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Xeroderma pigmentosum complementation group C protein (XPC) serves as a general sensor of damaged DNA

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Abstract

The *xeroderma pigmentosum* complementation group C protein (XPC) serves as the primary initiating factor in the global genome nucleotide excision repair pathway (GG-NER). Recent reports suggest XPC also stimulates repair of oxidative lesions by base excision repair. However, whether XPC distinguishes among various types of DNA lesions remains unclear. Although the DNA binding properties of XPC have been studied by several groups, there is a lack of consensus over whether XPC discriminates between DNA damaged by lesions associated with NER activity versus those that are not. In this study we report a high-throughput fluorescence anisotropy assay used to measure the DNA binding affinity of XPC for a panel of DNA substrates containing a range of chemical lesions in a common sequence. Our results demonstrate that while XPC displays a preference for binding damaged DNA, the identity of the lesion has little effect on the binding affinity of XPC. Moreover, XPC was equally capable of binding to DNA substrates containing lesions not repaired by GG-NER. Our results support an indirect read-out model for sensing the presence of lesions by human XPC and suggest XPC may act as a general sensor of damaged DNA capable of recognizing DNA containing lesions not repaired by NER.

Keywords

XPC; Nucleotide excision repair; base excision repair; lesion recognition; DNA binding; high-throughput assay

1. Introduction

Exposure to chemical carcinogens and environmental radiation results in DNA damage that, left unresolved, threatens genomic integrity. In order to guard against genetic instability cells have developed an arsenal of biochemical pathways to identify and repair damage as

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well as coordinate cellular functions such as gene transcription, DNA replication, and cell cycle progression. Critical to the function of these biochemical pathways is the ability to identify the presence of many different types of DNA damage against a high background of unmodified, nearly isomorphous nucleotides (reviewed in [1, 2]).

Nucleotide excision repair (NER) is a versatile repair pathway that removes bulky covalent lesions from DNA. The hallmark of NER is the ability to repair a wide variety of chemically distinct DNA lesions without the need for lesion-specific damage recognition factors [3]. Mutations in any of the seven genes encoding for the NER proteins in humans results in *Xeroderma pigmentosum* (XP), a spectrum of clinical disorders resulting in predisposition to the development of skin cancer, neurodegeneration, and accelerated aging [4]. NER occurs in four steps requiring the assembly and remodeling of protein-protein and protein-DNA complexes [5]. NER is initiated when the presence of a lesion is sensed in a specific DNA locus. The surrounding dsDNA is then unwound and NER proteins assemble on the NER bubble. Dual incision of the damaged strand by nucleases releases an oligonucleotide containing the lesion, and gap filling by a replicative DNA polymerase fills the resulting gap using the undamaged strand as a template, thereby restoring the original DNA sequence [5, 6].

NER is divided into two sub-pathways, transcription coupled NER (TC-NER) and global genome NER (GG-NER) (reviewed in [7]). TC-NER is initiated upon stalling of RNA pol II after encountering a DNA lesion and is therefore limited to actively transcribed DNA strands [8]. In contrast, GG-NER removes lesions throughout the entire genome irrespective of the transcriptional activity of the damage locus. XPC, a 940-residue DNA binding protein, is the primary GG-NER initiating protein in humans [9, 10]. In cells, XPC forms a complex with the HR23B [11, 12] and Centrin2 proteins [13], and XPC-HR23B has previously been shown to be necessary and sufficient to support NER activity *in vitro* [14–16]. Previous experiments have shown that XPC-HR23B binds both single stranded (ss) and double stranded (ds) DNA with affinity in the nanomolar range [17, 18], and appears to display a slight preference for binding dsDNA containing bulky nucleotide modifications [15]. Initial recognition of damage repaired by GG-NER is believed to occur via XPC sensing destabilization of dsDNA that is induced by the presence of the lesion [19–21]. The crystal structure of the *Saccharomyces cerevisiae* homolog, Rad4, bound to a duplex DNA substrate containing a cyclobutane pyrimidine dimer (CPD) has been determined and suggests an indirect read-out model for DNA damage sensing. In the crystal structure Rad4 makes extensive contact to the unmodified nucleotides opposite the lesion but has no direct contact with the CPD lesion itself, which was disordered and not observed in the structure [20].

In the current model of GG-NER, XPC continuously scans the genome for damage by transiently binding the DNA [9]. Binding to a damage site results in immobilization of XPC and signals recruitment of the basal transcription factor TFIIH, which unwinds the duplex surrounding the lesion to initiate the NER cascade [6, 22–24]. Although XPC is primarily active in the GG-NER pathway, recent reports suggest that it may also play a role in the base excision repair (BER) pathway (reviewed in [25]). Cells from XP-C patients display increased sensitivity to oxidative stress and reduced rates of repair by BER [26]. Additionally, XPC has been found to physically interact with several DNA glycosylases, including OGG1 and SMUG1 [27, 28].

