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Damage Repertoire of the *Escherichia coli* UvrABC Nuclease Complex Includes Abasic Sites, Base-Damage Analogues, and Lesions Containing Adjacent 5′ or 3′ Nicks†

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ABSTRACT: Using oligonucleotide synthesis, we demonstrate a rapid and efficient method for the construction of DNA duplexes containing defined DNA lesions at specific positions. These DNA lesions include apyrimidinic sites, reduced apyrimidinic sites, and base-damage analogues consisting of *O*-methyl- or *O*-benzylhydroxylamine-modified apyrimidinic sites. A 49 base pair DNA duplex containing these lesions was specifically incised by the UvrABC nuclease complex. The incision sites occurred predominantly at the eighth phosphodiester bond 5′ and the fifth phosphodiester bond 3′ to the lesion. Multiple incisions were observed 3′ to the lesion. The extent of DNA incisions was base-damage analogues > reduced apyrimidinic sites > apyrimidinic sites. Introduction of 3′ or 5′ nicks at the site of a base-damage analogue by treatment of these substrates with either endonuclease III or endonuclease IV reduced, but did not abolish, subsequent incision by the UvrABC complex, whereas introduction of a 3′ nick at an abasic site increased the incision efficiency of the UvrABC complex. These data demonstrate a convergence of base and nucleotide excision repair pathways in the removal of specific base damages.

Nucleotide excision repair represents a generalized enzymatic pathway for the removal of many types of DNA lesions, including UV-induced pyrimidine dimers and "bulky" chemical adducts such as psoralen-thymine monoadducts and cisplatin G-G intrastrand cross-links. *Escherichia coli* nucleotide excision repair is initiated by the UvrABC nuclease complex in a cascade of ordered reactions (Grossman et al., 1988; Sancar & Sancar, 1989; Van Houten, 1990). Prior to their interaction with DNA, the UvrA and UvrB subunits associate in solution in an ATP-dependent manner to form an $UvrA_2B$ complex (Orren & Sancar, 1989; Oh et al., 1989). During the damage recognition step, the $UvrA_2B$ complex probes the DNA helix for damage-induced distortions by transiently melting into the two strands during a limited helicase activity, in which ATP is absolutely required (Oh & Grossman, 1989a). This helicase activity is inhibited by the presence of a DNA lesion resulting in a stable protein-DNA complex (Husain et al., 1986; Seeberg & Steinum, 1982; Van Houten et al., 1987; Yeung et al.,

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1983, 1986). Several alterations in both the structure of the DNA and the composition of the enzyme complex accompany the formation of this preincision complex (Oh et al., 1987, 1988; Van Houten et al., 1987, 1988a,b). It has been suggested that the UvrA dimer is displaced during the formation of a long-lived UvrB–DNA complex (Orren & Sancar, 1989). This preincision protein–DNA complex facilitates the binding of the UvrC subunit (Backendorf et al., 1986; Grossman et al., 1988), which results in two incisions flanking the damaged nucleotide. These usually occur at the eighth phosphodiester bond 5′ and the fifth phosphodiester bond 3′ to the lesion (Van Houten, 1990). The combined action of helicase II (UvrD protein) and pol I is required for the release of the oligonucleotide containing the damage and the postincision Uvr subunits (Husain et al., 1986; Caron et al., 1986; Van Houten, 1988).

One of the intriguing aspects of the UvrABC nuclease complex is its broad substrate specificity. A recent survey listed over 20 different types of DNA damage which are acted on by this repair enzyme (Van Houten, 1990). Certain DNA lesions which are substrates for the UvrABC complex, such as pyrimidine dimers and cisplatin G–G intrastrand diadducts, induce significant distortion into the DNA helix, including strand unwinding, helix destabilization, and a site-specific bend (Husain et al., 1988; Pearlman et al., 1985; Rice et al., 1988; Sherman & Lippard, 1987). However, several recent reports suggest that these alterations may not be necessary for damage recognition, as other types of DNA damage which do not appear to introduce appreciable distortions into the DNA helix are also substrates for the UvrABC nuclease complex. Two such lesions are O^6-methylguanine (O^6-MeGua)[1] (Voigt et al., 1989) and thymine glycols (TG) (Lin & Sancar, 1989; Kow et al., 1990).

Genetic studies of *E. coli* were first to suggest that the UvrABC complex may bind to O^6-MeGua in vivo and interfere with the action of O^6-methyltransferase in the repair of this lesion (Chambers et al., 1985). Samson and co-workers (Samson et al., 1988) monitored the loss of O^6-MeGua using antibodies and reported that these lesions are lost at a slower rate in UvrB$^-$ cells as compared to UvrB$^+$ cells. Van Houten and Sancar (1987) demonstrated that DNA treated with *N*-methyl-*N*-nitronitrosoguanidine (MNNG) was a substrate for the UvrABC complex. In the same report, it was also shown that *E. coli* UvrA-deficient strains lacking one or more of the normal alkylation repair pathways were more sensitive to the killing effects of MNNG than their UvrA$^+$ counterpart. More recently, Voigt et al. (1989), using a defined DNA substrate containing an O^6-MeGua adduct at a specific site, have shown that this relatively nondistorting DNA lesion is recognized by the UvrABC complex in vitro, confirming the previous in vivo data (Chambers et al., 1985; Samson et al., 1988). Since simple alkylation damage is recognized by the UvrABC complex, it was of interest to determine whether base lesions, resulting from oxidative damage, are also substrates for this enzyme. Two common forms of oxidative damage are abasic sites and thymine glycols.

We have recently shown that PM2 DNA containing on the average one abasic site (AP) per molecule was not incised above background by the UvrABC complex, while DNA containing ring-opened reduced AP sites or alkoxyamine-modified AP sites was incised efficiently by the enzyme (Kow et al., 1990). We demonstrated that TG were recognized efficiently by the UvrABC complex, whereas the structurally similar lesion dihydrothymine was not a substrate for the enzyme. This report also presented genetic data which demonstrate a functional role of the UvrABC complex in the repair of TG in vivo. Efficient recognition of TG residues has been reported independently by Lin and Sancar (1989). They also reported that, in contrast to the above observations, covalently closed pBR322 or DNA fragments containing abasic sites appear to be relatively good substrates for the UvrABC nuclease complex.

