DNA–XPA interactions: a $^{31}$P NMR and molecular modeling study of dCCAATAACC association with the minimal DNA-binding domain (M98–F219) of the nucleotide excision repair protein XPA

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**ABSTRACT**

Recent NMR-based, chemical shift mapping experiments with the minimal DNA-binding domain of XPA (XPA-MBD: M98–F219) suggest that a basic cleft located in the loop-rich subdomain plays a role in DNA-binding. Here, XPA–DNA interactions are further characterized by NMR spectroscopy from the vantage point of the DNA using a single-stranded DNA nonamer, dCCAATAACC (d9). Up to 2.5 molar equivalents of XPA-MBD was titrated into a solution of d9. A subset of $^{31}$P resonances of d9 were observed to broaden and/or shift providing direct evidence that XPA-MBD binds d9 by a mechanism that perturbs the phosphodiester backbone of d9. The interior five residues of d9 broadened and/or shifted before $^{31}$P resonances of phosphate groups at the termini, suggesting that when d9 is bound to XPA-MBD the internal residues assume a correlation time that is characteristic of the molecular weight of the complex while the residues at the termini undergo a fraying motion away from the surface of the protein on a timescale such that the line widths are more characteristic of the molecular weight of ssDNA. A molecular model of the XPA-MBD complex with d9 was calculated based on the $^{15}$N (XPA-MBD) and $^{31}$P (d9) chemical shift mapping studies and on the assumption that electrostatic interactions drive the complex formation. The model shows that a nine residue DNA oligomer fully covers the DNA-binding surface of XPA and that there may be an energetic advantage to binding DNA in the 3′→5′ direction rather than in the 5′→3′ direction (relative to XPA-MBD α-helix-3).

**INTRODUCTION**

Genomic DNA is continuously being damaged. This damage may be induced by endogenous reactive species generated during events of normal cellular metabolism, such as hydrolysis, oxidation and methylation, or by reactive species that are exogenous in origin, such as UV and ionizing radiation (1,2). Mutagenesis, carcinogenesis, aging, genetic diseases and cell death are some of the possible deleterious cellular consequences (3,4). To prevent the potentially harmful outcomes of DNA damage, cells have developed efficient DNA repair mechanisms (2). One such mechanism is nucleotide excision repair (NER), a highly conserved DNA repair pathway found in the three major kingdoms of life (5). An interesting feature of NER is its versatility. Not only is it the primary defense against the carcinogenic effects of solar UV light, but it also acts on a wide variety of bulky, helix-distorting lesions (6,7) and to a lesser extent, more subtle DNA lesions such as AP-sites (8).

Six core NER repair factors coordinate the excision of damaged DNA from the genome in the form of a 24–32 bp oligomer around the lesion (7,9,10). Of the six repair factors (RPA, XPC–hHR23B, TFIIH, XPG, ERCC1–XP and XPA) both RPA and XPA are essential for NER activity (11–13). XPA interacts with RPA (11,14) and while human XPA has a moderately greater affinity for damaged DNA over undamaged DNA (15,16), an XPA–RPA co-complex has yet a greater affinity for damaged DNA (17,18). XPA also interacts with other NER proteins including ERCC1–XP and XPA (19) and XPC–hHR23B (21), and RPA interacts with the nucleases XPG and ERCCI–XP (17,22). It has been suggested that XPA, in association with RPA, plays a central, multifunctional role in NER, with the two proteins recognizing DNA damage and then recruiting and organizing the other NER proteins into position to remove the lesion (23,24).

Human XPA is a 31 kDa metalloprotein of 273 amino acid residues and one molecule of zinc (25,26). A 122 amino acid region between M98 and F219 (XPA-MBD) is the shortest known region of XPA that binds DNA (27). The solution