Although the DNA binding activity of XPC has been the subject of multiple studies, there is a lack of consensus regarding the lesion discrimination properties of XPC. XPC-HR23B is difficult to produce and poor yields of recombinant protein have limited investigations to one or a small number of lesion-containing DNA substrates [15, 17, 29]. Moreover,

experimental conditions vary considerably among previous studies limiting the ability to directly compare XPC-HR23B binding affinities determined for DNA substrates containing various types of lesions [15, 17, 18, 21, 30]. To address these uncertainties, we have performed a systematic study of human XPC-HR23B binding to different DNA substrates with a range of different lesions typically associated with DNA excision repair pathways. To minimize the utilization of the protein and maximize accuracy and reproducibility we developed a high-throughput fluorescence anisotropy assay to rapidly measure the DNA binding affinity of XPC-HR23B. Our results suggest that XPC-HR23B serves as a general sensor of DNA helix instability, and that it may play a role in sensing the presence of DNA lesions in DNA repair pathways other than NER.

2. Materials and Methods

2.1 Chemical Synthesis and Construction of Damaged DNA Substrates

DNA substrate sequences are shown in Figure 1. Unmodified oligonucleotides and 3 - fluorescein tagged oligonucleotides were purchased from Sigma. Chemically modified 12-mer oligonucleotides ($5'$ GCTAGCG*AGTCC $3'$) were either purchased or synthesized. 8-oxo-deoxyguanosine (8-oxo-dG) modified oligonucleotide was purchased from Midland Certified Reagent Co., tetrahydrofuran (THF) modified oligonucleotide was purchased from IDT. 12-mer oligonucleotides containing the 1, N^2 - -guanine [31], 7-(2-oxoheptyl)- -guanine [32], 8-hydroxy-1, N^2 - -guanine [33], 1, N^2 -propano-guanine [34], M₁G [34], and methyl-formamidopyrimidine (MeFapy) [35], and modified 11-mer oligonucleotide ($5'$ GGCAGA*TGGTG $3'$) containing the 7,12-dimethylbenz(a)anthracene-adenine (DMBA) [36] lesions were available from previous studies. 60 base pair (bp) and 42 bp substrates were constructed by ligation as described previously [37, 38] with the exception that fluorescein was incorporated on the damaged strand which allowed use of fluorescence instead of 32 P radioactivity as the probe. 36-mers were synthesized using a previously published sequence [17]. Duplexes were prepared by annealing equimolar amounts of complementary oligonucleotides in TNE buffer (10 mM Tris, pH 7.5, 100 mM NaCl, 1 mM EDTA), resolved by 8% native PAGE, and eluted in TE buffer (10 mM Tris, pH 7.5, 1 mM EDTA).

2.2 Expression of XPC-HR23B Complex

Full-length XPC and HR23B were amplified by PCR using indicated oligonucleotides (Supplemental Table S1) that incorporated a tobacco etch virus protease (TEV) cleavable N-terminal 6xHis tag in XPC, and a human rhinovirus 3C protease (HRV3C) cleavable N-terminal 6xHis tag in HR23B, and sub-cloned into the two multiple cloning sites in pFastBac Dual vector, respectively. The pFastBacDual/XPC-HR23B plasmid was verified by sequencing and transformed to *E. coli* DH10Bac to generate recombinant bacmid and subsequent baculoviruses in Sf9 insect cells. Suspension cultures of Sf9 cells were infected with a high-titer baculovirus encoding XPC and HR23B at a multiplicity of infection of 2, and cells were harvested 70 hours post infection.

2.3 Purification of XPC-HR23B Complex

Sf9 insect cells were lysed in ice-cold Buffer T1 (50 mM Tris pH 8.0, 0.5 M NaCl, 10% glycerol, 5 mM Imidazole, 1× Roche Protease Inhibitor cocktail, 1 mM PMSF), 6xHis-tagged proteins isolated by TALON chromatography and eluted in Buffer T1 containing 0.3 M Imidazole. Pooled fractions were diluted with Buffer D1 (50 mM Tris, pH 8.0, 10% glycerol) to 0.3 M NaCl and incubated with a combination of HRV3C and TEV proteases overnight at 4 °C. Cleaved XPC-HR23B complex was then immobilized on a 5 mL HiTrap Heparin column (GE Healthsciences) equilibrated in buffer H1 (50 mM Tris, pH 8.0, 0.3 M NaCl, 10% glycerol) and eluted using a linear gradient of buffer H2 (50 mM Tris, pH 8.0,