These observations raise two important questions regarding the action mechanism of the UvrABC complex: (i) What is its mode of DNA damage recognition and (ii) to what degree does nucleotide excision repair participate in the repair of relatively nondistorting base damage in vivo? To help address these questions, we have undertaken the present study. Specifically, we wanted to investigate the interaction of the UvrABC nuclease complex with base-damage analogues such as alkoxy-modified AP sites and to demonstrate directly whether AP sites are indeed substrate for the UvrABC complex. We have developed a rapid and relatively easy method for constructing DNA duplexes with defined DNA lesions at precise locations. These substrates include an AP site (ring-closed), a reduced AP site (ring-opened), and the base-damage analogues *O*-methylhydroxylamine- (MA) and *O*-benzylhydroxylamine-modified AP sites (BA). We report here that the UvrABC complex recognizes the base-damage analogues, MA residues and BA residues, more efficiently than the reduced AP site which in turn is recognized more efficiently than an AP site. Each of these lesions is incised by the UvrABC complex in the characteristic manner. We also provide evidence that these lesions are substrates for UvrABC nuclease even after being nicked either 5′ or 3′ by endonuclease IV or endonuclease III, respectively. In addition, it appears that AP sites once nicked by endonuclease III are better substrates for the UvrABC complex as compared to the unnicked substrate. A model for the interaction of the nucleotide and base excision repair pathways in the repair of specific types of DNA lesions is presented.

Materials and Methods

Chemicals. Tris base, boric acid, and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma; acrylamide, ammonium persulfate, *N*,*N*′-methylenebis(acrylamide), and urea were obtained from BRL; sodium borohydrate ($NaBH_4$), *O*-methyl- and *O*-benzylhydroxylamine were purchased from Aldrich.

Enzymes. All restriction and DNA-modifying enzymes were purchased from BRL unless otherwise noted.

The UvrA, UvrB, and UvrC subunits were purified from *E. coli* overproducer strain CH296 containing plasmids pUNC45, pUNC211, and pDR3274, respectively (obtained from A. Sancar, University of North Carolina). The purification procedure was as described previously (Sancar, 1987).

Endonuclease III and endonuclease IV were prepared as described previously (Breimer & Lindahl, 1984; Katcher & Wallace, 1983; Ljungquist, 1977).

Uracil glycosylase was prepared by the method of Lindahl (1977) using *E. coli* strain M72 carrying plasmid pBD15, which overproduces uracil *N*-glycosylase 200-fold (Lindahl, 1977).

Oligonucleotides and Preparation of Substrates. An oligonucleotide (49-mer) containing uracil at position 26 and the complementary 50-mer were synthesized on a Du Pont automatic DNA synthesizer (GENERATOR DNA synthesizer)

[1] Abbreviations: AP, apyrimidinic site; BA, *O*-benzylhydroxylamine-modified apyrimidinic sites; MA, *O*-methylhydroxylamine-modified apyrimidinic sites; O^6-MeGua, O^6-methylguanine; rAP, reduced apyrimidinic site; TG, thymine glycol(s).

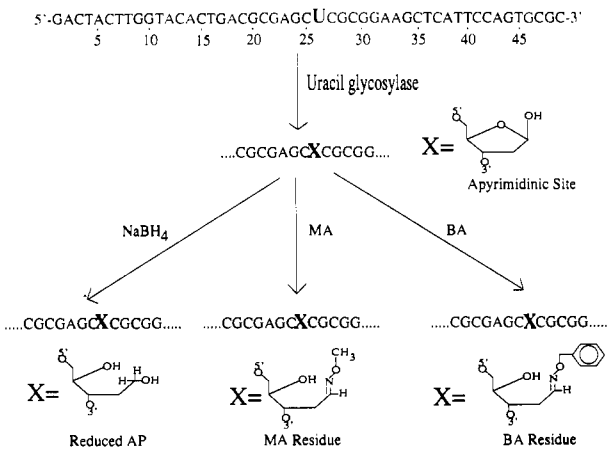

FIGURE 1: Flow diagram for the preparation of oligonucleotides containing defined lesions. A 49-base oligonucleotide containing uracil at position 26 was synthesized on a Du Pont oligonucleotide synthesizer and purified by gel electrophoresis. This 49-mer was annealed to a complementary 50-mer, and the uracil was removed by uracil glycosylase. The resulting apyrimidinic site was modified with either $NaBH_4$ or *O*-methyl- or *O*-benzylhydroxylamine. AP = apyrimidinic site, rAP = reduced apyrimidinic, MA = *O*-methylhydroxylamine, BA = *O*-benzylhydroxylamine.

and after detritylation were purified by electrophoresis on a 12% polyacrylamide sequencing gel under denaturing conditions. 3'-End-labeled oligonucleotide (49-mer) containing uracil was prepared by using a 3'-end-labeling kit ([α-^{32}P]-cordycepin) from Amersham and performed according to the suppliers instruction. 5'-End-labeled oligonucleotides were prepared by T4 polynucleotide kinase and [γ-^{32}P]ATP by using standard techniques. Duplex containing a defined uracil was prepared by adding 17 μg of either the 3'- or the 5'-end-labeled 49-mer (containing uracil) with 17 μg of the unlabeled 50-base complement in a buffer containing 10 mM Tris-HCl, pH 7.5, 1.0 mM EDTA, and 10 mM NaCl (TEN Buffer). The mixture was then heated to 90 °C for 5 min followed by slow cooling to room temperature to allow annealing of the two strands. The sequence of the duplex containing uracil is as follows:

5'-GACTACTTGGTACACTGACGCGAGCUCGCGGAAGCTCATTCCAGTGCGC-3'

3'-CTGATGAACCATGTGACTGCGCTCGAGCGCCTTCGAGTAAGGTCACGCGG-5'

DNA duplex containing a defined AP site (deoxyribose moiety) was prepared by incubating the 3'- or 5'-end-labeled duplex containing uracil at position 26 with 5 μL of the uracil glycosylase for 10 min at 37 °C, which resulted in quantitative conversion of the uracil to an AP site (Lindahl et al., 1977). Duplex containing a reduced AP site (deoxyribitol moiety) was prepared by $NaBH_4$ (0.6 M) reduction of the duplex containing the AP site (Kow, 1989). Duplex containing *O*-methyl- and *O*-benzylhydroxylamine residues was prepared by incubating the duplex containing the freshly prepared AP site with 10 mM *O*-methyl- or *O*-benzylhydroxylamine, respectively, at room temperature for 30 min (Kow, 1989). The duplexes containing the defined lesions including AP sites, reduced AP sites, or *O*-methyl- or *O*-benzylhydroxylamine residues were purified by electrophoresis on a native 12% acrylamide gel. The radioactive band containing the DNA was identified by autoradiography, excised from the gel, and electroeluted with an IBI electroeluter. The DNA was precipitated and resuspended in TEN buffer and stored at −20 °C. The procedures for the preparation of oligonucleotides containing defined lesions are summarized in Figure 1.