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structure for XPA-MBD has been determined using NMR-based methods: it consists of two subdomains joined together by a linker sequence, a C-terminal zinc-binding core and an N-terminal loop-rich subdomain (28–30). Because the 1H and 15N chemical shifts of the amide resonances of the polypeptide backbone are sensitive to the chemical environment of the nuclei at protein–ligand interfaces (31,32), it has been possible to map the surface of XPA-MBD which interacts with DNA. While the zinc in XPA is essential for binding DNA and for NER activity (33,34), the chemical shift perturbation studies indicate that the zinc-binding core does not play a direct role in DNA-binding (28–30). Instead, the DNA-binding region of XPA is located in the loop-rich subdomain along a surface of basic amino acid residues (28–30). Cisplatin-treated DNA (30), DNA containing dihydrothymidine or 6-4-thymidine-cytidine and undamaged single-stranded (ss)DNA (29) all produced a similar chemical shift perturbation pattern with 15N-enriched XPA-MBD indicating that XPA binds all DNA on the same surface with a low DNA-binding specificity (35). Such chemical shift mapping studies followed the DNA–XPA interactions from the vantage point of the protein’s amide backbone using 15N-labeled XPA-MBD. Here, we use 31P NMR spectroscopy to follow the DNA–XPA interactions from the vantage point of the DNA phosphodiester backbone using a nine residue, ssDNA oligomer, dCCATAACC (d9). This undamaged, ssDNA oligomer was used because the results of previous chemical shift perturbation studies with XPA-MBD and single-stranded, double-stranded, damaged and undamaged DNA were similar and because it is generally believed that one of the key features XPA and/or RPA recognizes in damaged DNA is single-stranded character (10,13). The 31P chemical shifts of the DNA are highly sensitive to the conformation of the phosphodiester backbone (36) and, hence, provide further insight into the structural basis for DNA recognition by XPA. To test if electrostatic interactions between the negatively charged phosphodiester backbone of DNA and the positively charged basic cleft in the loop-rich subdomain of XPA could account for XPA–DNA interactions, model calculations were performed by docking d9 onto the DNA-binding surface of XPA-MBD.

MATERIALS AND METHODS

Preparation of XPA-MBD and d9

Uniformly 15N-labeled XPA-MBD was expressed in Escherichia coli bacterial strain BL21(DE3)pLYsS (Novagen Inc., Madison, WI) as previously described (26,29) and the protein purified by a modified protocol. Frozen cells (~80°C) from a 750 ml culture were resuspended in 40 ml of 50 mM potassium phosphate, pH 7.5. The cell suspension was made 0.2 mM in phenylmethylsulfonyl fluoride immediately prior to three passes through a French Press (SLM Instruments Inc., Rochester, NY). Following 1 min of sonication the cell debris was centrifuged at 17,500 r.p.m. for 45 min in a JA-20 rotor in a Beckman Avanti J-25 centrifuge (Palo Alto, CA). After centrifugation, 10–20 ml supernatant was applied to a Bio-Rad hydroxyapatite column (Bio-Rad, Hercules, CA) attached to a BioCAD Sprint Perfusion Chromatography System (PerSeptive Biosystems, Framingham, MA). The column was washed with 10 mM potassium phosphate, 1.0 mM DTT pH 7.1 and eluted with a linear gradient of 0–400 mM potassium phosphate, 1.0 mM DTT, pH 7.1, over 10 column volumes. XPA-MBD eluted with ~150 mM potassium phosphate. The peak containing XPA-MBD was collected, pooled and concentrated to ~2 mM with a Centriprep-10 (Amicon Inc., Beverly, MA). The protein was further purified on a Superdex75 HiLoad column (Pharmacia, Piscataway, NJ) that, at the same time, exchanged XPA-MBD into NMR buffer (20 mM potassium phosphate, 100 mM KCl, 5 mM DTT and 50 µM Na3P, pH 7.3). SDS–PAGE and Coomassie Blue staining showed the NMR samples to be >95% pure.

The ssDNA nonamer, dCCATAACC (d9), was synthesized on an Applied Biosystems 392 DNA/RNA synthesizer (Foster City, CA) as described previously (29). NMR samples (1–2 mM) of d9 were prepared in 600 µl NMR buffer in D2O. All the chemicals used were purchased from the Sigma Chemical Company (St Louis, MO) except for the D2O (Cambridge Isotope Laboratories, Andover, MA). Protein concentrations were determined using the Bradford assay (Bio-Rad) and DNA concentrations were determined using UV absorbance at 260 nm (1 A260nm ssDNA = 37 µg/ml).

