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DNA containing a chemically reduced apurinic site is a high affinity ligand for the *E.coli* formamidopyrimidine-DNA glycosylase

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ABSTRACT

The *E. coli* Formamidopyrimidine-DNA Glycosylase (FPG protein), a monomeric DNA repair enzyme of 30.2 kDa, was purified to homogeneity in large quantities. The FPG protein excises imidazole ring-opened purines and 8-hydroxyguanine residues from DNA. Besides DNA glycosylase activity, the FPG protein is endowed with an EDTA-resistant activity which nicks DNA at apurinic/apyrimidic sites (AP sites). In contrast, DNAs containing chemically reduced AP sites are not incised by the FPG protein. However, the DNA glycosylase activity of the FPG protein is strongly inhibited in the presence of a purified synthetic 24 base-pair double-stranded oligonucleotide which contains a single apurinic site transformed chemically through borohydride reduction into a ring-opened deoxyribose derivative. The ability of the FPG protein to form a complex with this synthetically modified DNA was studied by electrophoresis in non-denaturing polyacrylamide gels. The FPG protein specifically binds the double-stranded oligonucleotide containing an apurinic site previously reduced in the presence of sodium borohydride. The complex was identified as a single retardation band on non-denaturing polyacrylamide gel electrophoresis. Complex formation is reversible and an apparent dissociation constant, $K_D$app, of 2.6×10^{-10} M was determined. In contrast, no such retardation band was obtained between the FPG protein and double-stranded DNA containing an intact apurinic site or single-stranded DNA containing either an intact or a reduced apurinic site.

INTRODUCTION

The Formamidopyrimidine-DNA glycosylase (FPG protein) of *Escherichia coli* was initially identified as a DNA glycosylase which excises the imidazole ring-opened form of N7-methylguanine (2, 6-diamino-4 hydroxy-5-N-methylformamidopyrimidine or FAPY residues) from DNA (1, 2). This enzyme also liberates the imidazole ring-opened forms of adenine and excises 8-hydroxyguanine residues from DNA treated with ionizing radiation or photosensitizers plus light (3, 4, 5, 6). Besides DNA glycosylase activity, the FPG protein is endowed with an EDTA-resistant activity which nicks DNA at apurinic/apyrimidic sites (7). The FPG protein catalyses the nicking of both the 3′ and 5′ phosphodiester bonds of abasic sites in DNA so that the base-free deoxyribose is replaced by a gap limited by 3′-phosphate and 5′-phosphate ends (7, 8). The FPG protein most likely catalyses a β-elimination followed by a δ-elimination (8). The biological role of the FPG protein, which is present in bacteria as well as in mammalian cells, is to repair DNA damage induced by alkylating agents (7, 8, 9), free radicals and oxygen-derived species such as singlet oxygen (4, 5).

The *E. coli fpg*$^+$ gene coding for the FPG protein has been cloned and the nucleotide sequence determined (10). The FPG protein is composed of 269 amino-acids and has a molecular weight of 30.2 kDa (10). In its active form, the FPG protein is a metallo-protein containing one zinc atom per monomer (11). The multifunctionality (DNA glycosylase and AP-lyase) and small size of this metallo-enzyme make it an interesting model for studying the interactions between DNA repair enzymes and damaged DNA.

Electrophoresis in non-denaturing polyacrylamide gels has been used extensively to study protein-nucleic acid complexes composed of repressors or transcription factors and DNA fragments containing target sequences (12, 13, 14). Other than this, little data has been reported until now about physico-chemical properties of DNA repair enzyme-modified DNA complexes (15, 16). In this paper, we show that the FPG protein forms a complex with an oligonucleotide which contains a single apurinic site which has been chemically reduced by sodium borohydride. The approach described in this paper might also be used to investigate the biochemical and structural properties

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of other protein-nucleic acid complexes involving DNA repair enzymes and modified DNAs which are not consumed by the protein.