DNA Sequencing. Sequencing of the uniquely 5' or 3' terminally labeled 49 bp duplex was performed by using standard Maxam–Gilbert procedures (Maxam & Gilbert, 1980).

UvrABC Nuclease Incision Reaction. Unless otherwise noted, UvrABC digestions were performed by incubating 1.1 pmol of UvrA, 2.1 pmol of UvrB, 5.5 pmol of UvrC, and approximately 2 ng (0.06 pmol) of 49 bp duplex in 50 μL of reaction containing 10 mM $MgCl_2$, 50 mM Tris-HCl (pH 7.5), 50 mM KCl, 2 mM ATP, 5 mM DTT (ABC buffer), and 100 μg/mL bovine serum albumin for 30 min at 37 °C. The DNA in buffer was prewarmed for 5 min at 37 °C prior to the addition of the UvrA, UvrB, and UvrC subunits. The reactions were stopped by freezing in a dry ice–ethanol bath, lyophilized to dryness, and resuspended in formamide plus tracking dyes (40 μL). The samples were heated to 90 °C and quick-cooled on ice, and a sample was analyzed by electrophoresis through 10% polyacrylamide sequencing gels under denaturing conditions (8.0 M urea) in a Tris-borate (pH 8.0) buffer. The gel was dried and analyzed as described below.

UvrABC Digestion following Endonuclease III or IV Treatment. Endonuclease reactions were performed by incubating 1–3 ng of the 49 bp duplex DNA and either 19.2 pmol of Endo III or 7.3 pmol of Endo IV in 50 μL of buffer containing 100 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.2 mM EDTA, and 0.2 mM β-mercaptoethanol for 30 min at 37 °C. A portion of this reaction (25 μL) was stopped by rapid freezing in a dry ice–ethanol bath. The other portion (25 μL) was brought up to 50 μL in ABC buffer, to which was added 1.1 pmol of UvrA, 2.1 pmol of UvrB, and 5.5 pmol of UvrC, and the reaction was incubated for 30 min at 37 °C. The reactions were stopped on dry ice–ethanol and lyophilized until dry. The DNA was analyzed on a 10% polyacrylamide gel under denaturing conditions.

Analysis of the Incision Products. The amount of radioactivity in the bands corresponding to the full-length substrate and the digestion products were quantitated by using a Betascope 603 blot analyzer (Betagen, Waltham, MA). The percentage of DNA incised by UvrABC was calculated as the fraction of radioactivity which migrated in the bands corresponding to the incision products as compared to the total radioactivity in both the full-length and the digestion products. Autoradiography was conducted by placing the dried gel onto Kodak XAR5 X-ray film for 15–30 h in the presence of intensifying screens at −70 °C.

RESULTS

Incision of Defined DNA Substrates Containing Abasic Sites or Base-Damage Analogues. In order to demonstrate directly that the UvrABC complex can incise abasic sites, it was necessary to construct a DNA duplex containing an apyrimidinic site at a defined position. We have developed a method to synthesize DNA duplexes containing one of four structurally related DNA lesions at a defined site; these include an apyrimidinic site (AP), a reduced AP site (rAP), an *O*-methylhydroxylamine-modified AP site (MA), and an *O*-benzylhydroxylamine-modified AP site (BA) (see Materials and Methods). Briefly, a 49-base oligonucleotide containing uracil at position 26 was prepared; subsequent treatment with uracil glycosylase resulted in the quantitative conversion to an AP site. AP sites are relatively unstable, and treatment with $NaBH_4$ or alkoxyamines results in stable ring-opened reduced AP sites (rAP) or alkoxyamine-modified AP sites (base analogues), respectively. The sequence of the 49-mer and the structure of the four DNA lesions are given under Materials and Methods and in Figure 1. These modified oligonucleotides were labeled 5' or 3' by standard methods and annealed to a complementary 50-base oligonucleotide. Fully

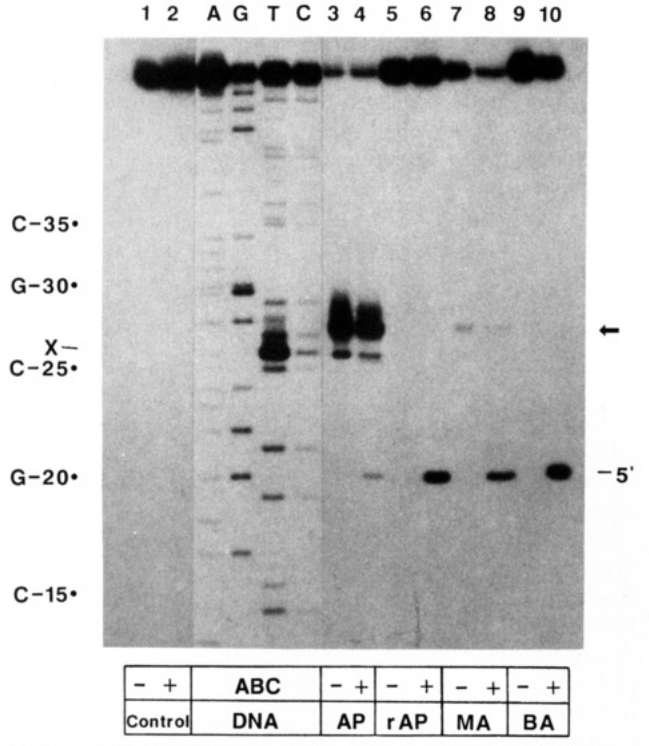

FIGURE 2: 5′ UvrABC incision sites at AP, rAP, MA, and BA lesions. The 49 bp duplex substrates containing a 5′-terminal label on the lesion-containing strand (see Materials and Methods) were incubated with 1.1 pmol of UvrA, 2.1 pmol of UvrB, and 5.5 pmol of UvrC for 30 min at 37 °C. Control DNA in lanes 1 and 2 contained uracil at position 26. The odd-numbered lanes were incubated in the absence of UvrABC and the even-numbered in the presence of UvrABC. All reactions were carried out in the presence of ATP. X denotes the band which results from Maxam–Gilbert cleavage of the uracil at position 26. The arrow indicates spontaneous strand breaks resulting from the abasic sites during the heating process prior to gel loading. The 5′ incision site is indicated and occurs at the eighth phosphodiester bond 5′ to the lesion as determined by the Maxam–Gilbert sequencing lanes. The modified strand is numbered in the 5′ to 3′ direction. Symbols are as those described in Figure 1.

duplexed substrates were purified by electrophoresis through native acrylamide gels.

