Human flap endonuclease-1 (FEN-1) is a member of the structure-specific endonuclease family and is essential in DNA replication and repair. FEN-1 has specific endonuclease activity for repairing nicked double-stranded DNA substrates that have the 5'-end of the nick expanded into a single-stranded tail, and it is involved in processing Okazaki fragments during DNA replication. Magnesium is a cofactor required for nuclease activity. We used small-angle x-ray scattering to obtain global structural information pertinent to nuclease activity from FEN-1, the D181A mutant, the wild-type FEN-1 34-mer DNA flap complex, and the D181A 34-mer DNA flap complex. The D181A mutant, which has Asp-181 replaced by Ala, selectively binds to the flap structure, but has lost its cleaving activity. Asp-181 is thought to be involved in Mg2+ binding at the active site (Shen, B., Nolan, J. P., Sklar, L. A., and Park, M. S. (1996) J. Biol. Chem. 271, 9173–9176). Our data indicate that FEN-1 and the D181A mutant each have a radius of gyration of ~26 Å, and the effect of Mg2+ on the scattering from the proteins alone is insignificant. The 34-mer DNA fragment was constructed such that it readily forms a 5'-flap structure. The formation of the flap conformation of the DNA substrate was evident by both the extrapolated I0 scattering and radius of gyration and was supported by NMR spectrum and nuclease assays. In the absence of magnesium, the FEN-1 34-mer DNA flap complex has an Rg value of ~34 Å, whereas the D181A 34-mer DNA flap complex self-associates, suggesting that a significant protein conformational change occurs by addition of the flap DNA substrate and that Asp-181 is crucial for proper binding of the protein to the DNA substrate. A time course change in the scattering profiles arising from magnesium activation of the FEN-1 34-mer DNA flap complex is consistent with the protein completely releasing the DNA substrate after cleavage.

The 5'-flap structure is a common DNA structural intermediate occurring during DNA replication, recombination, and repair (1). In eukaryotic DNA replication, displacement of an upstream primer by an incoming polymerase can result in the formation of a 5'-flap structure (2, 3). The 5'-flap intermediates are also formed during double-stranded break repair (4, 5), homologous recombination (6), and excision repair (7–10). In DNA repair and replication activities, structural recognition of the 5'-flap by specific DNA repair nucleases is essential. The importance of the DNA metabolic reactions, involving the structure-specific nucleases, is best illustrated by the human genetic disorder xeroderma pigmentosum (11–13). This disease, characterized by severe sensitivity to sunlight and a predisposition to skin cancer, results directly from defects in the nucleotide excision pathway. Mutation defects in the repair nucleases may be a point of breakdown in this DNA repair pathway.

The design of a model flap DNA structure, similar to those conjectured to occur in the nucleotide excision pathway, has led to the discovery of human flap endonuclease-1 (FEN-1),1 which structurally recognizes and cleaves the flap DNA structure (4, 14–17). FEN-1, an ~43-kDa Mg2+- or Mn2+-dependent enzyme, demonstrates both 5'-flap structure-specific endonuclease activity (1, 4, 7) and nick-specific 5’→3’ exonuclease activity (4, 14, 17, 18). The exonuclease activity of FEN-1 is similar to the function primer removal that is necessary for in vitro mammalian DNA replication. In its endonuclease role, FEN-1 recognizes the phosphodiester backbone of a 5'-flap single strand and tracks down this arm to the cleavage site, the junction where the two strands of duplex DNA adjoin a single-stranded arm (1, 3). FEN-1 does not cleave bubble substrates, single-stranded 3'-flaps, heterologous loops, or Holliday junctions, but acting as an exonuclease, FEN-1 will hydrolyze double-stranded DNA substrates containing a gap or 3'-overhang. FEN-1 endonuclease activity is independent of 5'-flap length, and endonuclease and exonuclease activities cleave both DNA and RNA without the need for accessory proteins (19). FEN-1 does, however, interact with other proteins at the replication fork, including a DNA helicase (20), the proliferating cell nuclear antigen (21–24), and possibly replication protein A (RPA) (25).