MATERIALS AND METHODS

Gel retardation electrophoresis

In these experiments the mixture (20 μl) containing radiolabeled *ds*-d[redAP] (2000 cpm) and the freshly thawed and diluted enzyme, was equilibrated for 15 min at 25°C in 25 mM HEPES/KOH pH 7.6, 100 mM KCl, 5 mM β-mercaptoethanol, 1 mM EDTA, 6% glycerol and then immediately loaded on a non-denaturing gel for electrophoresis.

For the DNA binding assays, the polyacrylamide gels (10%) contained acrylamide/N, N'-methylenebisacrylamide at a [19.76:0.24] ratio in 0.09 M/0.08 M tris/borate (pH 8.4), 0.6% ammonium persulfate, and 0.05% N, N, N', N'-tetramethyl-enediamine. Gels were prepared the day before use and pre-electrophoresed for 1 hour at 300 V (4°C) before loading the samples. For each equilibrium experiment, the gel was run for 3 hours at 200 V (4°C), fixed in a 10% acetic acid/10% methanol solution (v/v), dried on Whatman paper and then exposed to pre-flashed Kodak X-AR film at −80°C.

Estimation of the $K_D$app of the complex between FPG protein and double-stranded d[redAP] was made by counting the radioactivity of the bands from the dried gel directly with a scintillation optical fiber imager using a SOFI detector (17).

Enzyme

The *E. coli* FPG protein was purified as previously described (11). The enzyme was stored at −20°C in 70 mM Hepes/KOH buffer, pH 7.6, containing 300 mM KCl, 5 mM dithiotreitol and 50% glycerol. For the gel retardation assay, the stock protein was dialyzed against a 25 mM Hepes/KOH pH 7.6 buffer containing 200 mM KCl, 5 mM dithiotreitol, 10% glycerol and aliquots were stored at −20°C.

Oligonucleotide preparation and purification

The preparation of the modified nucleic acid required the five steps summarized in figure 1a.

(i) Synthesis of d[G] and d[C]. Synthesis of single-stranded complementary oligonucleotides of 24 bases d[G] and d[C] (Fig. 1b) was performed with a Milli Gene DNA synthetizer (Millipore) on a solid-phase (1 μmole column) using the Phosphoramidite Method. They were purified by high performance liquid chromatography in two steps: first on an anion-exchange column (Nucleogen DEAE 60-7 from Machery-Nagel) using a potassium chloride gradient in 20 mM sodium acetate (pH 6.5) and 20% acetonitrile and secondly on a reverse phase column (ODS-Ultrasphere from Beckman) using an acetonitrile gradient in 0.1 M triethylammonium acetate pH 7.0. This volatile buffer was used to allow evaporation of the solution to dryness without a desalting step. Single-stranded oligodeoxyribonucleotides were dissolved in sterile water and stored at −20°C.

(ii) Depurination of oligonucleotide d[G]. The unlabeled or [5'-^{32}P]-labeled d[G] was depurinated in 10 mM HCl at 65°C for 30 min. The incubation mixture was neutralized with 10 mM NaOH and then adjusted either to 25 mM Hepes/KOH, pH 7.6, 0.1 M KCl, 1 mM EDTA for the AP-nicking activity assay or to 400 mM potassium phosphate buffer, pH 6.5, for the sodium borohydride reduction at the AP site. To estimate the yield of the modifications, we incubated the [5'-^{32}P]-labeled depurinated molecules with an excess of the FPG protein. After phenol deproteination, analysis of the reaction mixtures were analyzed by denaturing 20% polyacrylamide gel electrophoresis followed by autoradiography showed that 50 to 70% of the d[G] molecules were depurinated.

(iii) Reduction of the oligonucleotide d[AP] at the AP site. The depurinated oligodeoxyribonucleotide d[AP] was treated with 1 M sodium borohydride in 400 mM potassium phosphate, pH 6.5, at room temperature for 1 hour and the resulting reaction mixture was desalted using a Sephadex G25 column. To estimate the yield of d[redAP] after reduction, we incubated the DNAs with FPG protein under the same conditions as previously described for d[AP]. The efficiency of the reduction step was better than 90%.