NMR spectroscopy

NMR data were collected on Varian 750- 600- or 500-Unity-plus spectrometers. Phosphorus spectra were collected exclusively at a 31P resonance frequency of 202 MHz. Standard phase sensitive (TPPI) homonuclear two-dimensional DQF-COSY (37), NOESY (38) and TOCSY (39,40) spectra were collected for d9 to assign the 1H resonances. HeteroCOSY (41) and heteroTOCSY (42) two-dimensional spectra were collected for d9 to verify the assignment of the 1H resonances and to assign the 31P resonances. For the 15N/1H HSQC titration experiments, aliquots of desalted d9 (0.03 µmol/µl) were added to 600 µl of 0.4 mM XPA-MBD. Two-dimensional 15N/1H HSQC (43,44) spectra (25°C) were recorded at d9:XPA-MBD molar ratios of 0.25, 0.50, 0.75, 1.0, 1.5, 2.0 and 2.5 (molar ratio = moles d9/moles XPA-MBD). For the 31P titration experiments, aliquots of XPA-MBD in 99% D2O (0.0025 µmol/µl) were added to 500 µl of 0.9 mM d9 in 99% D2O. One-dimensional proton decoupled 31P spectra (25°C) were recorded at d9:XPA-MBD molar ratios of 4.0, 2.7, 2.0, 1.0, 0.8, 0.67, 0.57, 0.50 and 0.44. To assist following the movement of the d9 31P resonances with the addition of XPA-MBD, two-dimensional 31P/H heteroCOSY spectra (15°C) were recorded on a 2.0 mM sample of d9 in 99% D2O at d9:XPA-MBD molar ratios of 10, 5, 3.3, 2.5 and 0.5. Both the 1H and 31P chemical shifts were referenced by the indirect referencing method (45,46).

Modeling calculations

A prominent feature observed in the solution structure of XPA-MBD is a basic cleft in the loop-rich subdomain (29,30). The charged head groups of these basic residues are distributed on the surface of the protein with a spacing of 5–12 Å, a distance that is approximately equal to one or two times the distance between phosphorous atoms in helical DNA (5–8 Å). Because of this correlation and the observation that ssDNA containing purine bases has a tendency to adopt elements of helical structure in solution (47), the starting structure of d9 used for the docking calculations was generated using NAMOT-2 (48) with d9 loosely restrained in a right-handed B-form helix. The
XPA-MBD used for the docking calculations was the average cobalt-refined solution structure deposited in the RCSB Protein Data Base (PDB) (1D4U). Residues K141, K148, K168, K179 and K204 form a nearly continuous loop in the basic cleft and, consequently, these residues were chosen as anchor points for docking the DNA onto the protein. Both the protein and the DNA were treated as rigid bodies. To prevent unfavorable contacts between the two molecules, a van der Waals penalty was applied where the energy between the two molecules was calculated with the AMBER parameters (49). A constraint energy term, Ec, was also applied to aid docking with Ec equal to \( [\Sigma(i)k \times (d - d_0)^2] \) when \( d > d_0 \) and equal to 0 when \( d < d_0 \). The force constant, \( k \), was arbitrarily set to 100 kcal/mol Å\(^2\) and \( d \) was the distance between the \( \alpha \)-carbon of the anchoring residue in the protein and the corresponding phosphorus atom in the DNA. The distance \( d_0 \) set to 6 Å corresponding to the range in values (5.0–6.4 Å) observed in lysozyme residues that formed hydrogen bonds with the phosphate backbone of DNA in the crystal structure of nucleosome (PDB code 1AOI) (50). The Metropolis Monte Carlo simulation method (51) was used to dock the DNA on to the protein. The docked complex was subjected to a short run (1000 cycles) of energy minimization using an AMBER force field (49). Hydrogen donor–acceptor pairs were identified and distance constraints were applied between these donor–acceptor pairs while subjecting the complex to 10 ps molecular dynamic simulation. The resulting complex was energy minimized (1000 cycles) using an AMBER force field. The calculations were performed with d9 oriented in the 3′→5′ and 5′→3′ direction (relative to XPA-MBD α-helix-3) and with and without right-handed, B-form helical restraints on the d9 starting structure during the docking calculations.