These substrates were digested with the UvrABC complex and analyzed using conditions described under Materials and Methods. Results of typical 5′ and 3′ incision reactions are displayed in Figures 2 and 3, respectively. The position of the 5′ incision by the UvrABC nuclease complex was the eighth phosphodiester bond 5′ to the lesion. The position of the 5′ incision can be inferred by the appearance of a new band which migrates 6.5 bases faster than the band resulting from Maxam–Gilbert cleavage reaction of the uracil-substituted DNA (position 26). Under these electrophoresis conditions, the fragment produced by the UvrABC incision reaction contained a 3′-hydroxyl group and migrated 1.5 bases more slowly than the same size fragment which results from Maxam–Gilbert chemistry, because the latter contains a 3′-phosphate.

The 3′ UvrABC nuclease incision occurred predominantly at the fifth phosphodiester bond 3′ to the modified site. This incision can be observed by a band migrating at position 30. Minor incisions were also observed at the sixth, seventh, and eighth phosphodiester bonds 3′ to the lesions (Figures 2 and 3). It is not known whether this staggering is inherent to the sequence or the type of DNA damage used in these experiments. Alternative incision patterns have been observed previously. Incision adjacent to UV-induced photoproducts appeared to be sequence dependent (Myles et al., 1987), while alternative incision at the fifth and sixth phosphodiester bonds 3′ to O^6-MeGua may be due to the relatively small size of the damage (Voigt et al., 1989). No incision was observed with control DNA duplex containing uracil at position 26 (Figure 2, lanes 1 and 2). Incision of these four substrates was dependent upon the addition of ATP (Figure 3A, lane 9) and only occurred in the presence of all three subunits (Figure 3B).

The extent of DNA incision was quantitated by scanning the dried sequencing gels containing UvrABC digestion products of the defined DNA substrates using a Betascope 603 blot analyzer (Betagen, Waltham, MA; see Materials and Methods). Histograms displaying the extent of 5′ and 3′ incisions from several experiments are displayed in Figure 4A. While significant variation of the overall incision efficiency was observed in different experiments, the overall trend was always the same: the DNA substrate containing an abasic site was incised less efficiently (2–10%) than the same sequence containing a reduced AP site (ring-opened) (12–22%). Modification of an AP site with an *O*-methyl- or an *O*-benzylhydroxylamine group further increased the incision efficiency of the UvrABC complex (20–42%). The difference in the extent of 3′ and 5′ incisions was a consequence of different preparations of substrates and was not a reflection of uncoupling of the 5′ incisions.

Abasic sites are relatively unstable and resulted in spontaneous strand scissions during heating prior to loading the DNA on the gels for electrophoresis. These strand breaks can be observed by a band migrating at the position marked by an arrow in lanes 3 and 4 and lanes 1 and 2 in Figures 3A and 4A, respectively. AP sites which have not spontaneously resulted in a nick are reactive with *O*-benzylhydroxylamine and will be protected from spontaneous strand scission. Treatment of the DNA substrates containing AP sites with *O*-benzylhydroxylamine (in ABC buffer) resulted in greater than 85% protection from heat-induced breakage (data not shown). These data suggest that breaks seen in Figure 2 (lanes 3 and 4) and Figure 3 (lanes 1 and 2) occurred during the heating step prior to electrophoresis and were not preexisting in the DNA.

The position of the UvrABC complex incision sites for DNA containing abasic sites or base analogue damage is displayed in Figure 5. Uncoupled 5′ or 3′ incisions in the absence of incision at the other site have been observed previously (Yeung et al., 1983; Walters, 1988; Selby, 1989). No uncoupling of 5′ or 3′ incision was observed in any experiment with these DNA substrates.

UvrABC Incision of Endonuclease III or Endonuclease IV Nicked Substrates. Having demonstrated that the UvrABC complex acts on DNA substrates containing abasic sites or base-damage analogues, it was of interest to determine whether the introduction of a nick either 5′ or 3′ to a damage site would affect the UvrABC incision efficiency or the position of the incision sites. Endonuclease III, a repair enzyme with both *N*-glycosylase and AP endonuclease activities, acts on AP sites and several types of base damage (Wallace, 1989). In addition, endonuclease III, a class I AP endonuclease, catalyzes the hydrolysis of the phosphodiester bond 3′ to the AP site. Endonuclease IV is a class II AP endonuclease which is capable of acting on a wide range of DNA damages including AP sites, rAP sites, and certain types of base damage (Wallace, 1989). Cleavage by endonuclease IV results in a nick 5′ to the lesion. Endonuclease III and endonuclease IV were used to generate a 3′ or a 5′ nick, respectively, into DNA duplexes containing AP sites or base-damage analogues. These nicked substrates were then subjected to UvrABC nuclease digestion.