(iv) Purification of the d[redAP] DNA. The d[redAP] oligonucleotide was incubated in the presence of saturating

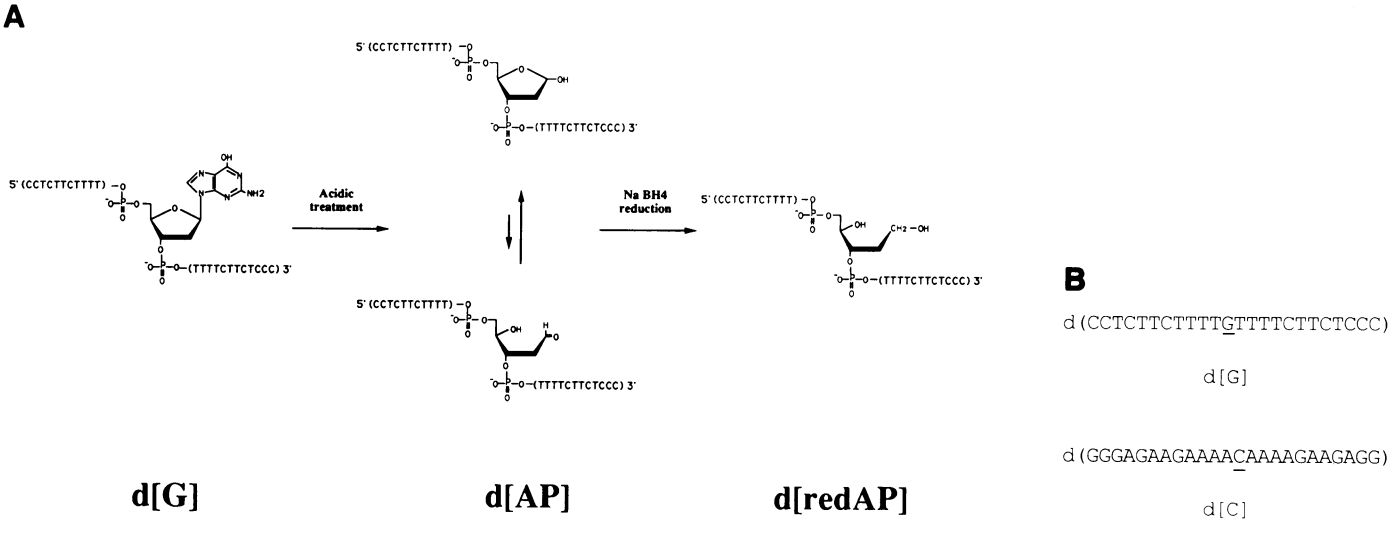

Fig. 1. (A) Chemical steps of the d[redAP] preparation and (B) sequences of the complementary parent oligonucleotides d[G] and d[C].

amounts of FPG protein to cleave the remaining d[AP] molecules and the remaining parent d[G] was separated by electrophoresis on a 20% non-denaturing polyacrylamide gel (19:1 acrylamide:bisacrylamide ratio). The band corresponding to the d[redAP] was cut out and eluted overnight in TE buffer at room temperature. The separation of the d[redAP] DNA from the parent d[G] is based upon a difference of mobility in non-denaturing gels. Since, these two single-stranded DNAs cannot be separated in 20% denaturing polyacrylamide gels. A possible explanation is that a stable conformational difference exists between the two molecules which is revealed by native gels. The d[G] and d[redAP] oligonucleotides may also be separated on a reverse phase column (ODS Ultrasphere) with an acetonitrile gradient.