### RESULTS AND DISCUSSION

#### Chemical shift mapping study with \( ^{15} \text{N} \)-labeled XPA-MBD

The \( ^1\text{H} \) and \( ^{15}\text{N} \) amide resonances in the \( ^{15}\text{N}/^1\text{H} \) HSQC spectrum of XPA-MBD have been previously assigned (29,30) and the average amide chemical shifts in XPA-MBD observed upon the addition of an equimolar quantity of d9 have been previously reported (29). Figure 1 plots the average chemical shift changes \( \Delta_{\text{ave}} = [(\Delta^{1\text{H}})^2 + (\Delta^{15\text{N}}/5)^2]/2 \) in the \( ^1\text{H} \) and \( ^{15}\text{N} \) resonances of XPA-MBD with the addition of excess DNA, at a d9:XPA-MBD molar ratio of 2.5. XPA-MBD residues marked with an asterisk in Figure 1 are \( ^{15}\text{N}/^1\text{H} \) HSQC cross peaks that disappear due to extensive line broadening upon the addition of d9. At 2.5 molar excess of DNA to protein no additional amide residues were significantly perturbed relative to those affected at an approximately equal molar ratio of d9:XPA-MBD. In Figure 2 the average chemical shift changes in the \( ^1\text{H} \) and \( ^{15}\text{N} \) resonances of the XPA-MBD residues most perturbed by the addition of DNA are plotted as a function of the d9:XPA-MBD molar ratio. For most of the residues the average \( ^1\text{H} \) and \( ^{15}\text{N} \) chemical shift change continued to increase with the addition of DNA suggesting that at 2.5 molar excess of DNA to protein, XPA-MBD was still not saturated with DNA. The most likely explanation for such an observation is that DNA binding to XPA is weak, as reported previously for larger DNA fragments (16,18,35).

#### NMR chemical shift assignments of d9

The phosphorus and proton resonances in d9 are labeled following the IUPAC-IUBMB-IUPAB nomenclature recommendation (46). For example, in the dinucleotide T(i-1)pC(i) the phosphorus is labeled (i). The d9 proton chemical shifts were assigned by inspection of the NOESY, TOCSY and DQF-COSY spectra (52,53). The H5′ was assigned deshielded relative to H5 according to Remin and Shugar (54) whereas the H2′/H2″ assignments were based on coupling constant arguments (55,56). The d9 phosphorus chemical shifts were assigned by inspection of the heteroCOSY and heteroTOCSY spectra on the basis of the \( ^1\text{H} \) chemical shift assignments (Table 1). In the heteroCOSY experiment, antiphase multiplet correlations are observed between \( ^{31}\text{P} \) and any three- or four-bond scalar coupled proton as well as additional correlations due to homonuclear \( ^1\text{H} \) coherences. The power of this method is illustrated in Figure 3, an expansion of the heteroTOCSY spectrum of d9 in the H3′ region at 15°C, a temperature where the chemical shifts of all eight \( ^{31}\text{P} \) atoms are...
Figure 3. A two-dimensional $^{31}$P/$^1$H heteroTOCSY spectrum of d9 at 15°C. The interresidues H3′(i) to P(i) cross peaks for C1–C8 and the intraresidue H3′(i) to P(i) cross peak for C9 are labeled. All eight phosphorus resonances have cross peaks to both inter- and intraresidue 3′ protons. The solid line is the construct following the sequential $^{31}$P to H3′ connectivities through the sequence.

The surface of XPA-MBD that interacts with DNA can be mapped by following perturbations to the 15N/$^1$H HSQC titration experiments where it was observed that the average chemical shifts of the 15N and $^1$H resonances for the amide residues at, or near, the DNA/XPA-MBD interface were still increasing at a d9:XPA-MBD molar ratio of 2.5. The explanation for such an observation is likely to be the same one proposed to explain the 15N/$^1$H titration data (Fig. 2); DNA-binding to XPA-MBD is weak (16,18,35) and the addition of excess DNA or protein helps push the equilibrium towards complex formation.

Figure 4 shows that the line widths of interior $^{31}$P resonances (A3 to C7) broaden before and to an extent greater than the terminal $^{31}$P resonances (C2, C8 and C9). This was also observed in heteroCOSY spectra of d9 collected at successively smaller d9:XPA-MBD molar ratios (data not shown). The A6 $^{31}$P heteroCOSY cross peaks disappeared first, followed by cross peaks for the T5 resonance and then the A4 and A7 resonances. At a d9:XPA-MBD molar ratio of 0.5 no heteroCOSY cross peaks were observed. Furthermore, although the data in Figure 4 were apodized similarly, the number of transients that needed to be collected in order to acquire a spectrum with similar signal-to-noise and line-shape features increased as the molar ratio of d9 to XPA-MBD decreased. The bottom-most phosphorus spectrum in Figure 4, collected in the absence of XPA-MBD, required 128 scans. At a DNA:XPA-MBD molar ratio of 0.44, 20 000 transients were required. The observation that all the $^{31}$P resonances became broader with the addition of XPA-MBD is most likely to be due to a shift in the equilibrium towards the complex in which the $^{31}$P resonance lines take on the correlation time (and broad lines) characteristic of the molecular weight of the complex. One consequence of a low DNA binding affinity is that even in the presence of excess protein, there is a population of free and bound d9. If the exchange rate between the free and bound forms of d9 is in the ‘intermediate’ regime on the NMR time scale, then the line widths of the $^{31}$P resonances will all
oligonucleotides are most sensitive to differences in the mobility of the interior backbone $^{31}$P resonances because the C2, C8 and C9 resonances appear to only get broader with each XPA-MBD addition. The shielding of at least one interior $^{31}$P resonance indicates that there must be some distortion of the structure in the shape of a cone. The XPA-MBD residues whose amide $^{15}$N and $^{1}$$H$ resonances were most perturbed by the addition of DNA are colored white.

