Asymmetric DNA recognition by the OkrAI endonuclease, an isoschizomer of BamHI

Éva Scheuring Vanamee1, Hector Viadiu1, Siu-Hong Chan2, Ajay Ummat1, Adrian M. Hartline2, Shuang-yong Xu2 and Aneel K. Aggarwal1,*

1Department of Structural and Chemical Biology, Mount Sinai School of Medicine, 1425 Madison Ave, New York, NY 10029 and 2New England Biolabs, Inc. 240 County Road, Ipswich, MA 01938-2723, USA

Received July 7, 2010; Revised August 12, 2010; Accepted August 17, 2010

ABSTRACT

Restriction enzymes share little or no sequence homology with the exception of isoschizomers, or enzymes that recognize and cleave the same DNA sequence. We present here the structure of a BamHI isoschizomer, OkrAI, bound to the same DNA sequence (TATGGATCCATA) as that cocrystallized with BamHI. We show that OkrAI is a more minimal version of BamHI, lacking not only the N- and C-terminal helices but also an internal 310 helix and containing β-strands that are shorter than those in BamHI. Despite these structural differences, OkrAI recognizes the DNA in a remarkably similar manner to BamHI, including asymmetric contacts via C-terminal ‘arms’ that appear to ‘compete’ for the minor groove. However, the arms are shorter than in BamHI. We observe similar DNA-binding affinities between OkrAI and BamHI but OkrAI has higher star activity (at 37°C) compared to BamHI. Together, the OkrAI and BamHI structures offer a rare opportunity to compare two restriction enzymes that work on exactly the same DNA substrate.

INTRODUCTION

Protein–DNA selectivity is a central event in many biological processes, ranging from transcription and replication to restriction and modification. Type II restriction endonucleases (REases) are ideal systems for studying selectivity because of their high specificity and great variety. More than 3800 Type II restriction enzymes representing 304 unique specificities have now been identified (1). They generally recognize DNA sequences that vary between 4 and 8 bp and require only Mg2+ as a cofactor to catalyze the hydrolysis of DNA (2). Their sequence specificity is remarkable. A single base pair change within the recognition sequence can lead to well over a million-fold reduction in activity (2). BamHI (from Bacillus amyloliquefaciens), is one of the best-studied REase, with structures of the free enzyme and complexes with cognate and non-cognate sites available (3–5). In addition, the co-crystal structures with divalent metals provide snapshots of the pre- and post-reactive states of BamHI (6). Together, these structures grant important insight into DNA recognition, selectivity and the mechanism of cleavage by this endonuclease.

REases share little or no sequence homology with the exception of isoschizomers, or enzymes that recognize and cleave exactly the same DNA sequence (7,8). We have succeeded in expressing and crystallizing a BamHI isoschizomer, OkrAI (from Oceanospirillum kriegii), which recognizes and cleaves the exact same DNA sequence as BamHI (5′–G#GATCC–3′). OkrAI is, however, smaller than BamHI (194 versus 213 amino acids), and from sequence comparisons appears to lack the equivalent of N- and C-terminal helices of BamHI. The absence of a C-terminal helix is particularly intriguing as this helix is crucial for BamHI function. We report here a structure of OkrAI bound to the same DNA fragment (TATGGATCCATA) as that cocrystallized with BamHI. Surprisingly, even though OkrAI lacks some of the secondary structural elements, its mode of DNA recognition is remarkably similar to that of BamHI, including asymmetric interactions via the C-terminal arms. The OkrAI and BamHI structures offer a rare opportunity to compare two REases that work on exactly the same DNA substrate.