The biological significance of the FEN-1 gene (RAD27 in Saccharomyces cerevisiae and rad2 in Schizosaccharomyces pombe) is emphasized by genetic analysis in yeast. The yeast FEN-1 mutants display severely impaired phenotypes such as UV sensitivity, deficient chromosome segregation, conditional lethality, and accumulation in S phase (15, 26–29). The yeast rad27 null mutant is a strong mutator, and the majority of mutations found are duplications. This is probably because

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* This work was supported by the Integrated Structural Biology Resource Program at Los Alamos National Laboratory, by Los Alamos National Laboratory Directed Research Development Grants 94205 (to M. S. P.) and 95623 (to G. A. Olah), by Department of Energy Grant KP1104-010 (to M. S. P.) and by National Institutes of Health Grant CA73764 (to B. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Dept. of Cell and Tumor Biology, City of Hope Medical Center and Beckman Research Inst., Duarte, CA 91010.

§ To whom correspondence should be addressed: Oklahoma State University College of Veterinary Medicine, 110 Veterinary Medicine, Stillwater, OK 74078. Tel.: 405-743-1887; E-mail: olah@okstate.edu.

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1 The abbreviations used are: FEN-1, flap endonuclease-1; SAXS, small-angle x-ray scattering; Rg, radius of gyration; Rr, radius of gyration of cross-section; I0, forward scatter, Pr, vector distribution function; dmax, maximum linear dimension.
unexcised flap strands in Okazaki fragments displaced by upstream DNA polymerization are subsequently annealed to the downstream complementary sequence. This part of the sequence will be duplicated in the next generation of DNA replication (30). FEN-1 activity requires a free 5′-end of the flap DNA strand. For instance, secondary structure formation of the single-stranded DNA into a hairpin structure is known to prevent the enzyme’s function (31). At risk motif sequences such as trinucleotide repeats have a higher probability to form these structures. Indeed, the same FEN-1 null mutant displays length-dependent CTG tract destabilization and a marked increase in expansion frequency (32, 33). Thus, FEN-1 is a key enzyme for maintaining genome integrity, and mutations in FEN-1 may give rise to a number of genetic diseases such as myotonic dystrophy, Huntington’s disease, several ataxias, fragile X syndrome, and cancer (30, 32).

As the role of FEN-1 in DNA replication and repair is becoming more clear, it is important to structurally characterize this enzyme to better understand how it functions either as an exo- or endonuclease. To examine the structure-function relationship of FEN-1 in its nuclease capacity, we have studied the effect of magnesium on its conformation in aqueous solution, as observed by small-angle x-ray scattering (SAXS). Experiments were also done with a D181A mutant of FEN-1, which still selectively binds to the 5′-flap DNA structure, but has lost its catalytic ability (34, 35). We show that no measurable structural change was evident in either FEN-1 or the D181A mutant due to the presence of Mg²⁺. A DNA fragment was constructed so that it readily adopts a 5′-flap conformation. When measurements of FEN-1 and the D181A mutant were performed in the presence of the 34-mer DNA flap fragment, the FEN-1-34-mer DNA flap complex was seen to be more compact than the D181A-34-mer DNA flap complex, which indicates that the wild-type FEN-1-34-mer DNA flap complex is in a cleavage-ready conformation. A time course scattering measurement showed that magnesium was able to activate the cleavage of the FEN-1-34-mer DNA flap complex, and the protein was found to completely release the remaining single- and double-stranded portions of the DNA products.

EXPERIMENTAL PROCEDURES

Protein Purification and Sample Preparation—Protein expression and purification were essentially carried out according to Nolan et al. (36). After FEN-1 was eluted from the column using elution buffer (20 mM Tris-HCl, pH 7.9, 0.5 mM NaCl, and 500 mM histidine), it was further dialyzed in Tris-HCl buffer, pH 7.9, containing 100 mM NaCl, 10 mM 2-mercaptoethanol, and 10% glycerol and then concentrated in a Centriprep-10 concentrator (Amicon, Inc.). Final protein concentrations used were ~6 mg/ml in the SAXS study for both the wild-type and D181A mutant proteins, in which 10 mM Mg²⁺ was either present or absent. Wild-type and D181A mutant protein concentrations were ~4.7 mg/ml for the protein-DNA flap complex studies, and the protein and DNA had a 1:1 stoichiometric ratio. Final concentrations were determined by the Bradford assay (46) using bovine serum albumin γ-globulin as a standard. Binding of the two proteins to the flap DNA substrate was confirmed by gel shift assay after labeling the 5′-end of the substrate with ³²P. The purity of the concentrated protein samples was checked by SDS-polyacrylamide gel electrophoresis and gave single bands as illustrated in Fig. 1a. The flap endonuclease activity of the protein was assayed via a flow cytometry-based nuclease assay system (36) before and after each SAXS measurement. Activity was also observed by time-resolved SAXS measurements by activating the endonuclease with Mg²⁺ in the presence of the flap DNA substrate.