1.0 M NaCl, 10% glycerol). Heparin-purified XPC-HR23B was re-passed over a TALON column to remove any residual protease or 6×His tag contaminants. Pooled protein was then resolved on an HR10/30 S200 size exclusion column (GE Healthsciences) and dialyzed against buffer S1 (50 mM Tris pH 8.0, 0.5 M NaCl, 50% glycerol, 1 mM EDTA, 1 mM DTT) overnight at 4°C. Protein concentration was determined by Bradford assay using the Bio-Rad Protein Assay Dye Reagent and confirmed by measuring UV absorbance at $\lambda=280$ nm. Dialyzed protein was divided into small aliquots, flash-frozen in liquid nitrogen, and stored at -80°C .

2.3 High-throughput DNA Binding Assay

XPC-HR23B and DNA substrates were diluted in binding buffer (25 mM HEPES, pH 7.8, 0.2 M KCl, 5% glycerol, 1 mM DTT). A Bravo automated liquid handling robot (Agilent Technologies) was used to perform a three-fifths serial dilution of XPC-HR23B in Corning #3676 low-volume 384-well microtiter plates and subsequently add the DNA substrates to each well. The plate was covered from light and incubated at room temperature with gentle agitation for 5 minutes prior to measuring fluorescence anisotropy to ensure samples were homogenized and had reached equilibrium. Fluorescence anisotropy was measured using an EnVision 2100 plate reader (PerkinElmer), plate reader settings were optimized to maximize sensitivity and allow the lowest possible concentration of fluorescein probe (2 nM). Binding measurements were performed in triplicate for each DNA substrate. Apparent dissociation constants (K_d) were determined for each individual titration by plotting fluorescence anisotropy against protein concentration and fitting to a simple two-state binding model using KaleidaGraph (v4.03). Percent active fraction of XPC-HR23B was determined by stoichiometric titration of the high-affinity bubble substrates (25 nM) as described previously [39]. Percent activity was defined as $([\text{DNA}]/[\text{XPC}_{\text{eq}}]) \times 100$, with $[\text{XPC}_{\text{eq}}]$ determined from the intersection of linear regression fits to the pre-saturation and saturation data points.

3. Results

3.1 Optimization of the High-throughput DNA Binding Assay for XPC-HR23B

In order to test the DNA binding properties of XPC under constant conditions, we developed a microtiter plate based high-throughput fluorescence anisotropy protocol to measure DNA binding activity using DNA substrates 3'-end labeled with fluorescein. An automated liquid handling robot was utilized to accurately and reproducibly prepare samples in 384-well microtiter plates. The full length XPC utilized for these experiments was co-expressed and purified with HR23B in Sf9 insect cells (Figure 2A). Our typical yield of protein was ~0.5–0.75 milligrams of pure XPC-HR23B per liter of insect cell culture. Stoichiometric titration of the high-affinity 36 bp bubble substrate (Figure 1) confirmed a 1:1 complex and revealed the preparation used for all of the DNA binding assays was ~80% active (Figure 2B). Our microtiter plate based method required 65 μg XPC-HR23B per 384-well plate, which allowed all DNA binding experiments to be performed using a single preparation of purified XPC-HR23B.

We performed extensive optimization of the experimental conditions in order to ensure the most accurate measurements of XPC-HR23B DNA binding activity. Preparations of XPC-HR23B were extremely sensitive to the ionic strength of the buffer system and solubility of purified protein was significantly enhanced in buffers containing NaCl in excess of 0.5 M. Therefore the DNA binding buffer reported by Trego *et al.* was chosen as the initial experimental condition for optimization as it was the higher ionic strength of the two buffers previously reported for fluorescence anisotropy experiments with human XPC-HR23B [17, 18]. Given the small volume of the reactions, we first optimized glycerol concentration to

reduce buffer viscosity that can influence anisotropy measurements. NaCl was then compared to KCl. Finally, the effects of buffer additives (EDTA, DTT, MgCl₂NP-40) were tested.