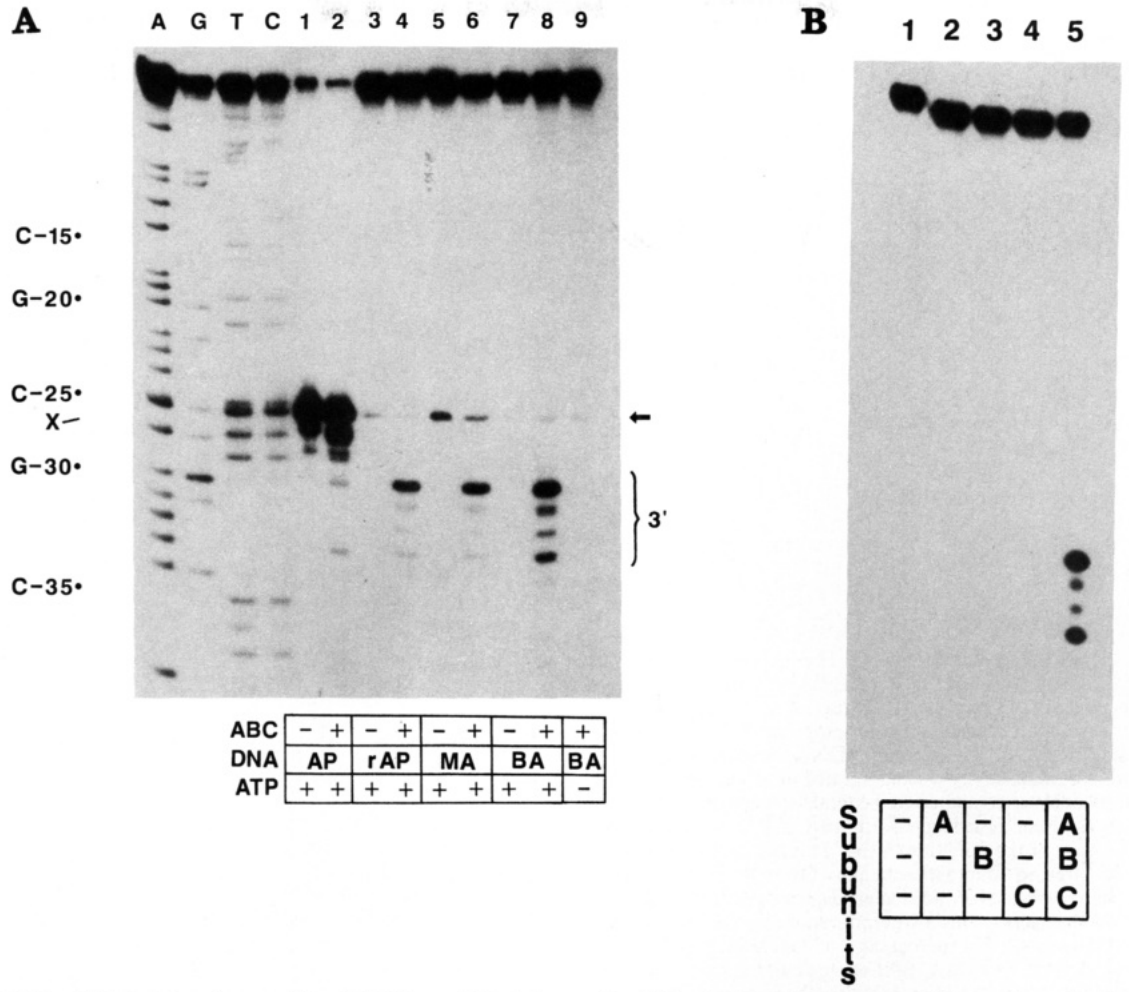

FIGURE 3: 3′ UvrABC incision sites at AP, rAP, MA, and BA lesions. The 49 bp duplex substrates containing a 3′-terminal label on the lesion-containing strand (see Materials and Methods) were incubated with 1.1 pmol of UvrA, 2.1 pmol of UvrB, and 5.5 pmol of UvrC for 30 min at 37 °C. (Panel A) UvrABC incisions at AP, rAP, MA, and BA lesions. Lanes 1, 3, 5, and 7 were incubated with ATP in the absence of UvrABC. Lanes 2, 4, 6, and 8 were incubated with ATP and UvrABC. Lane 9 is BA-modified duplex incubated with UvrABC in the absence of ATP as a control. The strand is numbered 5′ to 3′. The arrow indicates spontaneous strand breaks which occurred at abasic sites during the heating process prior to gel loading. The X denotes the band which results from Maxam–Gilbert cleavage of uracil at position 26. The 3′ incision site is indicated and occurs predominantly at the fifth phosphodiester bond 3′ to the lesion with other incisions occurring to a lesser extent at the sixth, seventh, and eighth phosphodiester bonds. (Panel B) BA-modified duplex incubated as above with either UvrA, UvrB, UvrC, or UvrABC as a control. Symbols are as those described in Figure 1.

The results of such an experiment are shown in Figure 6. As seen in this autoradiograph, 5′-labeled DNA containing a 5′ or a 3′ nick at a base-damage analogue is still a substrate for the UvrABC nuclease complex. On the basis of previous studies, digestion of AP sites with endonuclease III results in the generation of a didehydrodideoxyribose moiety (ring-closed) 3′ to the nicked site (Baily & Verly, 1987; Manoharan et al., 1988). Since this lesion resembles an AP site, it was expected that treatment with endonuclease III prior to the UvrABC complex would result in a decreased incision efficiency as compared to the BA-containing DNA. This was indeed the case, but surprisingly, endonuclease III processed BA-containing DNA (an AP site with a 3′ nick) was consistently incised by the UvrABC complex more efficiently than an AP site that did not contain a nick (Figures 6 and 7). These data also indicate that DNA substrates containing a 5′ nick introduced by endonuclease IV were incised less efficiently than the same substrate containing a 3′ nick induced by endonuclease III (Figure 7).

Analysis of the 3′ incision sites of the UvrABC complex using 3′ terminally labeled substrates containing a 3′-endonuclease III nick yielded the expected incision patterns (Figure 8, lanes 2 and 9). As was seen for the 5′-labeled substrates, UvrABC incised 3′-labeled DNA duplexes containing a 3′ nick (endonuclease III) more efficiently than a 5′ nick (endonuclease IV) (Figure 8, compare lanes 2 and 9 with lane 11; data not shown).

During the course of these experiments, it was routinely observed that DNA which had first been pretreated in endonuclease III buffer and then diluted into a buffer compatible with UvrABC resulted in more efficient incision than the UvrABC buffer alone (60% as compared to 20–40%). Additional experiments appear to indicate that this increased incision efficiency is most likely due to a higher concentration of KCl (100 mM) (data not shown).

Discussion

The purpose of this study was 3-fold: (1) to resolve whether abasic sites are substrates for the UvrABC nuclease complex; (2) to develop a model series of DNA lesions to examine the damage recognition process of the UvrABC complex; and (3) to investigate whether base and nucleotide excision repair enzymes may interact in the repair of specific types of base damage.

Damage Repertoire of the UvrABC Nuclease Complex. To investigate these objectives, four unique substrates each containing a different DNA lesion at the same site of a defined 49 bp sequence was constructed. These substrates have al-

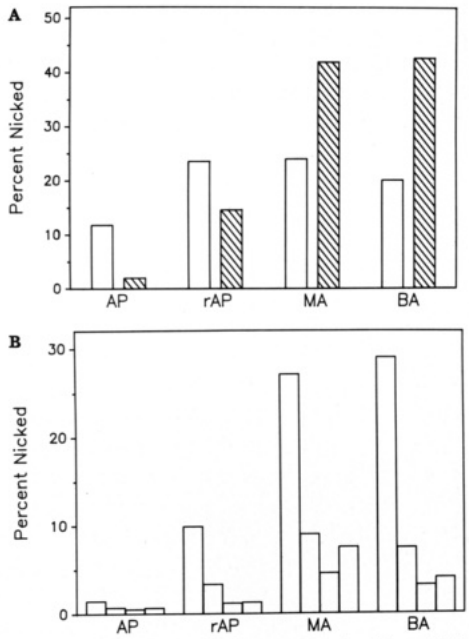

FIGURE 4: UvrABC incision efficiency. The incision efficiencies of UvrABC for the substrates AP, rAP, MA, BA were determined from several experiments as performed in Figures 2 and 3A. The extent of incision was quantitated on a radioisotope imaging facility (see Materials and Methods). The percent of DNA incised was calculated as the fraction of radioactivity which migrated in bands corresponding to digestion products as compared to the total radioactivity in the lane. Symbols are as those described in Figure 1. (Panel A) UvrABC incision efficiency of the four substrates. This histogram represents the mean of three to four experiments. Open bar = 5′ incision efficiency: hatched bar = 3′ incision efficiency. (Panel B) Relative frequency of 3′ incisions. This histogram represents the mean of two to three experiments. The percentage of DNA incised for each modified duplex (AP, rAP, MA, BA) was calculated as stated above at the fifth, sixth, seventh, and eighth phosphodiester bonds (represented from left to right in each group) 3′ to the lesion as seen in Figure 3.

