Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Atomic structure of the *Serratia marcescens* endonuclease at 1.1 Å resolution and the enzyme reaction mechanism

S. V. Shlyapnikov,[a] V. V. Lunin,[b] M. Perbandt,[c] K. M. Polyakov,[b] V. Yu. Lunin,[d] V. M. Levdikov,[b] Ch. Betzel[c] and A. M. Mikhailov[b]*

[a]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov Str. 32, Moscow 117984, Russia, [b]Institute of Crystallography of Russian Academy of Sciences, Leninsky Prospekt 59, Moscow 117333, Russia, [c]Institute of Physiological Chemistry, University Hospital, Macromolecular Structure Unit, c/o DESY, Building 22a, Notkestrasse 85, 22603 Hamburg, Germany, and [d]Institute of Mathematical Problems of Biology, Russian Academy of Sciences, Pushchino 142290, Russia

Correspondence e-mail: amm@biostr.crystal.msk.ru

The three-dimensional crystal structure of *Serratia marcescens* endonuclease has been refined at 1.1 Å resolution to an R factor of 12.9% and an $R_{\rm free}$ of 15.6% with the use of anisotropic temperature factors. The model contains 3694 non-H atoms, 715 water molecules, four sulfate ions and two Mg^{2+}-binding sites at the active sites of the homodimeric protein. It is shown that the magnesium ion linked to the active-site Asn119 of each monomer is surrounded by five water molecules and shows an octahedral coordination geometry. The temperature factors for the bound Mg^{2+} ions in the A and B subunits are 7.08 and 4.60 Å^2, respectively, and the average temperature factors for the surrounding water molecules are 12.13 and 10.3 Å^2, respectively. In comparison with earlier structures, alternative side-chain conformations are defined for 51 residues of the dimer, including the essential active-site residue Arg57. A plausible mechanism of enzyme function is proposed based on the high-resolution *S. marcescens* nuclease structure, the functional characteristics of the natural and mutational forms of the enzyme and consideration of its structural analogy with homing endonuclease I-*Ppo*I.

Received 14 December 1999
Accepted 29 February 2000

PDB Reference: endonuclease, 1ql0.

1. Introduction

Investigation of the primary, secondary, tertiary and quarternary structures of enzymes is aimed primarily at providing a better understanding of the role of the oligomeric and spatial arrangement, the packing of partial regular forms (such as 3_{10} helices, distorted helices and β-sheets) and amino-acid sequences in the realisation of the chemical mechanism of the function of the enzyme molecule. This, in turn, leads to the identification of catalytically important active-site residues, analysis of their reactive states and dynamics of interrelation, their mutual topographic orientation at the individual protein loci and the three-dimensional protein globule in its entirety.

In the past few years, the efforts of many enzymologists have been directed towards the study of the new family of structurally related magnesium-dependent endodepolymerases from different species of prokaryotic and eukaryotic organisms which catalyze the cleavage of nucleic acids between the 5′ phosphate and 3′ oxygen of the sugar moiety and are able to degrade both single-stranded and double-stranded DNA and RNA with similar efficiency without particular sequence preferences. The *S. marcescens* (*Sm*) endonuclease (E.C. 3.1.30.2) is a typical example of this protein family and is the only enzyme that has been extensively studied biochemically for which structural information is available (Guynn *et al.*, 1998).

© 2000 International Union of Crystallography
Printed in Denmark – all rights reserved

Some time ago, highly ordered crystal forms of *Sm* nuclease suitable for X-ray studies were grown by two independent groups of investigators (Miller *et al.*, 1991; Bannikova *et al.*, 1991) and the enzyme's three-dimensional structure was solved at 2.1 Å using isomorphous replacement (Miller *et al.*, 1994) and was then refined at 1.7 Å resolution (Lunin *et al.*, 1997). More recently, the magnesium-binding site was located in the protein (Miller *et al.*, 1999) and it was shown that the active site of *Sm* nuclease contains several residues which are conserved in homologous proteins. The role of these residues in the catalytic mechanism of nucleic acid cleavage has been analyzed by detailed biochemical and mutational analyses (Filimonova *et al.*, 1994; Friedhoff, Kolmes *et al.*, 1996; Friedhoff, Meiss *et al.*, 1996).