Preparation of the 34-mer DNA Flap Substrate—An oligonucleotide (5′-CCCCCCCATGCTAGTTTCTGTTATAGGTTTCTGTA-3′) was synthesized by the solid-phase phosphoramidite method using an Applied Biosystems synthesizer. The oligonucleotide was designed to form a 5′-flap substrate, which contains two Watson-Crick duplex arms folded by TTTT loops (37) and a 10-base-long 5′-flap single strand (see Fig. 1b). It was purified by eluting the material through a POROS R2H reverse-phase chromatography column, followed by eluting through a POROS HQ/M anion-exchange chromatography column if necessary, equipped with a Bio-Cad Workstation 7000E (PerSeptive Biosystems). The purity was analyzed using a Tris borate/EDTA-urea gel (Novex) and found to be >98% pure. NMR spectroscopic analysis was used to establish the formation of the flap structure as shown in Fig. 1b. Electrophoretic mobility shift and DNA footprinting assays were used to determine the suitability of the newly designed substrate for structural analysis.

SAXS Data Collection, Reduction, and Analysis—SAXS data were reduced as described elsewhere (38, 39) to give Io(Q) versus Q, where Io(Q) is the scattered x-ray intensity and Q is the amplitude of the scattering vector. For elastic scattering processes, Q is equal to 4πsinθ/λ, where θ is half the scattering angle and λ is the wavelength of the incident x-rays. Guinier (40) and the indirect least-squares transform (41) analyses were used to calculate Rg, forward scatter (I₀), and the vector distribution function (Pr(r)). Aggregation was ascertained from Io, which was expected to be proportional to the molecular mass (39). Lysozyme was used as a standard for scaling Io with the implicit assumption that lysozyme has the same mean scattering density as FEN-1 and the D181A mutant. Interparticle interference contribution to the scattering and at the concentrations used was assumed to be negligible since preliminary measurements of the samples at concentrations between 0.5 and 6 mg/ml scaled linearly and gave the same Rg values. Pr(r) is the frequency of all interatomic vectors within the scattering particle weighted by the product of their scattering powers. The zero and second momentals of Pr(r) are equal to I₀ and Rg, respectively. The maximum linear dimension of the scattering particle (dmax) was determined from the corresponding value at which the Pr(r) function goes to zero.

SAXS measurements were done using the instrument described elsewhere (38) at the Los Alamos National Laboratory. The instrument configuration used nickel-filtered 1.542 x-rays produced from a 1.5-kilowatt sealed tube copper target source and was line-focused by a single mirror giving a full width at a half-maximum of 0.74 mm and a full height of 26 mm as measured at the detector. A 4-inch-long position-sensitive linear detector (TEC Model 2100Q) was placed 64 cm from the sample. Measurements spanned a Q range of 0.015–0.27 Å⁻¹. The net scattering from the samples was calculated by subtracting a normalized buffer spectrum measured in the same sample cell. Time course SAXS measurements, whereby data were recorded for 30 min at subsequent intervals, was used to track endonuclease activity after addition of magnesium to protein/DNA substrate samples. Typically, measurements at these protein concentrations require ~6 h for sufficient statistics; however, 30-min scans were good enough for observing changes in I₀. SAXS from at least two separate sample preparations were measured for all measurements. Measurements were made at 10 °C.

Pr(r) analysis of the data collected included a deconvolution of the slit geometric contribution to the scattering. Omission of this correction results in a systematic ~0.3-Å smaller Rg for FEN-1, well within the statistics of the data measured in this report (0.5–1.0 Å; 1 S.D.). Guinier analysis of the data collected was not deconvoluted for instrument geometry.

RESULTS AND DISCUSSION

Magnesium is an essential cofactor for many enzymes involved in DNA metabolism such as DNA polymerases, nucleases, and ligases. This divalent metal, commonly ligated by acidic amino acid residues in nucleases, attacks water molecules to produce a nucleophile that can break phosphodiester bonds (34). Chelating of the metal ions out of human FEN-1 can inactivate the enzyme completely. It has been hypothesized that activation of the enzyme by adding Mg²⁺ to the reaction requires conformational changes in the enzyme before it can cleave the DNA substrate (36). To test this hypothesis, we performed small-angle x-ray scattering from wild-type FEN-1, the D181A mutant, and their complex with DNA substrate to determine their global structure and possible conformational changes upon addition to the Mg²⁺ cofactor.