MATERIALS AND METHODS

Isolation, expression and purification of OkrAI

An experimental search for BamHI isoschizomers was initiated by screening cultured microorganisms. OkrAI was discovered in Oceanospirillum kriegii (Polisson C., unpublished data, NEB strain collection). The methylase selection method (9) was used to clone the OkrAI...
methylase gene (okrAIM) and part of the OkrAI endonuclease gene (okrAIR). Inverse polymerase chain reaction (PCR) walk was carried out to obtain the entire okrAIR gene. A PCR fragment carrying the reaction (PCR) walk was carried out to obtain the entire OkrAI gene. The specific activity of OkrAI is 106 units of OkrAI per gram of wet cells in clarified cell extracts.

The enzyme was purified by chromatography through phosphocellulose (Whatman P11), hydroxylapatite (Bio-Rad), DEAE Sepharose (Pharmacia), heparin Sepharose (Pharmacia) and Affi-gel Blue (Bio-Rad) columns. The purified OkrAI protein was stored in the following buffer: 20 mM Tris–HCl (pH 7.5), 200 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% (v/v) glycerol. The specific activity of OkrAI is determined to be approximately 2 × 106 units/mg protein on λ DNA substrate.

Cocrystallization and structure determination

OkrAI was cocrystallized with the same palindromic 12-mer (TATGGATCCATA) used to grow the BamHI/DNA cocrystals. The OkrAI/DNA cocrystals grow under similar conditions as the BamHI/DNA cocrystals (from 15–17% PEG 8000, 0.2 M calcium acetate and 0.1 M sodium cacodylate at pH 6.7). All X-ray data were collected by setting excitation filter at 490 nm and emission filter at 520 nm. Each reaction sample (volume 200 μl) was resuspended in buffer with 10 mM Tris–HCl, pH 7.5 at 25°C, 100 mM KCl, 10 mM CaCl2, 1 mM EDTA and 5 mM DTT. The DNA+ labeled oligo was mixed with the unlabelled complementary strand and annealed to a final concentration of 50 μM. Experiments were performed using Panvera Beacon 2000 fluorescence polarization system (at 25°C). Fluorescence intensity data were collected by setting excitation filter at 490 nm and emission filter at 520 nm. Each reaction sample (volume 200 μl) consisted of 5 nM of 5'-Fluorescein labeled DNA and increasing concentrations of BamHI or OkrAI (0.1–400 nM) in the above-mentioned buffer. Individual reaction samples were equilibrated for more than 30 min before measuring the anisotropy values. The anisotropy values obtained were subtracted from the anisotropy value of the blank (buffer with no protein) and then divided with the maximum value of anisotropy value obtained for the experiment. This normalized anisotropy value yields the fraction of the DNA bound to the protein (y). The fraction of the DNA bound form (y) was plotted against the concentration of BamHI or Okra (x in nM) and fitted to the following equation (using non-linear regression in Origin 7, OriginLab):

\[ y = \frac{(k_d + C_{DNA} + x) - \sqrt{(k_d + C_{DNA} + x)^2 - 4C_{DNA}x}}{2C_{DNA}} \]

| Table 1. Crystallographic data collection and refinement |
|----------------------------------|
| A. Data collection and phasing statistics |
| Total no. of reflections | 131 820 |
| Resolution range (Å) | 20–2.3 |
| No. of unique reflections | 32 438 |
| R<sub>sym</sub> (last shell) | 0.042 (0.108) |
| Completeness (%) (last shell) | 89 (84) |
| I/σ(I) | 43.2 (20.8) |
| Redundancy (last shell) | 4.1 (4.0) |
| B. Refinement statistics |
| Resolution range (Å) | 20–2.3 |
| No. of reflections used in refinement | 30 786 |
| R<sub>cryst</sub>/R<sub>free</sub> | 0.16/0.23 |
| No atoms |
| Protein | 6007 |
| DNA | 972 |
| Water | 710 |
| Average B factors (Å<sup>2</sup>) | 14.2 |
| R.m.s. deviations |
| Bonds (Å) | 0.017 |
| Angles (deg.) | 1.9 |