X-ray diffraction studies of *Sm* nuclease, however, involved only free enzyme which did not contain any bound substrate. Therefore, information on the catalytic groups of the enzyme's active site was scarce and limited to indirect data; this was a principal cause of the proposal of mutually exclusive mechanisms of *Sm* nuclease catalysis with different amino-acid residues involved in the reaction (Miller *et al.*, 1994, 1999; Lunin *et al.*, 1997; Friedhoff, Meisss *et al.*, 1996). Here, we present the refinement of the *Sm* nuclease structure at 1.1 Å resolution, describe the enzyme's active site, analyze previously published results on the biochemical properties of the wild-type enzyme and its mutational analogues, draw an analogy to the structurally similar homing endonuclease and propose the most likely mechanism of enzyme action.

2. Materials and methods

The prismatic crystals of dimensions 0.7 × 0.3 × 0.2 mm used for high-resolution X-ray data collection and analysis were obtained as described previously (Bannikova *et al.*, 1991). X-ray data were collected using a MAR image-plate system at the BW7B wiggler beamline of DORIS/DESY, Hamburg, Germany. The crystals were directly frozen in an N_2 gas stream at 100 K using an Oxford Cryosystems Cryostream. Glycerol [25%(*v*/*v*)] was used as a cryoprotectant. The diffraction data were processed using the program *DENZO* (Otwinowski, 1993).

The crystal structure of *Sm* nuclease was refined using all data in the resolution range 20–1.1 Å. The anisotropic version of the *REFMAC* program was used (Murshudov *et al.*, 1997). Stages of refinement alternated with inspection of the Fourier synthesis and manual correction of the model using *FRODO* (Jones, 1978). The final crystallographic *R* factor for all reflections was 12.9% (R_{free} = 15.6%). Inspection of the structure of the nuclease using *PROCHECK* (Laskowski *et al.*, 1993) did not display serious errors. The data-collection and refinement statistics are summarized in Table 1.

The main-chain protein folding is almost identical to that in the 1.7 Å model (Lunin *et al.*, 1997), but 51 amino-acid residues (25 in the *A* subunit and 26 in the *B* subunit), including the catalytically important Arg57, have alternative side-chain conformations. The model contains 715 water molecules (131 in alternative conformations); there are no water molecules with *B* factors of greater then 55 Å^2. The model contains two Mg^{2+} ions with full occupancy (one in each subunit).

Table 1
Crystal data, refinement statistics and characteristics of *Sm* nuclease.

Space group	$P2_12_12$
Unit-cell parameters (Å)	
a	106.39
b	73.67
c	68.12
V_m (Å^3 Da^{-1})	2.1
Resolution range (Å)	20–1.1
No. of unique reflections	198103
R_{sym} (final shell)	0.041 (0.252)
Completeness (%)	91.5
No. of reflections used	184483
R factor (R_{free})	0.129 (0.156)
R.m.s. deviations from ideality	
Bond lengths (Å)	0.013
Bond angles (°)	1.4
Torsion angles (°)	4.5
Chiral volumes (Å^3)	0.113
Average *B* factors (Å^2)	
Protein atoms	10.84
Water O atoms	19.46
Distribution of dihedral angles on Ramachandran map (%)	
In allowed regions	91.2
In additional allowed regions	7.8

Modelling of DNA binding by *Sm* nuclease and the superposition of the structure of the *Sm* nuclease on the structure of I-*Ppo*I homing endonuclease (PDB code 1a73; Flick *et al.*, 1998) was carried out using *FRODO* and *Swiss-PdbViewer* (Guex & Petsch, 1997) in a similar way to that described previously (Miller *et al.*, 1999).

3. Results and discussion

According to the final model of *Sm* nuclease, the values of the torsion angles φ and ψ calculated with *PROCHECK* for the main chain are found in the allowed regions and all non-Gly residues adopt most favourable or allowed conformations. The model satisfies all the stereochemical requirements generally applied to high-resolution structures. The residues Ala10 and Asn177 are involved in γ-turns, which are nowadays also considered to be allowed conformations (Hutchinson & Thornton, 1996). It should be noted that Ala10 is located in a short loop (Cys-Ala-Val-Gly-Cys) which is stabilized by a disulfide bridge between the Cys residues and by the hydrogen bonds Cys9 O···Val11 N, Val11 O···Gly221 N and Gly12 O···Ala220 N. As indicated by statistical analysis (Bosharov, 1997), Asn residues are more often found in the correct part of the Ramachandran map than other residues.