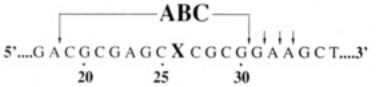

FIGURE 5: Summary of the 5′ and 3′ incision sites of UvrABC for DNA containing AP, rAP, MA, or BA residues. Incision sites occur at the eighth phosphodiester bond 5′ and predominantly at the fifth phosphodiester bond 3′ to the lesion as indicated by the larger arrows. These were determined in experiments as those shown in Figures 1 and 2. X denotes position 26 which was modified as shown in Figure 1 (see Materials and Methods). Minor incisions occur at the sixth, seventh, and eighth phosphodiester bonds 3′ as indicated by the smaller arrows.

lowed us to systematically introduce structurally similar DNA lesions with increasing bulkiness into the same sequence for the study of the damage recognition repertoire of the UvrABC nuclease complex.

Recent studies have suggested that base damage such as thymine glycols and O^6-methylguanine adducts, which do not appear to induce appreciable distortion into the DNA helix, are substrates for the UvrABC complex (Voigt et al., 1989; Linn & Sancar, 1989; Kow et al., 1990). Conflicting data have been presented as to whether AP sites are substrates for this enzyme (Lin & Sancar, 1989; Kow et al., 1990). We previously failed to observe any nicking above background when highly supercoiled PM2 DNA containing on the average one AP site per DNA molecule was incubated with the UvrABC nuclease complex (Kow et al., 1990), whereas Lin and Sancar (1989) have shown that supercoiled pBR322 or restriction fragments of pBR322 containing AP sites were recognized by the UvrABC complex.

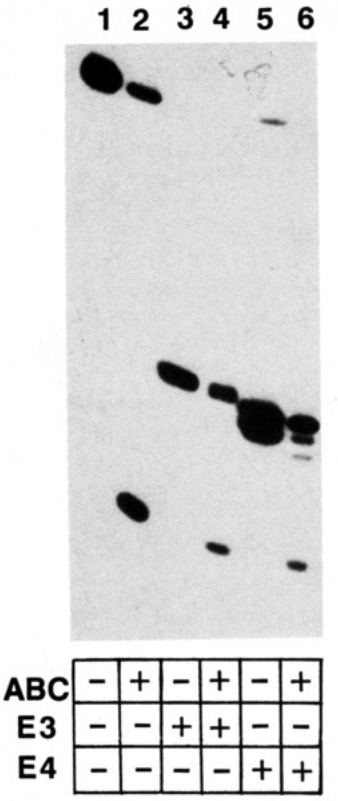

FIGURE 6: UvrABC complex incises a BA substrate containing endonuclease III or endonuclease IV generated nicks. The 49 bp BA containing duplex was 5′ terminally labeled on the lesion-containing strand and incubated with 19.2 pmol of endonuclease III (lane 3) or 7.3 pmol of endonuclease IV (lane 5) for 30 min at 37 °C. Half of these reactions were then incubated with 1.1 pmol of UvrA, 2.1 pmol of UvrB, 5.5 pmol of UvrC, and ATP-containing buffer for 30 min at 37 °C, lanes 4 and 6 respectively. Lanes 1 and 2 are BA modified duplex incubated for 30 min in the endonuclease buffer without endonuclease followed by incubation with UvrABC as above (lane 2). BA = *O*-benzylhydroxylamine-modified apyrimidinic site.

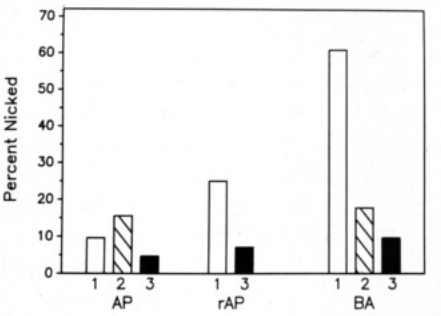

FIGURE 7: Relative efficiency of the UvrABC complex for DNA substrates containing nicks. Relative 5′ incision efficiencies were determined quantitatively by using a radioisotope imaging facility (see Materials and Methods). The percent of DNA incised was calculated as the fraction of radioactivity that migrated in bands corresponding to the UvrABC digestion products as compared to the total radioactivity in the lane. This histogram represents the mean of two to three experiments. 1 (open bar) = UvrABC incision alone in endonuclease and UvrABC buffers; 2 = UvrABC incision following treatment with endonuclease III; 3 = UvrABC incision following treatment with endonuclease IV. Symbols are as those described in Figure 1.

Construction of a defined substrate containing an AP site at a defined position helped resolve this apparent conflict. Our present study demonstrates that an apyrimidinic site is clearly a substrate for the UvrABC complex, although its overall incision efficiency was consistently the lowest of the four substrates tested. One possible explanation for the lack of detection with the PM2 substrate is that AP sites are recog-