Fluorescence anisotropy measurements

5'-Fluorescein (6-carboxyfluorescein)-labeled DNA (5'-TAT GAG CGG ATC CTT ACG AG-3') was purchased from IDT DNA Technologies. Oligos were re-suspended in buffer with 10 mM Tris–HCl, pH 7.5 at 25°C, 100 mM KCl, 10 mM CaCl<sub>2</sub>, 1 mM EDTA and 5 mM DTT. The DNA+ labeled oligo was mixed with the unlabelled complementary strand and annealed to a final concentration of 50 μM. Experiments were performed using Panvera Beacon 2000 fluorescence polarization system (at 25°C). Fluorescence intensity data were collected by setting excitation filter at 490 nm and emission filter at 520 nm. Each reaction sample (volume 200 μl) consisted of 5 nM of 5'-Fluorescein labeled DNA and increasing concentrations of BamHI or OkrAI (0.1–400 nM) in the above-mentioned buffer. Individual reaction samples were equilibrated for more than 30 min before measuring the anisotropy values. The anisotropy values obtained were subtracted from the anisotropy value of the blank (buffer with no protein) and then divided with the maximum value of anisotropy value obtained for the experiment. This normalized anisotropy value yields the fraction of the DNA bound to the protein (y). The fraction of the DNA bound form (y) was plotted against the concentration of BamHI or OkrAI (x in nM) and fitted to the following equation (using non-linear regression in Origin 7, OriginLab):

\[ y = \frac{(k_d + C_{DNA} + x) - \sqrt{(k_d + C_{DNA} + x)^2 - 4C_{DNA}x}}{2C_{DNA}} \]
where, $y$ is the fraction of the DNA bound to the protein, $C_{\text{DNA}}$ is the concentration of DNA used (5 nM), $K_d$ is the dissociation constant and $x$ is the concentration of BamHI or OkrAI.

**OkrAI cleavage activity assays**

Purified OkrAI and BamHI were serially diluted in 2-fold series in 20 mM Tris–HCl, pH 7.9, 50 mM NaCl before each assay. Five microliters of the diluted enzymes were incubated with 1 µg of λ DNA in 50 µl reactions containing 20 mM Tris–HCl, 100 mM NaCl, 10 mM MgCl$_2$, 1 mM DTT, pH 7.9. The reactions were carried out at the indicated temperature for 1 h. The reactions were stopped by the addition of a loading dye that gave a final concentration of 0.17% for sodium dodecyl sulfate (SDS) and 8.3 mM for EDTA. The cleavage products were analyzed by electrophoresis through 0.8% agarose gels. One unit of endonuclease activity is defined as the minimum amount of enzyme required for the complete cleavage of the substrate. Fidelity index (FI) is defined as the ratio of the maximum enzyme amount showing no star activity to the minimum amount needed to achieve 1 U of activity.

**RESULTS**

**Overall architecture**

Similar to BamHI, an OkrAI dimer approaches DNA from the major groove side (Figure 1). The subunit consists of a large six-stranded mixed β-sheet, which is sandwiched on both sides by α helices (3). Strands β$_3$, β$_4$ and β$_5$ are antiparallel and form a β meander; strands β$_2$, α helices are blue, β strands are magenta and loops are white. The BamHI C terminus that is α helical in the apo form and unstructured in the DNA bound form is highlighted in red.
\[\beta^5\] and \[\beta^3\] are parallel and resemble a Rossmann fold, with \(\alpha'\) and \(\alpha'\) acting as the crossover helices. The dimer interface is formed primarily by helices \(\alpha'\) and \(\alpha'\), which pair with the corresponding helices from the symmetry related subunit to form a parallel four-helix bundle (Figure 1). Compared to BamHI, some of the structural elements are either missing or are shorter in OkrAI (Figure 2). In particular, the first helix, \(\alpha'\), of BamHI is entirely missing in OkrAI and \(\beta^3\) is connected to \(\alpha'\) (the equivalent of \(\alpha'\) in BamHI) by a short loop. The loops between \(\beta^2\) and \(\alpha'\) and \(\alpha'\) and \(\beta^3\) are shortened by one residue. The short \(3_{10}\) helix \(\alpha'\) of BamHI between strands \(\beta^2\) and \(\beta^3\) is also missing in OkrAI and is connected by only a loop. Strands \(\beta^4\) and \(\beta^5\) are both shorter by three residues but connected by a slightly longer loop in OkrAI (four versus two residues). There is an insertion of five residues between a slightly longer loop in OkrAI (four versus two residues).