(v) Hybrid formation. The single stranded complementary oligonucleotides d[G], d[AP], d[redAP] (5 ìM each) were mixed with the complementary d[C] in 20 μl TE buffer, heated at 80°C for 5 minutes and slowly cooled for 3 hours to 4°C. The duplex concentration was measured either by direct Cerenkov counting of the [5′-^{32}P]-labeled oligonucleotide or by measurement of the absorbance at 260 nm.

[5′-^{32}P]-labelling of oligonucleotide d[G]

The d[G] oligonucleotide (14 pmoles), [γ^{32}P]-ATP (43 pmoles; 200 μCI) and 30 units of T_4 polynucleotide kinase, were incubated for 1 hour at 37°C in 30 μl of a solution containing 50 mM Tris/HCl, pH 7.6, 10 mM $MgCl_2$, 0.1 mM Na_2 EDTA, 10 mM β-mercaptoethanol and 100 mM NaCl. The reaction was stopped by the addition of EDTA to 20 mM followed by heating at 80°C for 5 min. The free [γ^{32}P]-ATP was removed by using the Mer Maid kit (Bio 101). Under these incubation conditions, 100% of the oligonucleotide was labeled. Quantitation was performed by direct Cerenkov counting.

Assay of [FAPY]-DNA glycosylase activity

The assay for [FAPY]-DNA glycosylase activity was performed as previously described (10). The incubation mixture (100 μl) contained 70 mM HEPES/KOH, pH 7.6, 100 mM KCl, 5 mM Na_2EDTA, 5 mM dithiothreitol (DTT), 10% glycerol, 2000 c.p.m. of [^{3}H]-[FAPY]-poly(dG-dC) (2) with a limiting amount of purified FPG protein. The mixture was incubated for 10 min at 37°C with increasing concentrations of several oligonucleotides which are potential competitors. The reaction mixtures were then ethanol precipitated and the soluble fraction quantitated by scintillation counting (2).

Apurinic-DNA nicking activity assay

The radiolabeled [5′-^{32}P]-d[AP] or -d[redAP] single- or double-stranded oligonucleotides and the FPG protein were incubated for 30 min at 37°C in 25 mM Hepes/KOH, pH 7.6, 100 mM KCl, 1 mM Na_2EDTA, 5 mM DTT, 5% glycerol (10 μl total volume). The reaction was stopped by the addition of an equal volume of denaturing buffer containing 30% ficoll (400,000 Mw), 0.1% SDS, 0.05% bromophenol blue and 0.05% xylene cyanol. Then, the DNA fragments from the enzyme digestion were separated on 20% polyacrylamide/ 8 M urea gels and detected by autoradiography.

RESULTS

Inhibition of the [FAPY]-DNA glycosylase activity of FPG protein by modified oligonucleotides

The FPG protein from *E. coli* is a bifunctional enzyme classified (7, 8) as a DNA glycosylase/AP-lyase (7, 8, 18). It has been established that DNA molecules containing reduced AP sites are not substrates for the FPG protein (7). This is not surprising since the reaction mechanism of AP nicking implies a β-elimination which is not possible when the aldehyde of the deoxyribose is reduced to an alcohol (7). The absence of DNA nicking at redAP sites suggests either that they are not recognized by the FPG protein or that the protein still binds the modified DNA but is no longer able to catalyze the DNA nicking reaction.

The results presented in figure 2 show the competitor effects of the various modified oligonucleotides upon the [FAPY]-DNA glycosylase activity of FPG protein. Parent oligonucleotides d[G] (single- and double-stranded) do not significantly affect the excision of FAPY residues (Fig. 2). In contrast, oligonucleotides containing an AP site and especially those containing a redAP site drastically inhibit the [FAPY]-DNA glycosylase activity, probably by competition with the FAPY substrate (Fig. 2). These results suggest that double- and single-stranded DNAs which contain reduced AP sites are specifically recognized by the FPG protein, although they are not substrates of the FPG protein.