DNA binding was initially measured and the experimental conditions optimized using two 36 bp DNA substrates (Figure 1) for which XPC-HR23B binding activity has been previously reported [17]. We monitored three parameters during optimization, reproducibility of the anisotropy measurements well-to-well, plate-to-plate reproducibility, and agreement with the apparent dissociation constants (K_d) previously determined for XPC-HR23B binding to the 36 bp substrates. Note that fluorescence intensity remained constant as protein concentration increased over the course of the titration indicating XPC-HR23B did not interact with the fluorescein probe. Glycerol has a very favorable effect on solubility and stability of XPC-HR23B, and we found that reducing the glycerol concentration below 5% resulted in loss of binding. Assays of 0.1–0.3 M KCl versus NaCl revealed both were effective in the range 0.15–0.25 M, but experiments performed in 0.2 M KCl had lowest well-to-well variability. Addition of DTT (1 mM) was also found to reduce variability in well-to-well measurements whereas neither EDTA (1–5 mM) nor MgCl₂ (5 mM) had any effect. In contrast, addition of NP-40 (0.05%) led to an increase in well-to-well variability. This may be due to problems with the liquid handling system pipetting small quantities of the detergent. The final optimized buffer conditions included 25 mM HEPES at pH 7.8, 0.2 M KCl, 5% glycerol, and 1 mM DTT.

XPC-HR23B bound tightly to both duplex DNA and a duplex containing a six-nucleotide mismatch with K_d values of 51 ± 1.7 nM and 5.6 ± 1.3 nM, respectively (Figure 2C). Our observed 9-fold increase in binding affinity for bubble versus duplex DNA is in excellent agreement with the reported literature value and demonstrates the robustness of our microtiter plate based protocol to measure differences in DNA binding affinity for XPC-HR23B. While we measured the same change in affinity between duplex and mismatch DNA substrates, the absolute K_d values differed from those reported by Hey *et al.* (10 ± 3 nM and 1.1 ± 0.3 nM, respectively) [17]. This observation further highlights the sensitivity of XPC-HR23B to experimental conditions and the necessity to measure DNA binding affinity under constant conditions.

3.2 Effects of DNA Length on XPC-HR23B Affinity for Bubble DNA Substrates

Although previous studies indicate full-length XPC-HR23B occupies a DNA footprint of ~40 nucleotides [10] it has been reported DNA substrates shorter than 60 bp may reduce the DNA damage specific binding activity of XPC [18, 40]. We first tested the effects of DNA length on the binding affinity of XPC-HR23B for a series of unmodified duplex and bubble DNA substrates (Figure 1). Figure 3 shows DNA binding curves for XPC-HR23B binding to either duplex (A) or bubble (B) substrates. XPC-HR23B bound 36 bp, 42 bp, and 60 bp duplex DNA with the same affinity ($K_d = 51 \pm 1.7$ nM, 56 ± 8.7 nM, and 48 ± 5.8 nM, respectively). XPC-HR23B formed 1:1 complexes with both 36 bp and 42 bp substrates containing 6 nucleotide mismatch bubbles, and bound the 36 bp substrate with 1.7 fold higher affinity than the 42 bp substrate ($K_d = 5.6 \pm 1.3$ nM and 9.3 ± 1.6 nM, respectively). However, we found XPC-HR23B formed a 2:1 complex when bound to the 60 bp substrate containing a 6 nucleotide bubble. Therefore a 60 bp substrate with a 2 nucleotide bubble was tested since the Rad4 crystal structure shows only 2 bases are displaced from the duplex stack [20]. Reducing the size of the mismatch resulted in a 1:1 complex with a K_d of 13 ± 2.0 nM. Together, these results indicate that the length of the DNA substrate has limited effect on the ability of XPC-HR23B to bind to bubble DNA substrates.

3.3 Affinity of XPC-HR23B for Chemically Modified DNA Substrates

In order to assay the ability of human XPC-HR23B to discriminate between different types of DNA lesions, a series of 60 bp DNA substrates were constructed with chemically modified bases. Eight DNA lesions (Table 1), including 5 repaired by NER and 3 repaired by BER, were initially selected for analysis and incorporated into a common duplex sequence. A 59 bp substrate containing the DMBA lesion was also tested. DMBA, a polycyclic aromatic hydrocarbon adduct, does not significantly disrupt the duplex structure [41] and is repaired by TC-NER but not GG-NER [42, 43]. Figure 4A shows representative binding curves for three 60 bp DNA substrates with different excision repair activities: - OH-propano-dG (NER), 8-oxo-dG (BER), and DMBA-dA (TC-NER only). Individual DNA binding curves for all 60 bp DNA substrates tested are shown in supplemental data (S1).