To perform SAXS experiments, it was necessary to develop an experimental approach to produce a large quantity of flap DNA substrate. Unfortunately, the conventional approach, which utilizes annealing of three independent oligonucleotides, was inadequate for this purpose due to its lowyield. To overcome this problem, we designed a single oligonucleotide that has a high propensity to form the flap DNA structure, as shown
in Fig. 1b (see “Experimental Procedures”).

We used NMR spectroscopy and gel mobility shift and flap endonuclease assays to determine that the newly designed oligonucleotide forms a predicted flap DNA substrate. NMR spectroscopy showed the presence of a double-stranded region registered by A=T and G=C base pairs and showed the presence of a TTTT loop, and wild-type FEN-1 was able to bind to DNA and yielded a correct cleavage product with an expected size of the released flap strand (10 bases) (data not shown). Based upon all of these results, we concluded that the new flap DNA substrate could be used for our further study described.

Fig. 2 shows Guinier plots calculated for FEN-1 and the D181A mutant as well as for FEN-1-34-mer DNA flap and D181A-34-mer DNA flap complexes. Each sample gives a Guinier region that can be fit with a straight line with reduced $\chi^2$ below 1 (Table I). Molecular masses calculated for the protein samples from $I_0$ and using the lysozyme standard were within 10% of the expected value of 43,416 Da. In addition, there was no significant upturn at low $Q$ in the scattering profiles, except for the D181A-34-mer DNA flap complex. The D181A-34-mer DNA flap complex sample had the same concentration as the FEN-1-34-mer DNA flap complex and gave an extrapolated $I_0$ value consistently 10% larger than that found for the FEN-1-34-mer DNA flap complex. This increase in $I_0$ indicates slight aggregation of the D181A-34-mer DNA flap complex samples. The $R_g$ and $d_{\text{max}}$ parameters in Table I were calculated from combinations of two to four scattering measurements using different sample preparations. Guinier plots of $\log(I/Q)$ versus $Q^2$ showed a linear $Q$ region (0.03–0.08 Å$^{-1}$) with a negative slope, from which the radius of gyration of cross-section ($R_g$) could be calculated. A linear region in such a plot suggests that the proteins have an elongated shape, at least in one dimension. The $R_g$ values are also tabulated in Table I.

Fig. 3 shows the $R_g$ values calculated from the $P(r)$ analysis of the individual measurements for the proteins alone in the presence and absence of magnesium. Also, a comparison of the $P(r)$ functions is given in Fig. 4. The $P(r)$ functions have a single peak at ~27 Å and are fairly symmetric, suggesting that the proteins are globular (ellipsoidal). Modeling the data with one ellipsoid using a Monte Carlo modeling method described elsewhere (42) gave dimensions for FEN-1 of $a = 13.6 \pm 0.2$ Å, $b = 32.4 \pm 1.0$ Å, and $c = 45.1 \pm 1.0$ Å for the best fit. The scattering profile generated from this model also gave an $R_g$ value consistent with the $R_g$ value determined from the Guinier plots. Within the statistics of $R_g$ measurements, it is evident that magnesium has no effect on FEN-1 or the D181A mutant in the absence of DNA. The fact that Mg$^{2+}$-induced conformational changes was not observed is probably because the Mg$^{2+}$ binding causes a localized instead of global conformational effect or the induced global conformational changes are quite small.

Next, we examine the effect of Mg$^{2+}$ on the DNA substrate by both scattering and modeling. Scattering profiles and $P(r)$ functions from the free 34-mer DNA fragment in the absence and presence of Mg$^{2+}$ are shown in Fig. 5. In the absence of Mg$^{2+}$, the $P(r)$ function showed a peak at ~16 Å and decreased approximately linearly out to ~60 Å. A simple two-cylinder model was constructed based on the 5'-flap DNA structure shown in Fig. 1b. The intent of this modeling was to add further support for the self-annealed structure by comparing the $P(r)$ function calculated from the model with the $P(r)$ function calculated...
X-ray Scattering from FEN-1 Complexed with Flap DNA and Mg$^{2+}$

