structure. In that case the overall structure of a particular restriction endonuclease should critically depend on its cleavage pattern, which is determined by the different location of the scissile phosphodiester bond. The idea that structural similarity of type II restriction enzymes may depend on the position of reactive phosphodiester bonds has been formulated earlier.[3] This has also been supported by recent finding that restriction endonucleases *Eco*RI (G/AATTC), *Mun*I (C/AATTG), and *Cfr*9I (C/CCGGG), recognizing different hexanucleotide sequences but exhibiting the same cleavage pattern as *Eco*RI, have homologic stretches of amino acids which correspond to the active site and sequence recognition elements in *Eco*RI.[14] Preliminary molecular modeling studies of *Mun*I (unpublished data) also suggest the presence of a structural element consisting of a central β-sheet sandwiched with two α-helices similar to that shared by *Eco*RI and *Eco*RV. However, more X-ray structures of restriction endonucleases are needed to answer the question if the five-stranded β-sheet sandwiched with two α-helices is a structural feature of most or only a particular family of restriction endonucleases.

CONCLUSIONS

It appears that a common structural element consisting of a central β-sheet sandwiched with two α-helices is conserved between restriction endonucleases *Eco*RI and *Eco*RV. This structural element is involved in the structural organization of the active site and also forms a core of the protein subunit in both enzymes. It might be expected that other restriction endonucleases share a similar structural element, however, their overall structures should critically depend on the location of the scissile phosphodiester bond in respect to the recognized nucleotide sequence.

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NOTE ADDED IN PROOF

Crystal structures of two other restriction endonucleases, namely *Bam*HI (Newman et al. Nature 368:660–664, 1994; Newman et al. Structure 2:439–452, 1994) and *Pvu*II (Athanasiadis et al. Nature Struct. Biol. 1:469–475, 1994; Cheng et al. EMBO J., in press) appeared after submission of this paper. Both enzymes also share α–β sandwich as a common structural element, while their overall structures differ. However, there are close structural similarities between enzymes having the same cleavage pattern: *Bam*HI (G/GATCC) structure is similar to *Eco*RI(G/AATTC) and that of *Pvu*II (CAG/CTG) is close to *Eco*RV (GAT/ATC).

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