**DNA conformation**

The OkrAI DNA retains a B-DNA conformation over the central 10 bp with average helical twist and rise of 32.26° and 3.33 Å, respectively, compared to a helical twist and rise of 33.44° and 3.43 Å for BamHI. There are no kinks in the OkrAI DNA axis of the type seen in EcoRV and EcoRI DNAs, or any major unwinding at the central base-pair step as seen in EcoRV (16), EcoRI (17) and BgIII (18) DNAs. The direction of curvature is the same as in most restriction enzyme complexes, namely away from the \(\alpha/\beta\) core. In contrast, dimeric transcription factors such as phage 434 repressor (19) or *E. coli* Trp repressor (20) bend DNA toward the body of the protein. As a consequence, the minor groove at the center of the DNA is significantly narrower (\(\sim 3\) Å) in the phage 434 DNA complex than in the OkrAI–DNA complex (\(\sim 7\) Å).

**DNA recognition**

The DNA is bound in the cleft formed by the two OkrAI monomers (Figure 1). There are extensive protein–DNA interactions, with side chain and main chain atoms, and tightly bound water molecules all contributing toward recognition of the GGATCC sequence. Interactions with DNA occur both in the major and minor grooves. In the major groove, every hydrogen bond donor and acceptor group takes part in direct or water-mediated hydrogen bonds with the protein (Figure 3). These interactions are remarkably similar to those observed in the BamHI cognate DNA complex. In particular, the major groove contacts are made primarily by regions at the N-terminal ends of helices \(\alpha'\) and \(\alpha'\), the equivalent of helices \(\alpha'\) and \(\alpha'\) in the BamHI complex. The outer G:C base pair (GGA) is contacted by Arg143 and Asp142 from the 140–146 loop that precedes helix \(\alpha'\), the equivalent of residues Arg155 and Asp154 in BamHI. The middle G:C base pair (GGA) is contacted by Asp142, Arg110 and Asn104, the equivalent of residues Asp154, Arg122 and Asn116 of BamHI. The inner A:T base pair (ATC) is contacted primarily through water mediated hydrogen bonds as well as by Asn104. Consistent with the structural data, mutating this amino acid residue in BamHI (N116H) resulted in a mutant enzyme that prefers to cleave a methylated site (GGN6mATCC) (21). In the minor groove, contacts to bases are made by the C-terminal arm of the R subunit. There is insufficient room to accommodate arms from both subunits if they were to lay symmetrically in the groove. The R arm makes specific interactions with bases in both DNA half-sites via residues Asp189, Gly190 and Arg191, the equivalent of residues Asp196, Gly197 and Met198 of BamHI. Thus, in a similar manner to BamHI, OkrAI makes contacts in the minor groove in an asymmetric fashion, with the arm from one subunit (R) entering the groove, and the arm from the other subunit (L) following and making contacts with the DNA sugar-phosphate backbone. Altogether, there are extensive interactions between OkrAI and the sugar-phosphate backbone, including contacts from residues at the NH2-terminal ends of helices \(\alpha'\), \(\alpha'\) and \(\alpha'\), the ordered region before strand \(\beta^5\) (residues 83–85), and the arm of the L subunit (residues 190 and 191).