As refinement of the *Sm* nuclease structure at 1.7 Å allowed us to describe more authentically the secondary structure of *Sm* nuclease (Lunin *et al.*, 1997), in this paper refinement of the enzyme structure at 1.1 Å lets us describe the enzyme molecule much more precisely. In contrast to earlier papers, the structure was refined using anisotropic approximation. Alternative conformations were defined for 51 residues in the nuclease dimer; it is important that alternative conformations

for the active-site residue Arg57 in both subunits were defined. The model also contains four sulfate ions, including the free sulfate ion in the active site of each subunit hydrogen bonded to the Mg^{2+}–water cluster.

According to the present data, the structure of *Sm* nuclease reveals essential inner symmetry. The protein secondary structure can be divided into three regions: a central β-sheet formed by six antiparallel β-strands, which is flanked by an α-domain consisting of four short α-helices and joining the N- and C-terminal parts of the molecule on one side, and an α/β conformational domain forming the upper layer of the globule, which consists of a double-stranded β-sheet embraced by a symmetrical system of eight helical fragments (Fig. 1).

The refined structure of *Sm* nuclease dimer at 1.1 Å resolution contains 3694 non-H atoms and 715 water molecules (131 in alternative conformations). Two Mg^{2+} ion binding sites are localized at the surface of the molecule within the α/β conformational domain and are shielded from behind by a central six-stranded β-sheet (Fig. 1). The Mg^{2+} ion acts as metal ion cofactor, makes contact directly with the side-chain O atom (OD1) of Asn119, shows an octahedral coordination geometry and is surrounded by five water molecules. The coordination distances for the water molecules and Asn119 OD1 are in the range 2.02–2.11 Å. The temperature factors for the bound Mg^{2+} ions in the *A* and *B* subunits are 7.08 and 4.60 Å^2, respectively, and the average temperature factors for surrounding water molecules and the Asn119 OD1 atom are 12.13 and 10.3 Å^2, respectively. Both sites are fully occupied and well defined in the $2F_o - F_c$ electron-density map (Fig. 2).

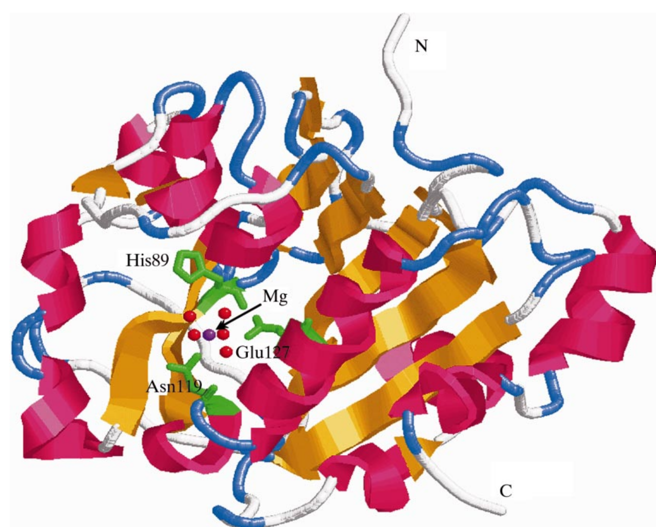

Figure 1
The secondary structure of the *Sm* nuclease monomer. The magnesium–water cluster (magenta Mg^{2+} and red water molecules) and active-site residues His89, Asn119 and Glu127 (green) are shown.

The principal goal of the *Sm* nuclease investigation is the clarification of the chemical mechanism of enzyme action and primarily involves identifying those amino-acid residues that are responsible in the framework of general acid/base catalysis for the activation of the water molecule which attacks the phosphodiester bond, the stabilization of the transition state and the promotion of the protonation of the nucleotide leaving group.

Based on structural and enzymological investigations, the enzyme DNA/RNA-binding cleft is located between the two main structural domains of the protein and the active site is formed by the invariant residues Arg57, Asp86, Arg87, His89, Asn119 and Arg131, several of which were thought to play important roles in the enzyme's catalytic function (Miller *et al.*, 1994, 1999; Lunin et al., 1997; Friedhoff, Kolmes *et al.*, 1996; Friedhoff, Meiss *et al.*, 1996).

We have suggested previously that Asp86 is involved in the chelation of the magnesium ion and that Glu127 acts as a general Lewis base for the activation of the water molecule (Lunin *et al.*, 1997), while Pingoud and coworkers have concluded that Glu127 interacts with the active-site magnesium and that His89 plays the role of general base (Friedhoff, Kolmes *et al.*, 1996; Friedhoff, Meiss *et al.*, 1996).