Formation of a stable *ds*-d[redAP]/FPG protein complex

The results described in the previous section suggest the formation of a complex between the FPG protein and oligonucleotides containing a reduced AP site. To substantiate this hypothesis,

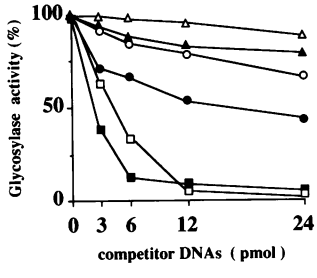

Fig. 2. Inhibition of the FPG protein [FAPY]-DNA glycosylase activity by various competitor oligonucleotides. The FPG protein (4 pmol) was assayed under standard conditions (as described in Materials and Methods) in the presence of increasing amounts of various unlabeled competitor DNA's. The competitors tested are: (△) and (▲) d[G] and *ds*-d[G]; (○) and (●) d[AP] and *ds*-d[AP]; (□) and (■) d[redAP] and *ds*-d[redAP], respectively. The 100% relative activity corresponds to the activity determined after an incubation at 37°C for 10 min.

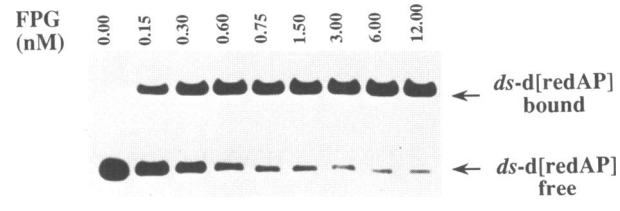

Fig. 3. Formation of the *ds*-d[redAP]/FPG protein complex as a function of FPG protein concentation. The *ds*-d[redAP] oligonucleotide (2000 cpm, [5′-^{32}P]-radiolabeled DNA) was incubated with the indicated concentrations of FPG protein at 25°C for 15 min in 20 μl reaction mixtures containing 25 mM Hepes/KOH pH 7.6, 100 mM KCl, 5 mM β-mercaptoethanol, 1 mM EDTA, 6% (v/v) glycerol. After incubation, the samples were applied to a 10% non-denaturing polyacrylamide gel which was prerun at 300 V for 1hour. After electrophoresis (200 V for 3 hours), the FPG-bound and free DNA bands were detected by autoradiography of the gel.

the affinity of the FPG protein for the redAP site was studied by electrophoresis in a non-denaturing polyacrylamide gels (12, 19). Figure 3 shows a gel retardation experiment which demonstrates the formation of a *ds*-d[redAP]/FPG protein complex. A single retardation band is obtained with the *ds*-d[redAP] which indicates that a stable protein-nucleic acid complex is formed. With an excess of FPG protein the free DNA band disappears completely and all the DNA is bound to the enzyme (Fig. 3).

Native FPG protein is required to obtain the retardation band. If the protein is treated with heat in the presence of Na_2 EDTA (20 mM) with *ds*-d[redAP] or by proteinase K before or after incubation, the complex is not observed. In contrast, the addition of a non-specific protein (bovin serum albumin) in the binding assay does not affect the formation of the complex (data not shown). We conclude that *ds*-d[redAP] forms a stable molecular complex with the native FPG protein.

At the incubation step, the complex formation seems rather insensitive to pH within the range 6.5 to 9.0. Below pH 6.5, the protein precipitates with the radiolabeled-DNA and does not migrate into the gel. Figure 4 shows the effects of ionic strength. A minimum of 20 mM KCl is required in the assay mixture with an optimum at 100 mM. At ionic strengths above this value the fraction of the complexed DNA decreases quite rapidly although a residual complex is still detectable at 600 mM KCl.

The pH of the gel, however, is a critical parameter for resolving the *ds*-d[redAP]/FPG protein complex. The electrophoresis of the complex was performed at pH 8.4 which corresponds to the isoelectric point of the free protein as shown by isoelectric focusing gels (11). Thus, under these conditions, the migration of the complex depends only on the charge of the DNA. Further at the isoelectric point the dissociation by the electric field is minimized whereas at a lower pH, the protein and nucleic-acid would migrate in opposite directions.