The structural similarity between OkrAI and BamHI in recognizing the cognate GGATCC sequence is reflected in their similar binding constants (with a \(K_d\) of 12.9 and 8.3 nM for OkrAI and BamHI, respectively), as measured by fluorescence anisotropy (Figure 4A). However, when we compare the DNA cleavage properties of the two enzymes there are differences in how they cleave non-cognate or ‘star’ sites at different temperatures. We find that both enzymes have reduced star activity at 25°C as compared to 37°C (Figure 4B), but OkrAI has a significantly higher star activity (4-fold higher) at 37°C compared to BamHI.

**Active site**

Most members of the PD-(D/E)XK family of restriction endonucleases, to which BamHI and OkrAI belong, utilize Mg2+ for cleavage. The active site geometry of the R subunit of the OkrAI–DNA complex is almost identical to that of the R subunit of the BamHI cognate complex (Figure 3), with residues of Glu71, Asp86, Glu99 and Glu101 aligning with Glu77, Asp94, Glu111 and Glu113 of BamHI. Other than BamHI and its isoscizomers (OkrAI, Bsp98I and DdsI) only one other REase, BtsCI is (22) known to contain a glutamate at the last catalytic residue position (Glu113 in BamHI and Glu101 in OkrAI), whereas the majority of restriction enzymes contain a lysine at this position. Interestingly, there is a severe loss of cleavage activity when Glu113 in BamHI is substituted by a lysine residue (23) or conversely, when Lys92 in EcoRV (24) or Lys113 in EcoRI (25) is substituted by a glutamate residue, even though the geometry of the active sites of these restriction enzymes is generally similar. Based on the structures of the pre- and post-reactive complexes of BamHI (6), a two-metal mechanism has been proposed for BamHI, in which metal A activates the attacking water molecule, while metal B stabilizes the buildup of negative charge on the leaving O3'.
atom. At the same time, both metals (acting as Lewis acids) are proposed to help stabilize the pentacovalent transition state. In the OkrAI complex, we observe two calcium ions at positions almost identical to the metal A and metal B sites in BamHI, suggesting that OkrAI also utilizes a two-metal mechanism for cleavage. Curiously, in the pre- and post-reactive complexes of BamHI (6), only the R subunit contains metals, the L subunit does not.

Interestingly, in OkrAI the L subunit contains a single metal site roughly halfway between metal A and metal B. Thus, in BamHI and OkrAI, the binding of two metals is correlated to the active site of the subunit that has its C-terminal arm in the minor groove. This asymmetry in DNA binding and the binding of metals have been suggested to correlate with an observed sequential DNA cleavage mechanism in BamHI (26).
DISCUSSION

The OkrAI and BamHI structures offer a rare opportunity to compare two REases that work on exactly the same DNA substrate. To our knowledge, the only other pair of isoschizomers whose structures have been determined are the homing endonucleases I-CreI and I-MsoI (27). However, unlike OkrAI and BamHI, these two enzymes show considerable divergence in residues that partake in DNA recognition and the protein–DNA interface as a whole is strikingly different in the two structures. In contrast, OkrAI emerges here as essentially a more minimal version of BamHI. OkrAI lacks not only the N- and C-terminal helices but also the β helix between strands β3 and β8, and containing β-strands (β4 and β5) that are shorter than those in BamHI. Despite these structural differences, OkrAI recognizes the GGATCC sequence in a remarkably similar manner to BamHI. All of the DNA recognition residues (as well as the catalytic residues) are conserved between the two enzymes and make the same pattern of hydrogen bonds to bases in the major and minor grooves. Not all of the BamHI isoschizomers may be so similar, however; with several isoschizomers having been identified (28–32) that differ in size from 22 kDa to 43 kDa and ranging from monomer (28) to tetramer (31). It will be interesting to compare the structures of these isoschizomers to know which structural elements are conserved and which have diverged over the course of the evolution of these type II restriction enzymes.