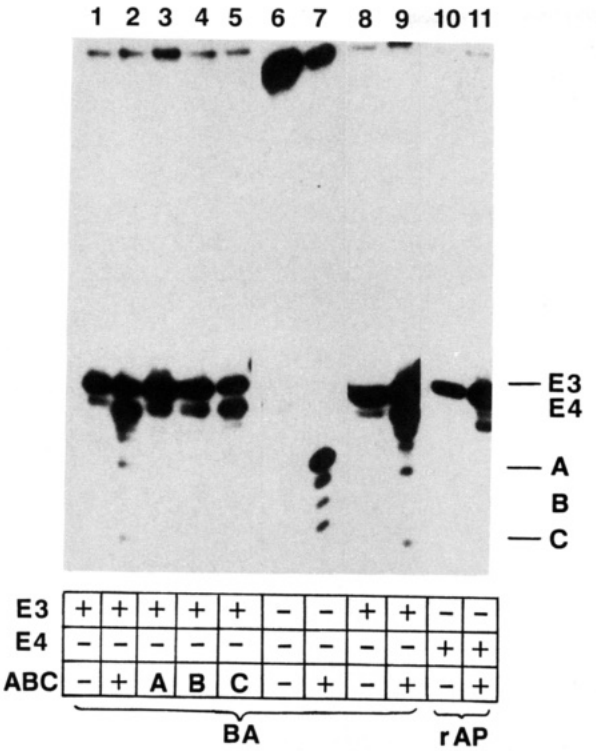

FIGURE 8: 3′ incision sites of the UvrABC complex for substrates containing endonuclease III or Endo IV generated nicks. The 49 bp duplex containing a BA or rAP residue was 3′ terminally labeled and incubated with 19.2 pmol of endonuclease III or 7.3 pmol of endonuclease IV for 30 min at 37 °C. Half of these reactions were then incubated with 1.1 pmol of UvrA, 2.1 pmol of UvrB, and 5.5 pmol of UvrC in ATP-containing UvrABC buffer for 30 min at 37 °C. Lanes 1–5 are BA-modified duplex treated with endonuclease III followed by UvrABC (lanes 2), UvrA (lane 3), UvrB (lane 4), and UvrC (lane 5). Lane 6 and 7 are BA-modified duplex ± UvrABC without endonucleases in endonuclease and ATP buffers. Lane 8 is BA treated with endonuclease III followed by UvrABC (lane 9). Endonuclease IV treatment of rAP-modified duplex is shown in lane 10 followed by UvrABC digestion (lane 11). The bands resulting from endonuclease III, endonuclease IV, and UvrABC digestion products are indicated. Symbols are as those described in Figure 1.

nized very inefficiently in highly supercoiled DNA. Furthermore, if the binding affinity of the UvrAB complex for an AP site is relatively weak as compared to nonspecific DNA binding affinity, the relatively low amount of protein used in our previous assays may not have been able to detect one AP site in 10 kb of highly supercoiled PM2 DNA.

By construction of four DNA substrates each containing a structurally related lesion within an identical sequence, the size of the lesion and the extent of the UvrABC incision could be compared directly for the first time. Under the conditions used in this study, we demonstrated that the UvrABC nuclease incision efficiency is greatest for the base analogues *O*-methylhydroxylamine- and *O*-benzylhydroxylamine-modified AP sites, followed by reduced AP sites, and AP sites were incised least efficiently. This incision efficiency agrees with our previous study in which these lesions were randomly introduced on the average of one per supercoiled PM2 molecule of DNA (Kow et al., 1990). DNA containing uracil instead of thymidine was clearly not a substrate for the UvrABC complex (see Figure 2, lanes 1 and 2).

The incision of these rAP-, MA-, and BA-containing substrates appeared to be greater than what was previously seen for a 45 bp duplex containing O^6-MeGua or 48 bp duplex containing a pyrimidine dimer at a defined site (Voigt et al., 1989). The incision efficiency that was observed with a 137 bp duplex containing a psoralen monoadduct (Van Houten et al., 1986a) was about the same as what was presented here. However, a direct comparison between the four substrates presented here and the O^6-MeGuan, pyrimidine dimer, and psoralen-containing substrates is impossible due to the differences in sequence context, as well as differences in substrate and enzyme preparations.

The position of the incision sites occurred predominantly at the eighth phosphodiester bond 5′ and the fifth phosphodiester bond 3′ to the lesion. It might be expected that the size of the DNA lesion might influence the position of the UvrABC incision sites. Nonstandard incision patterns have been observed for psoralen interstrand cross-links (Van Houten, 1987), UV-induced photoproducts (Myles et al., 1987), O^6-MeGua (Voigt et al., 1989), and anthramycin adducts (Walter et al., 1988). This study did not show any effect of lesion size on the position of the incision sites. These substrates displayed heterogeneity in the incision sites 3′ to the lesion, with minor incision occurring at the sixth, seventh, and eighth phosphodiester bonds 3′ to the lesion site.

Future investigations will be devoted to kinetic and thermodynamic studies of the interaction of the UvrABC subunits with these four and other structurally related lesions. By making systematic changes in the chemical modifications of the lesion, we hope to make discrete changes in the structure of the DNA helix. These structural changes, monitored through physical and molecular modeling techniques, will help achieve a description of the structural alterations which are important for the damage recognition properties of the UvrABC complex.

DNA Lesions Containing 5′ or 3′ Nicks Are Substrates for UvrABC. Since the substrates described in this report are also recognized by base excision repair enzymes from *E. coli*, we could, therefore, examine whether DNA lesions which have been nicked by either endonuclease III or endonuclease IV can be further processed by the UvrABC complex. We demonstrated that BA residues, a base-damage analogue, containing a 5′ nick (endonuclease IV) or a 3′ nick (endonuclease III), were incised by the UvrABC complex, albeit at a reduced level. Interestingly, we found that an AP site which had been incised by endonuclease III was consistently a better substrate for the UvrABC complex than the AP site alone (Figures 6 and 7). Pieters et al. (1989) have recently analyzed the thermodynamic and structural changes which accompany cleavage of the phosphate backbone of normal B-form DNA. They found that there is appreciable local distortion of the two bases flanking the nick site and that loss of a phosphate increases this distortion. This observation, combined with our present findings, suggests that an AP site containing either a 3′ nick or a ring-opened sugar (rAP site) is a more distorting lesion than an AP site. Physical studies as well as molecular modeling and energy minimization studies will help to correlate structural perturbations with UvrABC incision efficiency.

The UvrABC complex appears to incise a DNA lesion containing a 3′ nick more efficiently than a 5′ nick (Figures 6–8). One possible explanation for this behavior is that endonuclease IV may stay bound following the production of a 5′ nick and might, therefore, interfere with the binding of the UvrABC complex. Alternatively, we have recently found that endonuclease IV can remove an additional 5′ base with a 3′ → 5′ gapping activity (Kow & Van Houten, 1990). This base (5′ to the lesion) may provide important contacts for the formation of the preincision Uvr–DNA complex.