$R_g$ and $R_c$ were calculated from the scattering data using Guinier analysis. $R_g$ and $d_{\text{max}}$ were calculated using $P(r)$ analysis. FEN-1 and the D181A mutant alone give approximately the same $R_g$, $R_c$, and $d_{\text{max}}$ values in both the absence and presence of Mg$^{2+}$, suggesting that Mg$^{2+}$ either induces no conformational change or induces a localized or global conformational change that is not measurable within the precision of these measurements. The 34-mer DNA in the presence of Mg$^{2+}$ aggregates as shown by a 16.6% increase in $I_o$, therefore, the larger $R_g$ and $d_{\text{max}}$ values observed relative to the case without Mg$^{2+}$ are not necessarily attributable only to a Mg$^{2+}$-induced conformational change in the DNA substrate. The D181A·34-mer DNA flap complex has a consistently ~10% larger $I_o$ relative to the FEN-1 · 34-mer DNA flap complex, suggesting that slight aggregation occurs in the mutant · DNA substrate complex. Thus, the scattering data show that the D181A mutant possibly binds and interacts differently with the DNA fragment compared with wild-type FEN-1.

### Table I

| Sample | Conc (mg/ml) | Guinier analysis | $P(r)$ analysis |
|---|---|---|---|
| FEN-1 | | | |
| $-\text{Mg}^{2+}$ | 5.8 | $26.4 \pm 0.4$ | $26.8 \pm 0.7$ | 82 |
| $+\text{Mg}^{2+}$ | 5.9 | $26.6 \pm 0.9$ | $26.2 \pm 0.4$ | 84 |
| D181A | | | |
| $-\text{Mg}^{2+}$ | 6 | $26.4 \pm 0.5$ | $26.1 \pm 0.5$ | 86 |
| $+\text{Mg}^{2+}$ | 6 | $25.9 \pm 0.6$ | $26.1 \pm 0.7$ | 88 |
| 34-mer DNA | | | |
| $-\text{Mg}^{2+}$ | 2 | $20.4 \pm 0.5$ | $20.2 \pm 0.4$ | 60 |
| $+\text{Mg}^{2+}$ | 2 | $21.8 \pm 0.6$ | $21.4 \pm 0.3$ | 70 |
| FEN-1 · DNA (1:1), $-\text{Mg}^{2+}$ | 4.7 | $34.4 \pm 0.9$ | $34.4 \pm 0.7$ | 104 |
| D181A · DNA (1:1), $-\text{Mg}^{2+}$ | 4.7 | $40.6 \pm 0.9$ | $43.9 \pm 0.8$ | 138 |

![Figure 3](image1.png)

**Figure 3.** Radius of gyration for wild-type FEN-1 and the D181A mutant in the absence of the 34-mer DNA flap substrate and in the presence or absence of Mg$^{2+}$. The $R_g$ values are the same within the precision of these measurements for both wild-type (WT) FEN-1 and the D181A mutant and in the presence or absence of Mg$^{2+}$. Conformation changes induced by Mg$^{2+}$ are not present or are localized or global, but smaller than the precision of the SAXS measurements described in this report.

![Figure 4](image2.png)

**Figure 4.** $P(r)$ functions for FEN-1 (a) and the D181A mutant (b) in the absence of DNA. Black is in the absence of Mg$^{2+}$, and gray is in its presence. The $P(r)$ functions are fairly symmetric, indicating that the two proteins are globular (ellipsoid shape) in solution and are not affected by the presence of Mg$^{2+}$ or by replacement of Asp-181 by alanine. Single ellipsoid modeling against FEN-1 showed it to have dimensions of $a = 13.6 \pm 0.2 \text{ Å}$, $b = 32.4 \pm 1.0 \text{ Å}$, and $c = 45.1 \pm 1.0 \text{ Å}$. The Mg$^{2+}$ causes change in the scattering profile and, more important, an increase by 16.6% in the extrapolated $I_o$ scattering. This intensity increase suggests that Mg$^{2+}$ causes the DNA to aggregate. Aggregation of the DNA substrate in the presence of Mg$^{2+}$ is not surprising since the interatomic interaction of the cation with the negatively charged phosphate groups in the DNA backbone would decrease electrostatic repulsion between DNA molecules in solution and allow weaker attractive interactions to dominate.