One of the most surprising features of the OkrAI structure is asymmetric DNA binding. This was unexpected because OkrAI appeared to lack, from sequence alignments, the equivalent of a C-terminal helix in BamHI, which is present in ‘free’ BamHI and when the enzyme binds to non-cognate DNA, but which unfolds on binding to cognate DNA to form long partially disordered arms, with the arm from one subunit (R) fitting into the minor groove and the arm from the other subunit (L) following the DNA sugar-phosphate backbone. As such, the C-terminal residues in BamHI are believed to fulfill a dual role: first, as helices, to aid in the initial binding and the diffusion of the enzyme on nonspecific DNA; second, by unfolding to increase the lifetime of the specific complex for the subsequent cleavage reaction. The lack of a C-terminal helix in OkrAI lends to C-terminal arms that are much shorter than in BamHI, and also, unlike BamHI, they are likely to be unstructured in free OkrAI or when the enzyme binds to non-cognate DNA. However, despite the truncated arms, OkrAI recognizes cognate DNA in the same asymmetric manner as BamHI, with one arm making base contacts in the minor groove and the other making contacts with the DNA backbone. As in the case of the BamHI–DNA complex, there is insufficient room in the minor groove to accommodate arms from both subunits. Asymmetric DNA binding is not limited to BamHI and OkrAI but extends to other proteins, including the transcription factor HAP1 (33). In HAP1 the two subunits bind in a head-to-tail fashion to a symmetric recognition site resulting in a dramatically asymmetric complex. A different kind of asymmetry is observed when monomeric MspI endonuclease binds its palindromic DNA sequence (34,35), utilizing different parts of the monomer to recognize symmetric half sites of the DNA.

Curiously, OkrAI and BamHI have increased ‘star’ activity at higher temperatures that may be related to the flexibility of their C-terminal arms. That is, at the higher temperature (37°C versus 25°C), we envisage that the BamHI C-terminal helix is more prone to unfolding and thereby in inducing a pseudo-specific complex competent for cleavage at the ‘star’ sequences; in free BamHI,
the C-terminal helix is the most mobile secondary structural element (with an average B factor of 33 Å²). Interestingly, at this higher temperature (37°C), OkrAI has even higher star activity than BamHI, which may be due to the lack of a C-terminal α-helix and the presence of shorter arms that are already unfolded and, as such, require lower activation energy to induce a pseudo-specific complex for cleavage at star sequences. Mutational studies are under way to test the role of the C-terminal residues on OkrAI and BamHI cleavage activities. Taken together, OkrAI and BamHI provide for the first time a structural framework for comparing two type II restriction enzymes that work on exactly the same DNA substrate.

**ACCESSION NUMBER**
Coordinates have been submitted to the RCSB Protein Data Bank with accession code 3ODH.

**ACKNOWLEDGEMENTS**
We thank the staff at CHESS and at the APS for help with data collection. We thank Elinor Vinecombe for assistance with the structure determination; Jim Samuelson, Jianping Xiao and Becky Kucera for technical assistance; Ira Schildkraut for initiation of the OkrAI cloning project; Don Comb for support and encouragement. OkrAI

---

**Figure 4.** Binding and cleavage data. (A) Fluorescence anisotropy results for OkrAI and BamHI. The fraction of the bound DNA is plotted against the concentration of the protein. The data points for BamHI are shown as black half squares and for OkrAI as red half circles, and the fitted curves as solid lines. (B) Cleavage activity data for OkrAI (top) and BamHI (bottom) at 25°C and 37°C using λ DNA as the substrate. The fidelity index (FI) is defined as the ratio of the maximum enzyme amount showing no star activity to the minimum amount needed to achieve 1 U of activity.
expression strain request should be directed to xus@neb.com.

FUNDING

National Institutes of Health grant GM44006 (to A.K.A.). Funding for open access charge: National Institutes of Health grant GM44006.

Conflict of interest statement. None declared.