Along with the Mg^{2+}–water cluster coordinated to Asn119, there are seven nearby amino-acid residues in the active site of *Sm* nuclease, including Arg57, Asp86, His89, Gln114 and Glu127 (Fig. 3), and additionally Asn106 and Gln120 (not shown); the former are conserved in the family of homologous proteins, while the latter are variable.

From the results of the nuclease structure refinement, it is now clear that Asn106 interacts with His89 and that the carbonyl O atom of Asn106 and His89 NE2 are hydrogen bonded. In turn, Gln120 ND2 is involved in a hydrogen bond to Asp86 OD2, whereas Asp86 OD1 participates in hydrogen bonding to the side chain of Asn119.

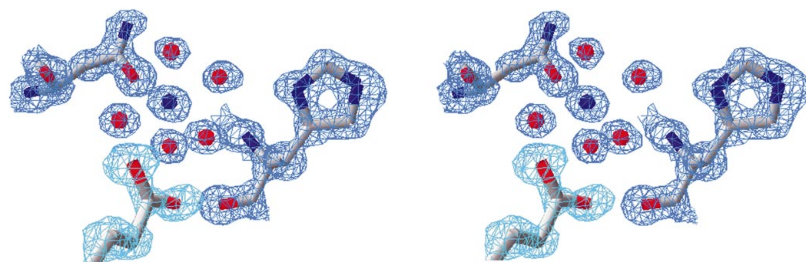

Figure 2
Stereoview of the $2F_o - F_c$ electron-density map and the active-site residues His89 (right), Asn119 (top left) and Glu127 (bottom left) as well as the Mg^{2+}–water cluster. Mg^{2+} is in blue, waters are in red.

The remainder of the above-mentioned residues are linked by hydrogen bonds to the coordinating water molecules. For example, one of the Glu127 carboxylate O atoms is hydrogen bonded to H_2O*b* and the other is bonded to H_2O*d*; Asn119 OD1, in addition to coordinating to the Mg^{2+} atom, is hydrogen bonded to H_2O*a* and H_2O*c*, while H_2O*d* interacts with the main-chain carbonyl O atom of Asn119. H_2O*a* and H_2O*e* interact with the main-chain amide of His89; H_2O*a* makes a hydrogen bond to the ND1 of His89 imidazole and H_2O*e* interacts with the main-chain carbonyl O atom of His89 (Table 2;

Table 2
Interatomic distances in *A* (*B*) subunits of the active site of *Sm* nuclease and values of temperature factors.

Atom 1	*B* factor (Å^2)	Atom 2	*B* factor (Å^2)	Distance (Å)
Mg^{2+}	7.08 (4.60)	Asn119 OD1	14.34 (11.09)	2.07 (2.01)
His89 NE2	10.37 (8.83)	Asn106 OD1	10.05 (9.03)	2.78 (2.81)
Asp86 OD1	13.42 (13.10)	Asn119 ND2	14.76 (11.43)	2.89 (2.83)
Gln120 NE2	18.93 (17.54)	Asp86 OD2	13.53 (11.98)	2.89 (2.84)
His89 ND1	9.64 (8.25)	H_2Oa O	8.38 (9.44)	2.73 (2.72)
Asn119 OD1		H_2Oa O		3.09 (3.05)
His89 N	8.07 (7.35)	H_2Oa O		2.97 (2.95)
Glu127 OE2	12.56 (9.49)	H_2Ob O	13.12 (9.82)	2.49 (2.59)
Asn119 OD1		H_2Oc O	9.87 (13.66)	3.10 (3.10)
Asn119 O	10.96 (10.83)	H_2Od O	13.17 (9.81)	2.90 (2.82)
Glu127 OE1	12.74 (10.03)	H_2Od O		2.70 (2.67)
His89 O	8.02 (7.65)	H_2Oe O	11.18 (8.75)	2.92 (2.87)
Gln114 NE2	8.14 (7.73)	H_2Oe O		2.58 (2.55)
His89 N		H_2Oe O		3.10 (3.17)
Arg57 NH2	19.03 (17.67)	Sulfate S	20.02 (13.09)	3.72 (3.68)
Mg^{2+}		Sulfate S		4.83 (4.71)
His89 ND1		Sulfate S		4.47 (4.32)
Arg57 NH2†	20.39 (17.48)	H_2Oa O†	16.12	2.59

† Alternative conformation (for H_2Oa only available in *A* subunit).

Fig. 3). Such a combination of interactions is necessary in order to provide binding and correct positioning of the Mg^{2+} cofactor and the relative location of the enzyme's catalytically important residues.