A comparison of the scattering from the two proteins bound to the 34-mer DNA substrate is shown in Fig. 6. Lysozyme could not be used as a standard for molecular mass determination for the protein-DNA complexes as done for the proteins alone since the mean scattering density for DNA is different.
from that for protein. Nevertheless, we can still estimate the expected $I_o$ for the protein-DNA complexes by comparison with $I_o$ for the protein alone samples. We can write the zero-angle scattering for the protein-DNA complex in terms of the two components as follows (Equation 1),

$$I_o = n (f_{protein} \Delta \rho_{protein} dV_{protein} + f_{DNA} \Delta \rho_{DNA} dV_{DNA})^2 \quad \text{(Eq. 1)}$$

where $n$ is the particle number density, $\Delta \rho_x$ is the scattering density of component $x$, and $dV_x$ is the integration volume element over component $x$. Assuming specific volumes of 0.73 ml/g for the protein and 0.56 ml/g for the nucleic acid, we expect, for our measurements, that the contribution of the DNA would increase $I_o$ by $\sim$60% above the protein-alone measurement. This calculated increase in $I_o$ takes into account the concentration differences of the samples ($\sim$4.7 instead of $\sim$6 mg/ml). We observed this increase for the FEN-1-34-mer DNA flap complex samples, suggesting that these samples are monodisperse. In addition, as expected for monodisperse solutions, we obtained good agreement between the $R_g$ values obtained using the Guinier and $P(r)$ analyses, 34.4 Å (see Table 1). The major peak in the $P(r)$ function for the wild-type complex is $\sim$28 Å, and $d_{max}$ is $\sim$104 Å. However, a difference was observed between the $R_g$ values for the D181A-34-mer DNA flap complex samples obtained from the Guinier and $P(r)$ analyses, 40.6 and 43.9 Å, respectively, suggesting that the D181A-34-mer DNA flap complex samples may be aggregated. The $I_o$ value is more meaningful in evaluating the degree of aggregation of the D181A-34-mer DNA flap complex and is $\sim$10% above the $I_o$ value for the FEN-1-34-mer DNA flap complex samples, suggesting that aggregation is present, but not severe. In addition, the $d_{max}$ value calculated for the mutant complex samples is $\sim$34 Å longer than that calculated for the wild-type complex. It is possible that the D181A mutant complex is a slightly more extended structure; however, drawing this conclusion is precarious due to the slight aggregation problem. The scattering data from the complexes and from the free proteins taken together suggest that the D181A mutant binds and interacts differently with the DNA substrate compared with wild-type FEN-1.

The 5’-nuclease domains of E. coli and Taq DNA polymerases are functional homologs of human FEN-1 that possess two Mg$^{2+}$-binding sites. The amino acid sequences of 10 5’-nuclease domains from DNA polymerases in the polymerase I family and viral nucleases were compared by Gutman and Minton (43). The results showed six highly conserved sequence motifs containing 10 conserved acidic residues. In earlier work, we extended this sequence comparison to eight additional sequences of XPG/FEN-1 nuclease family and confirmed that seven acidic amide residues are very conserved in all 18 sequences (35). According to the crystallographic structures of Taq DNA polymerase (44) and T4 RNase H1 (45), these residues (Asp-34, Asp-86, Glu-158, Glu-160, Asp-179, Asp-181, and Asp-233 in human FEN-1 based on the superimposition between FEN-1 and T4 RNase H1) cluster within a sphere of 7-Å
radius around the metal ions. Some of these residues ligate one Mg$^{2+}$ ion, whereas others ligate the second Mg$^{2+}$ ion. Our mutagenesis work (34) further confirmed that Asp-181 is a critical amino acid that ligates the Mg$^{2+}$ and is involved in the cleavage process of the flap endonuclease activity only, but does not appear to affect DNA binding. In this report, we found from SAXS that Asp-181 is actually important for proper binding of the DNA and is not only required for cleavage activity. In fact, proper binding proceeds enzymatic activity whereby the proper orientation of the DNA in the catalytic site is required before cleavage can ensue.

Measurement of the time course change in scattering after initiating cleavage activity by adding magnesium to FEN-1-34-mer DNA flap complex samples required slowing down the kinetics. Typically, for the protein and substrate concentrations used in this report, activity rates are such that it would require only minutes for DNA cleavage completion, too fast to allow for measurements at 10 °C to slow down the nuclease activity. Typically, for the protein and substrate concentrations used in this report, activity rates are such that it would require only minutes for DNA cleavage completion, too fast to allow for measurements at 10 °C to slow down the nuclease activity. After 6 h, $I_0$ did not decrease any further. In b, we show $I_t$ (black circles) versus time fitted to an exponential function of the form $I_t = A + B \cdot e^{-\alpha t}$, where $A$ and $B$ are constants to be determined in the fit and $\alpha$ is the reaction rate, also to be determined in the fit. The fitting procedure was iterative so as to account for the underestimation of the extrapolated $t = 0$ measurement. Note the underestimation of the first measurement, which lies below the iterative exponential fit.