REFERENCES

1. Roberts,R.J., Vinoye,T., Posfai,J. and Macelis,D. (2010) REBASE – a database for DNA restriction and modification: enzymes, genes and genomes. Nucleic Acids Res., 38, D234–D236.
2. Roberts,R.J. and Halford,S.E. (1993) In Linn,S.M., Lloyd,R.S. and Roberts,R.J. (eds), Nucleases. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
3. Newman,M., Strzelecka,T., Dorner,L.F., Schildkraut,I. and Roberts,R.J. and Halford,S.E. (1993) In Linn,S.M., Lloyd,R.S. and Roberts,R.J. (eds), Nucleases. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
4. Newman,M., Strzelecka,T., Dorner,L.F., Schildkraut,I. and Roberts,R.J. (1994) Structure of restriction endonuclease BamHI and its relationship to EcoRI. Nature, 368, 660–664.
5. Newman,M., Strzelecka,T., Dorner,L.F., Schildkraut,I. and Aggarwal,A.K. (1995) Structure of Bam HI endonuclease bound to DNA: partial folding and unfolding on DNA binding. Science, 269, 656–663.
6. Viadu,H., Kucera,R., Schildkraut,I. and Aggarwal,A.K. (2000) Crystallization of restriction endonuclease BamHI with nonspecific DNA. J. Struct. Biol., 130, 81–85.
7. Viadu,H. and Aggarwal,A.K. (1998) The role of metals in catalysis by the restriction endonuclease BamHI. Nat. Struct. Biol., 5, 910–916.
8. Roberts,R.J. and Macelis,D. (1991) Restriction enzymes and their isoschizomers. Nucleic Acids Res., 19(Suppl.), 2077–2109.
9. Szomolanyi,E., Kiss,A. and Venetianer,P. (1980) Cloning the modification methylase gene of Bacillus sphaericus R in Escherichia coli. Gene, 10, 219–225.
10. Brunger,A.T., Adams,P.D., Clore,G.M., DeLano,W.L., Gros,P., Grosse-Kunstleve,R.W., Jiang,J.S., Kuszewski,J., Nilges,M., Pannu,N.S. et al. (1998) Crystallography & NMR system: A new software suite for macromolecular structure determination. Acta Crystallogr. D Biol. Crystallogr., 54(Pt 5), 905–921.
11. CCP4. (1994) The CCP4 suite: programs for protein crystallography. Acta Crystallogr. D Biol. Crystallogr., 50, 760–763.
12. Vagin,A.A., Steiner,R.A., Lebedev,A.A., Potterton,L., McNicholas,S., Long,F. and Murshudov,G.N. (2004) REFMAC5 dictionary: organization of prior chemical knowledge and guidelines for its use. Acta Crystallogr. D Biol. Crystallogr., 60, 2184–2195.
13. Laskowski,R.A., MacArthur,M.W., Moss,D.S. and Thornton,J.M. (1993) PROCHECK: a program to check the stereochemical quality of protein structures. J. Appl. Cryst., 26, 283–291.
14. Lu,X.J. and Olson,W.K. (2003) SDNA: a software package for the analysis, rebuilding and visualization of three-dimensional nucleic acid structures. Nucleic Acids Res., 31, 5108–5121.
15. Lee,B. and Richards,F.M. (1971) The interpretation of protein structures: estimation of static accessibility. J. Mol. Biol., 55, 379–400.
16. Winkler,F.K., Banner,D.W., Oefner,C., Tsernoglou,D., Brown,R.S., Heathman,S.P., Bryan,R.K., Martin,P.D., Petratos,K. and Wilson,K.S. (1993) The crystal structure of EcoRV endonuclease and of its complexes with cognate and non-cognate DNA fragments. EMBO J., 12, 1781–1795.