From a structural viewpoint, in addition to Asn119, there are at least three further residues important for enzyme activity, namely Arg57, His89 and Glu127, which are not only located in close proximity to each other, but mutagenesis of any one of them results in a drastic reduction in the catalytic properties of *Sm* nuclease (Friedhoff, Kolmes *et al.*, 1996; Kolmes *et al.*, 1996), although until now their chemical function has remained under question.

As has already been mentioned, in the studies of the *Sm* nuclease structures which have been solved crystallographically, we are dealing with the enzyme without substrate and are therefore restricted to indirect data about enzyme–substrate interactions.

The crystallographic structures, at 1.8 Å, of intron-encoded homing endonuclease I-*Ppo*I from *Physarum polycephalum* complexed with DNA from *P. polycephalum* (without Mg^{2+} ions) and its post-cleavage product (in the presence of Mg^{2+}; Flick *et al.*, 1998) have been published. It was shown that I-*Ppo*I and *Sm* nucleases have similar biochemical properties and share quite similar active-site geometries (Miller *et al.*, 1999; Friedhoff *et al.*, 1999; Figs. 4*a* and 4*b*). Both enzymes bind catalytic Mg^{2+} ions with a single Asn119 ligand; the catalytic His89/His98 (*Sm*/I-*Ppo*I), Asn119 and metal–water cluster superimpose quite well (Table 3), which suggests that DNA around the scissile bond might be bound in a similar way by *Sm* nuclease as by the nuclease I-*Ppo*I. This suggestion is supported, as seen in the superimposed nuclease structures, by the close proximity of the 3′- and 5′-phosphate groups of the scissile DNA phosphodiester bond to the protein residues Arg87 and Arg131, which are invariant in the *Sm* nuclease family and are important for the substrate-binding and enzyme activity (Friedhoff, Kolmes *et al.*, 1996; Flick *et al.*, 1998; Kolmes *et al.*, 1996).

In the I-*Ppo*I product–DNA complex, the terminal 3′-deoxyribose O atom is coordinated to the magnesium ion; if this is the case for *Sm* nuclease, we would expect that the 3′ O atom from bound DNA occupies the position of the H_2Oc molecule in the magnesium-coordinated cluster of the enzyme. The water molecule from the hydration sphere of the Mg^{2+} ion bound to Asn119 protonates the leaving group of the nucleotide (Fig. 3). The direct binding 3′-OH group of the substrate to the magnesium ion suggests an inner-sphere mechanism of activation by magnesium (Cowan, 1998), which is not trivial for nucleases. Asp86, Gln114 and Gln127 of *Sm* nuclease are indirect participants in this process; their role is probably restricted by the correct positioning of the Mg^{2+}–water cluster. At the same time, Glu127 (as proposed previously; Lunin *et al.*, 1997), as well as magnesium-bound water (hydroxide), cannot be involved in the *Sm* nuclease catalysis as the general base. The Glu127Ala mutant of *Sm* nuclease retains about 0.1% of the residual activity of wild-type enzyme using DNA as substrate, but cleavage of deoxythymidine 3′,5′-bis-(*p*-nitrophenyl) phosphate is not at all affected by this mutation, as the protonation of the leaving group is not necessary in this case (Kolmes *et al.*, 1996). The fact that magnesium may not be required for hydrolysis of this artificial nucleotide by wild-type nuclease or the Glu127Ala mutant assumes that a water molecule from the coordination sphere of the magnesium ion could indeed be the general acid which protonates the leaving group during the cleavage of nucleic acids by *Sm* nuclease.

Figure 3
A schematic representation of the active-site structure of *Sm* nuclease. The substrate 3′ O atom occupies the position of H_2Oc in the magnesium coordination sphere. Hydrogen bonds are shown as broken lines. The mechanism of the phosphodiester-bond cleavage is shown by arrows. H_2Oa, activated by His89, attacks the phosphodiester bond to be cleaved.

From the above reasoning, it is clear that the only principal candidate for the general base function of *Sm* nuclease is His89. This residue cannot be a general acid in the mechanism of nucleic acid cleavage by *Sm* nuclease (as we assumed previously; Lunin *et al.*, 1997), because if this were the case the His89Ala mutant should be able to hydrolyze the previously mentioned synthetic nucleotide substrate; however, it does not do so (Kolmes *et al.*, 1996).