Through the analysis of the $^{31}$P chemical shifts of many short dsDNA sequences a general trend has been observed; that the $^{31}$P chemical shifts of the phosphates at the end of the helix tend to be more deshielded than the $^{31}$P chemical shifts of phosphates in the interior of the helix (36,60,61). The latter observations have been interpreted in terms of two different types of B-DNA geometries, B1 and B2, with different torsional and bond angles at the phosphorus atom. The phosphates in the interior of the DNA double helix are primarily in the B1 (approximately –4.5 p.p.m.) conformation while those at the ends of the helix are a mixture of B1 and B2 (approximately –3.0 p.p.m.) due to greater terminal conformational freedom associated with ‘fraying’ (36,59). While d9 is a ss random-coil DNA oligomer, it is interesting to note that the two most deshielded $^{31}$P resonances are C2 and C9 phosphates at the 5′ and 3′ ends of d9, respectively.

**Molecular modeling: docking of d9 on to the DNA-binding surface of XPA-MBD**

The lysine and arginine side chains in the loop-rich subdomain of XPA form a nearly continuous line with an interresidue distance of 5–12 Å between adjacent positively charged head groups, a distance compatible with the 5–8 Å distance between phosphate groups on the backbone of helical DNA. To determine if electrostatic interactions between the positively charged surface of XPA with the negatively charged phosphodiester backbone of DNA is a practical mechanism to account for DNA-binding, d9 was modeled onto the surface of the NMR-derived solution structure of XPA-MBD. The maximal number of electrostatic contacts between the DNA and protein were allowed. To assess if there was any directionality to the DNA-binding, the DNA was modeled onto the surface of XPA-MBD in both the 3′→5′ and 5′→3′ directions. The results of the molecular modeling are displayed in Figures 5 and 6.

Figure 5A is a space-filled representation of the average solution structure determined for XPA-MBD (PDB code 1D4U) (29) and shows that XPA-MBD adopts an extended structure in the shape of a cone. The XPA-MBD residues whose amide $^{15}$N and $^{1}$$H$ resonances were most perturbed by the addition of DNA are colored white.
the addition of undamaged ssDNA (d9, Fig. 1), undamaged dsDNA and DNA containing the lesions dihydrothymidine and the 6-4-thymidine-cytidine photoproduct (29) are highlighted in blue with the affected, positively charged residues colored dark blue. Figure 5A shows that the blue colored residues are all clustered in the loop-rich subdomain of XPA-MBD at the top of the cone. Figure 5B is a space-filled rendition of the result of modeling d9 bound to XPA-MBD orientated in the 5′→3′ direction (using α-helix-3 of XPA-MBD as the reference point). The DNA, colored grey, fits nicely onto the exterior of XPA-MBD with the phosphodiester backbone near the surface of the protein. A similar arrangement of the protein and DNA is observed when the modeling is performed with the DNA orientation reversed to the 5′→3′ direction. In both instances a nine residue nucleotide sequence appears to fully cover the surface of XPA-MBD.