17. Kim,Y.C., Grable,J.C., Love,R., Greene,P.J. and Rosenberg,J.M. (1990) Refinement of Eco RI endonuclease crystal structure: a revised protein chain tracing. Science, 249, 1307–1309.
18. Lukacs,C.M., Kucera,R., Schildkraut,I. and Aggarwal,A.K. (2000) Understanding the immutability of restriction enzymes: crystal structure of BglII and its DNA substrate at 1.5 A resolution. Nat. Struct. Biol., 7, 134–140.
19. Aggarwal,A.K., Rodgers,D.W., Drottar,M., Ptashne,M. and Harrison,S.C. (1988) Recognition of a DNA operator by the repressor of phage 434: a view at high resolution. Science, 242, 899–907.
20. Otwinowski,Z., Schevitz,R.W., Zhang,R.G., Lawson,C.L., Joachimiak,A., Marmorestein,R.Q., Luisi,B.F. and Sigler,P.B. (1988) Crystal structure of trp repressor/operator complex at atomic resolution. Nature, 335, 321–329.
21. Whitaker,R.D., Dorner,L.F. and Schildkraut,I. (1999) A mutant of BamHI restriction endonuclease which requires N6-methyladenine for cleavage. J. Mol. Biol., 285, 1525–1536.
22. Too,P.H., Zhu,Z., Chan,S.H. and Xu,S.Y. (2010) Engineering Ni.BstCI and Nb.BstCI nicking enzymes and applications in generating long overhangs. Nucleic Acids Res., 38, 1294–1303.
23. Xu,S.Y. and Schildkraut,I. (1991) Isolation of BamHI variants with reduced cleavage activities. J. Biol. Chem., 266, 4425–4429.
24. Selent,U., Ruter,T., Kohler,E., Liedtke,M., Thielking,V., Alves,J., Oegschlager,T., Wolfes,F. and P Georgetown,A. (1992) A site-directed mutagenesis study to identify amino acid residues involved in the catalytic center of the restriction endonuclease EcoRV. Biochemistry, 31, 4808–4815.
25. Grabowski,G., Jeltsch,A., Wolfes,F., Maass,G. and Alves,J. (1995) Site-directed mutagenesis in the catalytic center of the restriction endonuclease EcoRI. Gene, 157, 113–118.
26. Hensley,P., Nardone,G., Chirikjian,J.G. and Wasmuth,M.E. (1990) The time-resolved kinetics of superhelical DNA cleavage by BamHI restriction endonuclease. J. Biol. Chem., 265, 15300–15307.
27. Chevalier,B., Turmel,M., Lemiex,C., Monnat,R.J. Jr and Stoddard,B.L. (2003) Flexible DNA target site recognition by diverging homing endonuclease isoschizomers I-CreI and I-MsoI. J. Mol. Biol., 329, 253–269.
28. Murakami,M., Mizuno,H. and Yamada,Y. (1990) The restriction endonuclease GceinI of Gluconobacter cerinus IFO 3260, an isoschizomer of BamHI, has a monomeric structure. J. Biol. Chem., 265, 219–225.
29. Grabowski,G., Jeltsch,A., Wolfes,F., Maass,G. and Alves,J. (1995) Site-directed mutagenesis in the catalytic center of the restriction endonuclease EcoRI. Gene, 157, 113–118.
30. Grones,J. and Turna,J. (1992) ApaCI, an isoschizomer of BamHI, isolated from Acetobacter pasteurianus. J. Biol. Chem., 267, 38609–38614.
31. Xu,Q.S., Roberts,R.J. and Guo,H.C. (2004) An asymmetric complex of restriction endonuclease MspI on its recognition sequence. J. Mol. Biol., 337, 111–120.
32. Xu,Q.S., Roberts,R.J. and Guo,H.C. (2005) Two crystal forms of the restriction enzyme MspI-DNA complex show the same novel structure. Protein Sci., 14, 2590–2600.
33. Holm,L. and Rosenström,P. (2010) Dali server: conservation mapping in 3D. Nucleic Acids Res., 38, W545–549.