Relying on the present data, His89 is neutral in the crystallographic structure of *Sm* nuclease; only the NE2 atom of His imidazole is protonated (Fig. 2) and His89 is hydrogen bonded to Asn106 and two water molecules H_2Oa and H_2Oe. In the structure of I-*Ppo*I, the His residue is located optimally to position a water for an in-line attack on the phosphodiester bond to be cleaved. There are two mechanistic schemes for the participation of the His89 residue in nucleotide cleavage by *Sm* nuclease in which either an unligated solvent water molecule or a magnesium-bound water molecule is activated by His (Miller *et al.*, 1999).

From our point of view, it is reasonably safe to suggest that only the second mechanism, involving activation of H_2Oa by His89, is realistic. This mechanism serves as a further indication of the catalytic role of Asn119 in the mechanism of phosphodiester-bond cleavage of deoxythimidine 3′,5′-bis-(*p*-nitrophenyl phosphate) by *Sm* nuclease. This nucleotide does not require protonation of its leaving group and is cleaved by wild-type enzyme without Mg^{2+} ion, but it is not at all affected by the Asn119Ala analogue of *Sm* nuclease (Kolmes *et al.*, 1996). This means that Asn119, even in the absence of the Mg^{2+}–water cluster, is involved in multipoint fixation of the water molecule attacking the phosphodiester bond and the logical deduction is made that it is the same H_2Oa molecule as in the presence of the magnesium cluster. In fact, the crystallographic structures of *Sm* nuclease active sites in the presence or absence of magnesium cofactor are identical within the accuracy of the X-ray analysis. Moreover, in both the structure of the DNA-I–*Ppo*I complex and the refined crystallographic *Sm* nuclease structure without Mg^{2+} ions the His89 (His98 in I-*Ppo*I)–Asn119 hydrogen-bonded water molecule is observed and, moreover, occupies the same position in the active sites as the Mg^{2+}–H_2Oa cluster.

Table 3
Interatomic distances in the superimposed structures of *Sm* and I-*Ppo*I endonucleases.

Sm nuclease	I-*Ppo*I nuclease	Distance (Å)
Mg^{2+}	Mg^{2+}	0.58
His89 ND1	His98 ND1	0.32
Asn119 OD1	Asn119 OD1	0.36
H_2Oa O	H_2Oa O	0.61
H_2Ob O	H_2Ob O	1.08
H_2Oc O	H_2Oc O	1.27
H_2Od O	H_2Od O	1.51
H_2Oe O	H_2Oe O	0.91

† H_2Oc position is identical to the position of the nucleotide 3′ O atom in the nuclease–substrate complex.

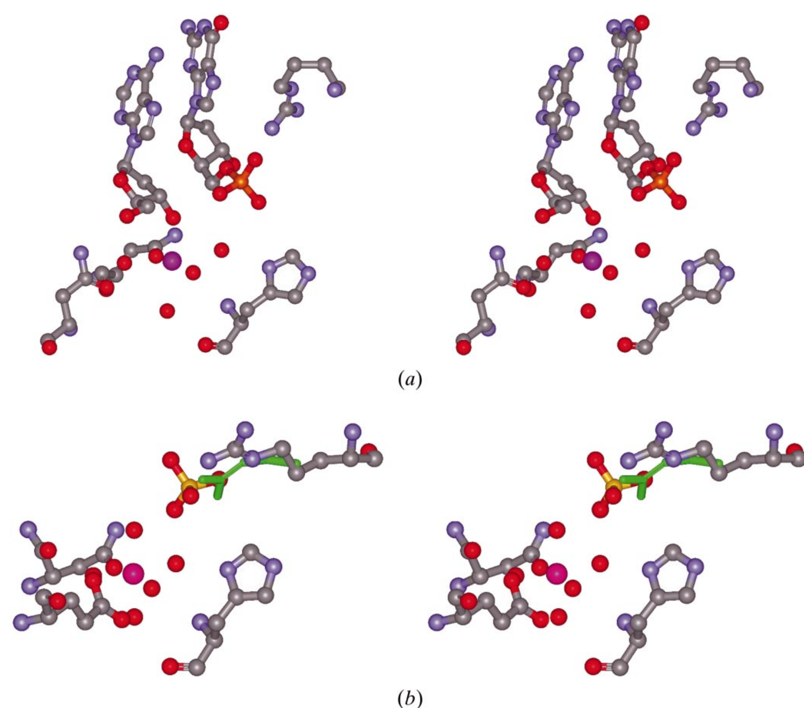

Figure 4
Stereoview of the active sites of I-*Ppo*I nuclease with cleavage products complex (*a*) and *Sm* nuclease (*b*). The only two nucleotides between which bond cleavage occurs are shown in (*a*). In (*b*), both conformations of the Arg57 side chain are shown for *Sm* nuclease (conformation with occupancy 0.4 is shown by green lines). Mg^{2+} is in magenta; the sulfate ion is in yellow/red.