Specificity and reversibility of the *ds*-d[redAP]/FPG protein complex

Competition among various DNAs and *ds*-d[redAP] for complex formation with the FPG protein was also investigated (Fig. 5). Two types of competition experiments were done: (i) simultaneous incubation of the FPG protein with [5′-^{32}P]-labeled *ds*-d[redAP] and with the cold competitor followed by gel retardation electrophoresis; (ii) preformation of the complex with the radiolabeled *ds*-d[redAP] followed by incubation with the cold competitor. Under conditions which lead to 100% complex formation, d[G] and *ds*-d[G] have no effect (Fig. 5: lanes 7 and 8). In contrast, d[AP] and *ds*-d[AP] result in a shift of the radioactivity from the retardation band towards the free DNA band (Fig. 5: lanes 9 and 10). d[redAP] is also able to compete with the *ds*-d[redAP] DNA for complex formation (Fig. 5: lanes 3 and 4). Cold *ds*-d[redAP] is able to displace the labeled DNA in the chase assay and this is an additional proof of the reversibility of formation of the complex (Fig. 5: lane 6).

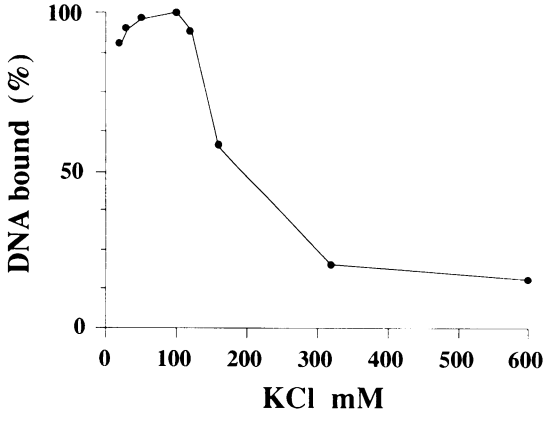

Fig. 4. Effect of the ionic strength on the *ds*-d[redAP] / FPG protein complex formation. The assays were carried out under the same conditions as described in the legend of figure 3 with 12 nM of FPG protein and 20, 40, 60, 120, 160, 320 and 600 mM KCl in 20 µl of reaction mixture. Quantitation of the gel autoradiogram was done with a Joyce densitometer (Model MK III C.).

Determination of the apparent dissociation constant

There is no direct evidence for a [1:1] stoichiometry for the complex. However, since there is only one DNA site which is specifically recognized by the FPG protein, we may assume that the complex involves one FPG protein molecule for one *ds*-d[redAP] oligonucleotide molecule.

Using this assumption, the apparent dissociation constant, $K_D$app, for FPG protein binding to *ds*-d[redAP] was estimated by defining the experimental conditions (see Fig. 6 caption) and employing the following approximations (20): f is the fraction of free DNA ($[DNA]_{free}$ / $[DNA]_{total}$), and [FPG] is the concentration of free protein at equilibrium. The dissociation constant is then given by:

$$K_D = ([DNA]free \times [FPG]) / [DNA\text{-}FPG\ complex] = (f \times [FPG]) / (1-f)$$

Since the concentration of DNA is less than 20 pM in each reaction mixture, we assume that the total concentration of protein $[FPG]_0$ (at least 100 pM) is close to that of the free protein concentration [FPG]. Under these conditions, the expression of the apparent dissociation constant may be simplified to:

$$K_D app = (f \times [FPG]_0) / (1-f)$$

The $K_D$app for the ds-d[redAP]/FPG protein complex is determined by incubating increasing amounts of the enzyme with a constant amount of *ds*-d[redAP]. The results of the experiment