Figure 5C is a MOLSCRIPT (62) rendition of Figure 5B that illustrates how the elements of secondary structure are organized in relation to the DNA. The DNA essentially sits on top of the triple-strand β-sheet and α-helix-1 in the loop-rich subdomain. There is likely to be no major change in the relative orientation of the units of secondary structure upon DNA-binding because, as reported previously, the NOE patterns observed in the three dimensional 1H-15N-edited NOESY-HSQC spectra of XPA-MBD and XPA-MBD bound to d9 and other DNAs were identical (29). Ikegami et al. (30) recognized that the loop-rich subdomain of XPA-MBD contained a hydrophobic pocket in addition to the basic cleft on the surface of the protein. The side chains of the residues that form the hydrophobic pocket are shown in yellow. In both models, K167 is too distant from the DNA to make contact with the phosphodiester backbone of the DNA. Note that K168 was chosen over K167 in the modeling because (i) the chemical shifts of the K168 backbone amide resonances are effected more by the addition of DNA (Fig. 1) and (ii) K168 lined up better with the other basic residues. A few differences were observed in the results of the docking calculations with the starting DNA orientated in different directions relative to α-helix-3. Most noticeably, one fewer salt bridge (11 versus 10) can form between the positively charged basic XPA side chains and the negatively charged DNA phosphodiester backbone with the DNA orientated in the 5′→3′ direction. Salt bridges may provide up to 15 kcal/mol of stabilization energy per salt bridge (64) and, as illustrated in Figure 6, K141 is too distant from the DNA to form such a salt bridge when the DNA is orientated 5′→3′. In addition to salt bridges, DNA–protein associations may be stabilized by 2–10 kcal/mol per hydrogen bond (65). Although not illustrated in Figure 6, 17 hydrogen bonds can form between the phosphodiester backbone of DNA and side chain residues of XPA-MBD in the model with the DNA positioned in the 3′→5′ direction. On the other hand, only nine hydrogen bonds can form with the DNA positioned in the 5′→3′ direction. Docking calculations performed in the absence of restraints on the conformation of the DNA also proposed that DNA-binding to XPA might be stabilized by hydrophobic interactions between the bases of ssDNA (damaged or undamaged bases) with the hydrophobic pocket in the loop-rich subdomain (30,63). Figure 5D shows that the bases on the 3′ end of d9 are in a position to potentially interact with the basic cleft. However, no NOEs between the DNA bases of d9 (and other DNA oligomers with and without lesions) and the side chain residues of XPA have been observed experimentally, and consequently, no hydrophobic restraints were introduced into the modeling calculations. Note that the reason(s) such experiments were unsuccessful may be due to the use of non-ideal DNA sequences and/or because DNA-binding to XPA is weak.

Figure 6 is a more detailed illustration of the interactions at the DNA–protein interface as observed by modeling the DNA onto the surface of XPA-MBD in the 5′→5′ and 5′→3′ directions. The positively charged head groups of XPA-MBD side chains are illustrated as blue spheres and the DNA bases nearest the hydrophobic pocket are shown in yellow. In both models, K168 was chosen over K167 in the modeling because (i) the chemical shifts of the K168 backbone amide resonances are affected more by the addition of DNA (Fig. 1) and (ii) K168 lined up better with the other basic residues. A few differences were observed in the results of the docking calculations with the starting DNA orientated in different directions relative to α-helix-3. Most noticeably, one fewer salt bridge (11 versus 10) can form between the positively charged basic XPA side chains and the negatively charged DNA phosphodiester backbone with the DNA orientated in the 5′→3′ direction. Salt bridges may provide up to 15 kcal/mol of stabilization energy per salt bridge (64) and, as illustrated in Figure 6, K141 is too distant from the DNA to form such a salt bridge when the DNA is orientated 5′→3′. In addition to salt bridges, DNA–protein associations may be stabilized by 2–10 kcal/mol per hydrogen bond (65). Although not illustrated in Figure 6, 17 hydrogen bonds can form between the phosphodiester backbone of DNA and side chain residues of XPA-MBD in the model with the DNA positioned in the 3′→5′ direction. On the other hand, only nine hydrogen bonds can form with the DNA positioned in the 5′→3′ direction. Docking calculations performed in the absence of restraints on the conformation of the DNA also proposed that DNA-binding to XPA might be stabilized by hydrophobic interactions between the bases of ssDNA (damaged or undamaged bases) with the hydrophobic pocket in the loop-rich subdomain (30,63). Figure 5D shows that the bases on the 3′ end of d9 are in a position to potentially interact with the basic cleft. However, no NOEs between the DNA bases of d9 (and other DNA oligomers with and without lesions) and the side chain residues of XPA have been observed experimentally, and consequently, no hydrophobic restraints were introduced into the modeling calculations. Note that the reason(s) such experiments were unsuccessful may be due to the use of non-ideal DNA sequences and/or because DNA-binding to XPA is weak.