In addition, the refined crystallographic *B* factors give us some indication of the flexibility or disorder of the protein structure. This is particularly important for the residues in the active site, because the *B* values will give some estimate of which residues are predestined for changes of position during the process of substrate binding and cleavage. A near-atomic resolution is sufficient to refine every single atom in anisotropic mode, which was performed in this case.

Arg57 of the *Sm* nuclease active site is rather conspicuous. The overall *B* factors for Arg57 as a rough approximation were doubled compared with all other residues at 1.7 Å resolution. Owing to the high resolution and the use of anisotropic *B* factors, we were able to locate the alternative conformation of its side chain. According to our data, Arg57 has alternative conformations of the side chain with occupancies 0.6 and 0.4 in both molecules of the dimer. In one conformation Arg57 is hydrogen bonded to a sulfate ion (occupancy 0.6) and in the other conformation Arg57 is bound to the Mg^{2+}–water cluster (Fig. 4*b*)

Results of *Sm* nuclease–DNA binding modelling show that it is almost possible to fit the nucleotide substrate into the active site in a similar manner as observed in I-*Ppo*I nuclease in

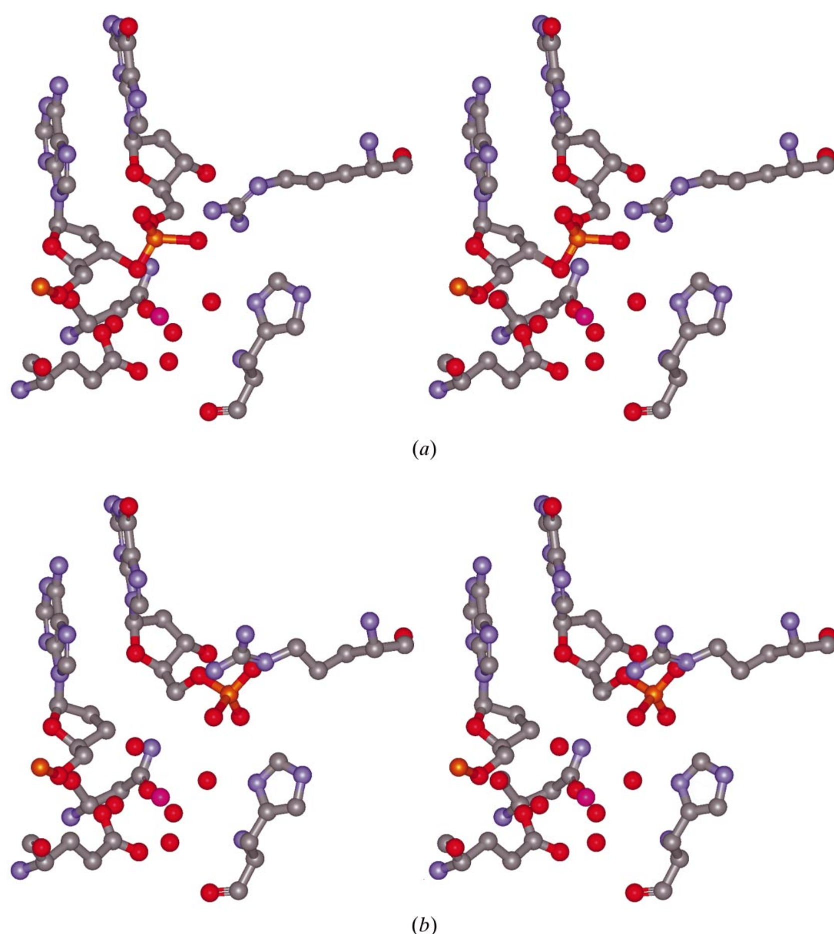

Figure 5
Stereoview of a modelled complex of *Sm* nuclease with DNA (only the dinucleotide fragment of bound DNA in the active site is shown). (*a*) Arg57 (right) binds the phosphate group of substrate before cleavage; (*b*) Arg57 in the alternative conformation, binding the phosphate group after cleavage. The phosphate group is in the centre of the figure in orange. Asn119 is located behind the Mg^{2+}–water octahedral cluster.

complex with cognate substrate. Because of the two possible locations of the side chain of Arg57, we can see two states of the active site: in the first case, with no sulfate present, Arg57 is in contact with the 5′-phosphate of the former scissile phosphodiester bond; in the second case, the altered position of the Arg57 side chain lets the phosphate group move away from the 3′ O atom after cleavage of the DNA (Figs. 5*a* and 5*b*). This is supporting evidence that Arg57 is likely to be involved in the positioning and polarization of the phosphate and may be also involved in transition-state stabilization during the process of phosphodiester-bond cleavage.