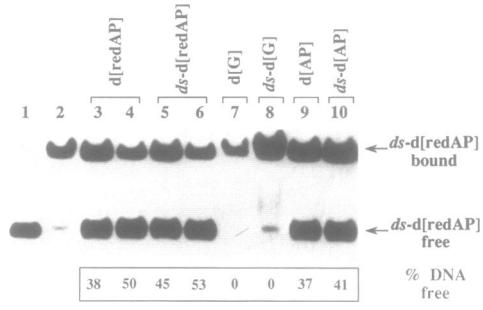

Fig. 5. Specificity and reversibility of the *ds*-d[redAP] / FPG complex formation. Native retardation gel electrophoresis was performed using the same conditions as described in the legend of figure 3 with [FPG] = 12 nM and the indicated cold DNA competitor (6 nM). (1) no FPG and no competitor; (2) FPG protein and no competitor; (3) and (5) co-incubation of FPG protein and the cold competitors d[redAP] and *ds*-d[redAP] (competition); (4) and (6) preincubation of the radiolabeled *ds*-d[redAP] followed by incubation with the cold competitors d[redAP] and *ds*-d[redAP] (chase); (7), (8), (9) and (10) co-incubation of FPG protein and the cold competitors d[G], *ds*-d[G], d[AP] and *ds*-d[AP] (competition). The ratios ([DNA]free / [DNA]total)×100 were measured after quantitation of the gel as already described in the fig. 4 legend.

are shown in figure 6. In practice, $[FPG]_0$ is known and f is derived directly from the electrophoresis data (Fig. 6A). Given the conditions described above, the $[FPG]_0$ concentration needed for half-maximal binding is very close to the $K_D$app. The experimental curve (Fig. 6B) yields an apparent dissociation constant, $K_D$app, of 2.6×10^{-10} M.

DISCUSSION

Few reports have been published about the physico-chemical properties of the recognition between the DNA glycosylase enzyme and modified DNA (15, 16). The lack of chemically well-defined substrate analogs that are not consumed by the FPG protein and which are capable of forming stable protein-nucleic acid complexes has been, until now, an obstacle to further mechanistic investigations. In this report, we describe the preparation of a stable, specific and chemically homogeneous complex between a DNA containing a unique reduced AP site and the FPG protein of *E. coli*..

Inhibition studies of the [FAPY]-DNA glycosylase activity show that single- and double-stranded oligonucleotides, which contain unique reduced abasic sites are strong inhibitors of the [FAPY]-DNA glycosylase activity of the FPG protein. A protein/DNA ratio of approximately [1:1] at 100% inhibition strongly suggests that complex formation is achieved by the binding of one molecule of FPG protein to one molecule of DNA ligand. Single- and double-stranded oligonucleotides which contain unique abasic sites, that are themselves substrates, also inhibit the enzyme activity, probably by competition with the FAPY substrate. The FPG protein is able to nick single- and double-stranded DNA substrates at AP sites with a greater specificity towards double-stranded DNA. These synthetic DNAs will be suitable tools for the investigation of the kinetic properties and for the study of the mechanism of the enzyme. They will allow us to define appropriate conditions for measuring the K_M and for determining the K_Is of inhibitor DNAs with respect to the two activities of the FPG protein.