During the course of these studies, we have also observed that the UvrABC nuclease complex appears to perform its 5′ incisions more efficiently than the 3′ incisions for substrates

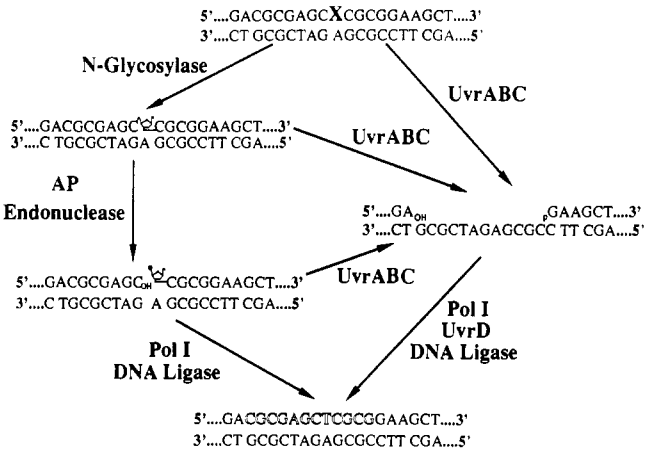

FIGURE 9: Model for the interaction of base and nucleotide excision repair pathways in the repair of specific base damage. A schematic diagram of potential pathways for how specific base lesions may be repaired in *E. coli* (see text for additional details). Specific base damage can be repaired by either base excision repair proteins or the UvrABC nuclease complex. In addition, intermediates in base excision repair are also substrate for the UvrABC complex.

containing a 5′ or 3′ nick at the site of the substrate. This apparent uncoupling could be due to the nature of the incision process. For example, in a scenario in which the 5′ UvrABC incision precedes the 3′ incision, the following might occur: once the 5′ incision is made on the nicked substrate, the resulting six- or seven-nucleotide oligomer may be sufficiently destabilized at 37 °C so that it dissociates, making it impossible for the Uvr complex to make a 3′ incision.

Potential Interaction of Base and Nucleotide Excision Repair Pathways. This report provides clear evidence that certain DNA lesions are substrates for both base and nucleotide excision repair enzymes. This convergence of repair pathways for specific DNA lesions is shown diagrammatically in Figure 9. Base excision repair proceeds by two general pathways. In one pathway, a damage-specific endonuclease such as endonuclease IV produced an incision 5′ to the damaged site, whereas in the other pathway a base-damaged-specific glycosylase removes the damaged base, generating an AP site. This AP site could then be (i) processed by the UvrABC nuclease complex or (ii) incised by a type I or type II AP endonuclease. Incision by endonuclease III or endonuclease IV leads to a 3′ or 5′ nick at the AP site, respectively. This current study provides evidence that these nicked AP sites can be further processed by the UvrABC complex. If the AP site had been incised by a type II endonuclease, such as endonuclease IV or exonuclease III, generating a 5′ nick adjacent to the AP site, then DNA polymerase I could remove the AP site by nick translation and insert a small repair patch. If, however, the AP site is nicked by the action of a type I AP endonuclease such as endonuclease III, generating a 3′ nick, the lesion must then be removed by the action of either a type II endonuclease or, as we have presented in this report, the UvrABC nuclease complex. It would appear that a critical step in the repair of most base damage would be the generation of an abasic site.

Recent evidence by Cunningham and co-workers suggests that a *uvrA xth nfo* triple mutant is inviable (Saporito et al., 1989). This observation suggests that this mutant may be completely defective in the removal of AP sites. Since normal respiration can lead to oxidative DNA damage and depurination occurs spontaneously at normal physiological temperatures and pH, AP sites would be expected to accumulate within a cell containing a *uvrA nfo xth* triple mutation. This accumulation would probably lead to cell death. We would therefore like to propose that base and nucleotide excision repair should not be viewed as separate repair pathways and that the repair of specific types of base damage might occur in an intricate manner involving the UvrABC complex and enzymes from the base excision repair pathway.

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Evaluation of Selected Benzoquinones, Naphthoquinones, and Anthraquinones as Replacements for Phylloquinone in the A_1 Acceptor Site of the Photosystem I Reaction Center†

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ABSTRACT: Selected substituted 1,4-benzoquinones, 1,4-naphthoquinones, and 9,10-anthraquinones were investigated as possible replacement quinones in spinach photosystem I (PSI) preparations that had been depleted of endogenous phylloquinone by extraction with hexane/methanol. As a criterion for successful biochemical reconstitution, the restoration of electron transfer was determined by measuring P-430 turnover at room temperature from flash-induced absorbance transients. Restoration of complete electron transfer between A_0^- and P-430 (terminal iron–sulfur centers, F_AF_B) was demonstrated by using phylloquinone, 2-methyl-3-decyl-1,4-naphthoquinone, 2-methyl-3-(isoprenyl)$_2$-1,4-naphthoquinone, and 2-methyl-3-(isoprenyl)$_4$-1,4-naphthoquinone. All other quinones tested did not restore P-430 turnover but acted as electron acceptors and oxidized A_0^-. It is concluded that the specificity of the replacement quinone for interaction with the primary acceptor, A_0^-, is low but additional structural constraints are required for the quinone occupying the A_1 site to donate to the iron–sulfur center, F_x. It is suggested that the 3-phytyl side chain of phylloquinone and the 3-alkyl tails of the three naphthoquinones that restored P-430 turnover may be required for interaction with a hydrophobic domain of the A_1 site in the PSI core to promote electron transfer to F_x and then to F_AF_B.

In green plant and cyanobacterial photosynthesis, the primary charge separation in photosystem 1 (PSI)[1] ($P\text{-}700^+A_0^-$) is stabilized by successive electron transfers through the redox centers referred to as A_1, F_x, and F_AF_B (Golbeck, 1987). This report is concerned with the center A_1, which was originally postulated to be a quinone on the basis of EPR measurements (Gast et al., 1983; Thurnauer & Gast, 1985). Their provisional identification was recently supported by comparison of the electron-spin-polarized EPR K-band spectrum of $P\text{-}700^+A_1^-$ with that of $P\text{-}870^+Q^-$ in iron-depleted bacterial reaction centers (Peterson et al., 1987). Because of the apparently exclusive localization of phylloquinone in PSI (Takahashi et al., 1985; Schoeder & Lockau, 1986), they suggested that phylloquinone may be the acceptor A_1.

Evidence for the participation of phylloquinone (vitamin K_1) on the reducing side of PSI was provided by Brettel et al. (1986), following a detailed analysis of absorbance transients in the UV at low temperature, and from quinone-depletion and biochemical reconstitution studies using phylloquinone (Biggins & Mathis, 1988; Itoh & Iwaki, 1989; Iwaki & Itoh, 1989, 1990).

Despite some anomalies regarding the EPR behavior of phylloquinone-depleted PSI preparations that still show electron transfer to terminal FeS centers at cryogenic temperatures (Sētif et al., 1987; Biggins et al., 1989), the functional role of phylloquinone in mediating electron transfer

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[1] Abbreviations: PSI, photosystem I; PSII, photosystem II; BPh^-, reduced bacteriopheophytin; Ph^-, reduced pheophytin; TMPD, *N,N,N′,N′*-tetramethylphenylenediamine.

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