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**Figure 6.** Expansions of the interfacial region between d9 and XPA-MBD. The DNA is orientated in the 5′→5′ (left) and 5′→3′ (right) direction relative to α-helix-3. The positively charged head groups of XPA-MBD side chains of amide H′P and 15N chemical shifts that were perturbed by the addition of DNA are illustrated as blue spheres. The DNA is illustrated with a ball and chain diagram and bases near the hydrophobic pocket (Fig. 5D) are colored yellow.

**Table 1.** Proton chemical shift assignments for dCCAATAACC at 15°C in D2O determined at a 500 MHz 1H frequency

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<th>Base</th>
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<th>H1′</th>
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<th>H3′</th>
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showed the 3′→5′ orientation to be energetically more favorable; nine versus eight salt bridges and 19 versus 15 hydrogen bonds. In all models seven of the eight phosphate groups of d9 participate in salt bridge formation and all eight phosphate groups participate in hydrogen bond formation with XPA-MBD.

It has been suggested that hydrophobic contacts between the DNA and XPA also contribute stabilizing energy to the protein–DNA complex (30,63,66). If hydrophobic interactions do contribute to stabilize the DNA–protein complex, then the DNA bases are in a better position to interact with the hydrophobic core of the loop-rich subdomain in the 3′→5′ orientation than in the 5′→3′ orientation. As illustrated in Figure 6, the DNA bases are exposed away from the hydrophobic core when modeled onto XPA in the 5′→3′ direction. Note that such differences largely remain if helical restraints on the confirmation of d9 are removed during the docking calculations.

At d9:XPA-MBD molar ratios <1.0, one of the interior 31P resonances of d9 was observed to become more shielded (Fig. 4) suggesting that the phosphodiester backbone of the DNA was being distorted when bound to XPA-MBD. Such a change in the phosphorus chemical shift is usually due to changes in the torsional angles (α, β, ε and ζ) and the O–P–O bond angle (36). Measurement of these angles in the model structures for d9 bound to XPA-MBD indicate that many of these angles do change substantially in all the calculated models (data not shown).

CONCLUSION

Although XPA has been shown to be essential to NER, its precise role in DNA damage recognition and/or verification is still unclear. Wakasugi and Sancar (18) propose that XPA and RPA verify the damage during the formation of a preincision complex. While the precise role of XPA in NER remains unresolved, it is clear that XPA interacts directly with DNA either during DNA damage recognition or the formation of the preincision complex. In the preincision complex, XPA is most likely to direct the positioning of the 5′-endonuclease ERCCI–XPF heterodimer (19). The data presented here provide both direct evidence that XPA binds DNA and Insight into the basis of the interaction. The 1H/1H HSQC and the 31P NMR titration data follow DNA-binding from the vantage points of the protein and the DNA, respectively. Both experiments showed that XPA-MBD binds d9 and the latter experiment showed that XPA-MBD binds d9 in a way that perturbs the phosphodiester backbone of DNA. At concentrations between 0.5 and 2.0 mM, the NMR spectra of both ligands, d9 and XPA-MBD, continued to change when one ligand was in >2-fold molar excess, indicating that DNA-binding to XPA-MBD is in the millimolar range. 31P resonances in the interior of d9 broadened and/or shifted before 31P resonances at the termini, suggesting that when d9 is bound to XPA-MBD the motion of the DNA is more restricted in the interior of the sequence than at the termini. The shielding of one of the interior 31P resonances of d9 indicates that the phosphodiester backbone became distorted upon association with XPA-MBD. Molecular modeling of the complex between d9 and XPA-MBD showed that a nine residue oligomer fully covers the DNA-binding surface of XPA-MBD. DNA-binding is possible through electrostatic interactions between seven negatively charged phosphate groups on the backbone of d9 and positively charged side chains of residues in the loop-rich subdomain of XPA. Regardless of the restraints placed on the DNA, modeling calculations suggest that there may be an energetic advantage to binding in the 3′→5′ direction rather than the 5′→3′ direction (relative to XPA-MBD α-helix-3). With or without restrictions on the conformations of the DNA, the potential for hydrophobic interactions between the hydrophobic core of the loop-rich subdomain of XPA and the DNA bases is greatest when the d9 is modeled in the 3′→5′ orientation.

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