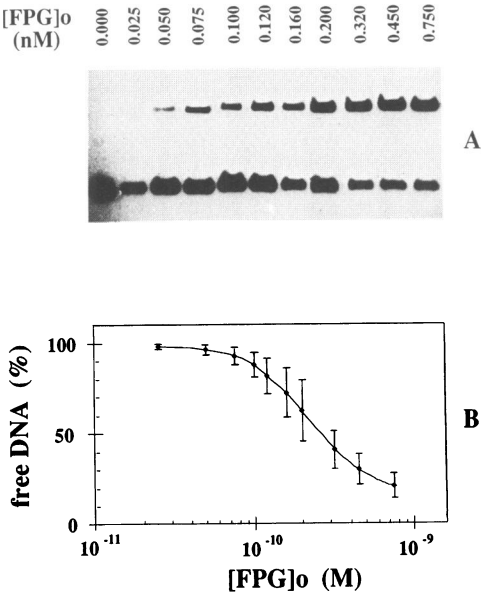

Fig. 6. Determination of the apparent dissociation constant of the *ds*-d[redAP]/FPG complex. The stock FPG protein solution was thawed and freshly diluted on ice into 20 µl of 25 mM HEPES/KOH pH 7.6, 100 mM KCl, 5 mM β-mercaptoethanol, 6% (v/v) glycerol and ~2000 cpm (<20 pM in the assays) of purified [5'^{32}P]-*ds*-d[redAP]. Reaction mixtures were equilibrated at 25°C for 15 min and then loaded onto the gel with a siliconized capillary. The electrophoresis conditions are those described in Materials and Methods. The standard binding conditions gave reproducible $K_D$app values. (A) Autoradiogram of gel assay. (B) Average and standard deviations of three independent experiments involving the titration of the *ds*-d[redAP] by FPG protein. A $K_D$app of 2.6×10^{-10} M is obtained from this average curve.

The gel retardation experiments with the *ds*-d[redAP] presented here show that this DNA most probably works like an analog of the *ds*-d[AP] and traps the FPG protein in an intermediate state of the reaction. However, no such complex can be obtained with the single-stranded d[redAP] under the conditions defined for the *ds*-d[redAP]. This result is not in agreement with the inhibition of [FAPY]-DNA glycosylase activity. The fact that single-stranded d[redAP] still inhibits the [FAPY]-DNA glycosylase activity indicates that the protein interacts with this ligand. The complex, therefore, is very probably unstable under the conditions of the gel electrophoresis experiments. This interpretation is also supported by the observation of a partial inhibition of complex formation (*ds*-d[redAP]/FPG protein complex) in the presence of the single-stranded d[redAP] as competitor. As single- and double-stranded d[AP] are recognized and cleaved by the enzyme, no complex can be observed with these substrates by electrophoresis.

Assessment of the stability of the complex provided novel structural and biological information. The amount of complex formed is optimal at 100 mM KCl and decreases at greater concentrations although it still can be detected at 600 mM KCl. This indicates relatively strong non-ionic interactions between the two molecules. Besides, divalent cations are not needed for the two enzyme activities (glycosylase and AP-lyase activities) (21). Furthermore, this metallo-protein containing one zinc atom per molecule has an EDTA resistant AP-nicking activity (7) and the complex formation with *ds*-d[redAP] is insensitive to the presence of EDTA. Conversely addition of EGTA, which is a stronger chelating agent than EDTA, partially inhibits the complex formation (data not shown). This suggests that the bound zinc atom of the protein is essential for maintaining the native conformation of the enzyme.

The $K_D$app value of the *ds*-d[redAP]/FPG protein complex is 2.6×10^{-10} M. This value is close to the dissociation constant measured for another DNA glycosylase-AP endonuclease, the T_4 endonuclease V, which binds to 14- or 18-mer duplexes containing unique pyrimidine photodimers (1.1 and 1.2×10^{-9} M respectively) (22). In our case the $K_D$app value compares well with those of other types of DNA/protein complexes for which the specificity of the recognition is contained in a DNA target sequence (*E.coli trp* repressor/operator DNA complex: $K_D$app = 5×10^{-10} M) (23). This shows that the FPG protein recognizes the *ds*-d[redAP] with very high affinity. This affinity is probably directed mostly towards the redAP site since no such complex is observed between the parent oligonucleotide d[G] and the FPG protein.

In light of these results, we now have at our disposal an *in vitro* model to study the interaction between a DNA-repair enzyme and a non-metabolized modified DNA ligand. Several approaches have been proposed for the incorporation of abasic sites into DNA at preselected positions (24, 25, 26). Although requiring several steps, the method presented here allows the preparation of large quantities of DNA containing reduced AP sites. We will now be able to investigate the conditions for crystallizing such nucleic acid/protein complexes.

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