The protein-DNA complex terms in Equations 3 and 4 would include a scattering cross-term as similarly expressed in Equation 1. From Equations 1–4, we would expect 0, 43.9, 32.5, and 15% decreases in $I_o$ scattering, respectively. The “apparent” $I_o$ scattering decreased by ~24–29% for two independent measurements. This apparent decrease was determined by comparing the first measurement made within 1 h of activation and a final measurement made after 6 h of activation after which no further decrease in $I_o$ was observed. At first glance, this measured decrease in intensity appears most consistent with FEN-1 remaining bound to the single-stranded portion of the DNA fragment after cleavage. However, because our measurements were for 30 min, the initial $t = 0$ measurement is underestimated. A crude estimate on the order of magnitude of the error in underestimating the $t = 0$ measurement can be made by assuming that the reaction is completed by 6 h and that it proceeds exponentially. A nonlinear least-squares “best” fit to a plot of $I_o$ with respect time can be made using the form $I_o = A + B \cdot e^{-\alpha t}$, where $A$ and $B$ are constants to be determined in the fit and $\alpha$ is the reaction rate, also to be determined in the fit. We assume a considerably larger error for the earliest measurement (average $t = 15$ m) relative to the later measurements and iterate the fit by successively replacing the earliest measurement until the fit converges and then extrapolate to $t = 0$. Using this simple and crude approximation gives the exponential fit to the $I_o$ data as shown in Fig. 7b. We find that, under the conditions of this time course experiment, $I_o$ at $t = 0$ is underestimated by at least 21%. Taking this into account, the correction in the decrease in $I_o$ would then be ~37–42% due to nuclease activity, more consistent with total dissociation of the protein from its DNA substrate. It is worth pointing out that, regardless of error estimates, we can conclude with certainty that the protein is not bound solely to the remaining nicked double-stranded DNA component since the measurement at $t = 0$ can be only underestimated, not overestimated. Similar measurements were made after Mg$^{2+}$ was added to D181A-34-mer DNA flap complex samples. In this case, no change in the scattering intensity with respect to time was observed.

FEN-1 nuclease is critical in the maintenance of genome stability and mutational avoidance. Yeast null mutants displayed a unique mutational spectrum derived from the failure of RNA primer removal during the lagging strand DNA synthesis. The SAXS study reported here provides a global structure of this unique DNA replication and repair enzyme and confirms the interaction of enzyme/flap DNA substrate/Mg$^{2+}$...
cofactor. It also suggests the critical role of Asp-181 in the enzyme/ flap DNA substrate interaction to ensure a proper conformation and a cleavage-ready status of the enzyme. A time course change in the scattering profiles arising from magnesium activation of the FEN-1 DNA flap substrate complex is consistent with the enzyme being freed from both the single- and double-stranded DNA product portions after cleavage. Given the critical role of FEN-1 in DNA replication and repair, this study illustrates initial understanding of molecular dynamics common to the structure-specific nuclease family that are central to processes influencing genome stability and early events that modulate cancer susceptibility and tumorigenesis.

Acknowledgments—We are indebted to Dr. Jill Trehwella for providing beam time on the small-angle x-ray scattering station at Los Alamos National Laboratory (Los Alamos, NM). We give special thanks to Dr. S. V. Santhana Mariappan (Los Alamos National Laboratory) for advice on DNA substrate design, NMR analysis for the synthesized flap DNA substrate, and proofreading. We thank Dr. John Nolan for providing us with flap DNA-beads needed for the flow cytometry assay used to test FEN-1 activity and David Eckhart for assistance in FEN-1 protein expression.

Note Added in Proof—During review of the manuscript, two crystallographic structures of archaeobacterial FEN-1s have been published (Horfield, D., Mol, C. D., Shen, B., and Tainer, J. A. (1998) Cell 95, 135–146; Hwang, K. Y., Baek, K., Kim, H.-Y., and Cho, Y. (1998) Nat. Struct. Biol. 5, 707–713).

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