Thus, the phosphodiester-bond cleavage by *Sm* nuclease is carried out by nucleophilic substitution at the P atom as an addition–elimination reaction of a water molecule to form a pentacoordinated intermediate having a trigonal bipyramidal geometry with the P atom in the centre, the attacking and leaving groups at the vertices and three oxygen substituents in the base plane. The mechanism of the nucleophilic substitution implies a maximal concentration of positive charge on the central P atom in the transition state when the anionic leaving group is departing. The reaction is accompanied by inversion of the configuration at the P atom and occurs by an in-line mechanism, *i.e.* the attacking nucleophile approaches from the site opposite to the leaving group. His89 of *Sm* nuclease and the magnesium water cluster are directly involved in this process and the metal ion could participate in more than one of its possible roles and could serve to activate the electrophile, stabilize the transition state and protonate the leaving group.

This work was supported by Grants BMBF (MP; SFB 344-D6) and RFBR (SS; 97-04-49772). We thank Professor Dr N. Genov for helpful discussion.

References

Bannikova, G. E., Blagova, E. V., Dementiev, A. A., Morgunova, E. Yu., Mikhailov, A. M., Shlyapnikov, S. V., Varlamov, V. P. & Vainshtein, B. K. (1991). *Biochem. Int.* **23**, 813–822.
Bosharov, M. A. (1997). *Biofizika*, **42**, 753–764.
Cowan, J. A. (1998). *Chem. Rev.* **98**, 1067–1087.
Filimonova, M. N., Krause, K. L. & Benedik, M. J. (1994). *Biochem. Mol. Biol. Int.* **23**, 1229–1236.
Flick, K. E., Jurica, M. S., Monnat, J. R. & Stoddard, B. L. (1998). *Nature (London)*, **394**, 96–101.
Friedhoff, P., Franke, I., Krause, K. L. & Pingoud, A. (1999). *FEBS Lett.* **443**, 209–214.
Friedhoff, P., Kolmes, B., Gimadutdinov, O., Wende, W., Krause, K. L. & Pingoud, A. (1996). *Nucleic Acids Res.* **24**, 2632–2639.
Friedhoff, P., Meiss, G., Kolmes, B., Pieper, U., Gimadutdinov, O., Urbanke, C. & Pingoud, A. (1996). *Eur. J. Biochem.* **241**, 572–580.
Guex, N. & Peitsch, M. C. (1997). *Electrophoresis*, **18**, 2714–2723.
Guynn, L. J., Dai, W. & Benedik, M. J. (1998). *J. Bacteriol.* **180**, 2262–2264.
Hutchinson, E. G. & Thornton, J. M. (1996). *Protein Sci.* **5**, 212–220.
Jones, T. A. (1978). *J. Appl. Cryst.* **26**, 268–272.
Kolmes, B., Franke, I., Friedhoff, P. & Pingoud, A. (1996). *FEBS Lett.* **397**, 343–346.
Laskowski, R. A., MacArthur, M. W., Moss, D. C. & Thornton, J. M. (1993). *J. Appl. Cryst.* **26**, 283–291.
Lunin, V. Yu., Levdikov, V. M., Shlyapnikov, S. V., Blagova, E. V., Lunin, V. V., Wilson, K. S. & Mikhailov, A. M. (1997). *FEBS Lett.* **412**, 217–222.
Miller, M. D., Benedik, M. J., Sallivan, M. C., Shipley, N. S. & Krause, K. L. (1991). *J. Mol. Biol.* **222**, 27–30.
Miller, M. D., Cai, J. & Krause, K. L. (1999). *J. Mol. Biol.* **288**, 975–987.
Miller, M. D., Tanner, J., Alpaugh, M., Benedik, M. J. & Krause, K. L. (1994). *Nature Struct. Biol.* **1**, 461–468.
Murshudov, G., Vagin, A. & Dodson, E. (1997). *Acta Cryst.* D**53**, 240–255.
Otwinowski, Z. (1993). *DENZO. An Oscillation Data Processing Program for Macromolecular Crystallography*. Yale University, New Haven, Connecticut, USA.