XPC-HR23B bound tightly to all DNA substrates presented, including unmodified duplex DNA with apparent K_d values in the low nanomolar range (Table 1). XPC-HR23B bound tightest to DNA containing a THF lesion that mimics the presence of an abasic site (11 ± 2.1 nM), which is repaired by the BER pathway. Conversely, XPC bound DNA containing the DMBA lesion with the same affinity as unmodified DNA (47 ± 2.8 nM and 48 ± 5.8 nM, respectively), consistent with the observation that this lesion does not significantly disrupt dsDNA [41]. In contrast to our results for binding to the highly distorted bubble DNA, we observed only 2–4 fold differences in affinity of XPC-HR23B for the various damaged duplex DNA substrates (Table 1). Interestingly, XPC-HR23B bound to the BER substrates 8-oxo-dG [44] and methyl-FAPY [45] with the same affinity as bona fide NER targets such as propano-dG and M₁dG [46–48].

In order to further investigate the length dependence of the DNA substrate on the specificity of XPC-HR23B, a series of 42 bp damaged DNA substrates was generated. Representative binding curves are shown in Figure 4B for the same three substrates shown in Figure 4A. Individual DNA binding curves for all 42 bp DNA substrates are shown in supplemental data S2). XPC-HR23B bound to the chemically modified 42 bp DNA substrates with nearly identical affinity as to the 60 base pair substrates containing the same lesions (Table 1), consistent with the binding measurements for bubble DNA substrates. Therefore the length of the DNA substrate tested has no significant effect on the binding affinity of XPC-HR23B for chemically modified duplex DNA, counter to the previous proposal of a requirement for 60 bp of DNA to observe DNA damage specificity [18, 40].

4. Discussion

Although XPC is currently accepted as the primary protein responsible for initiating GG-NER, the concept of hierarchal lesion recognition by XPC remains controversial. Problems associated with producing recombinant protein, use of different techniques and experimental conditions to measure DNA binding affinity, and highly variable DNA substrate sequence has led to confusion regarding the absolute affinity of XPC for DNA substrates containing various types of DNA lesions. These uncertainties have been addressed by our systematic study of XPC binding undamaged and damaged DNA using a library of chemically unrelated lesions in a common DNA sequence under constant experimental conditions. The use of a high-throughput approach that minimized the amount of purified protein required per titration and the time required for each titration enabled testing of each group of DNA substrates against a single preparation of XPC-HR23B protein.

XPC-HR23B displayed a 2–4 fold increase in affinity for chemically modified dsDNA relative to unmodified duplex. However, neither the length of the DNA substrate nor the type of DNA lesion present influenced the affinity of XPC-HR23B for the damaged substrates. This suggests that XPC will bind tightly to a wide range of damaged DNA,

consistent with its role in GG-NER. XPC-HR23B bound DNA substrates containing well-established BER lesions with the same approximate affinity as substrates containing lesions specifically repaired by NER. These results suggest that although XPC-HR23B binding to damaged DNA is necessary to initiate GG-NER it does not determine the eventual repair fate of the lesion, highlighting the critical role of downstream repair proteins in the damage recognition process.

In the co-crystal structure of the *S. cerevisiae* Rad4 homolog of XPC bound to a DNA substrate containing a CPD lesion [20], Rad4 makes contacts with the dsDNA downstream of the lesion and the two extra-helical undamaged bases opposite the damaged nucleotides. However, it makes no contact with the lesion itself. This model suggests Rad4 (and XPC) only senses the localized helix destabilization induced by the presence of the lesion. Our data shows XPC binds substrates containing 2 or 6 nucleotide bubbles with the same affinity, consistent with the unstacking of only two base pairs upon insertion of the γ -hairpin between the DNA strands observed in the crystal structure. Moreover, the lack of contact between the protein and DNA lesion in the Rad4 structure is consistent with our observation that the chemical identity of the DNA lesion does not impact the equilibrium DNA binding affinity of XPC. Taken together, these results support the model in which helix destabilization induced by the lesion is the determining factor in DNA binding affinity, not the identity of lesion.

The similarity of XPC DNA binding affinity for lesions repaired by different pathways suggests a generic sensing mechanism to detect aberrant DNA structure. In this model a lesion need only destabilize the duplex sufficiently to facilitate insertion of the γ -hairpin into the helix and recruitment of downstream repair proteins [20, 49]. Such a model is supported by pre-steady state binding experiments, which demonstrated that the increased equilibrium binding affinity of human XPC-HR23B for cisplatin damaged dsDNA is primarily the result of an increased rate of association with the substrate [18].

Our DNA binding data illustrate that XPC is equally capable of sensing the presence of smaller oxidative lesions as well as bulky covalent modifications in duplex DNA. These results support a growing body of evidence suggesting a role for XPC in BER [25–28, 50]. XPC^{-/-} cells show reduced rates of BER [26] and XPC stimulates the activity of several glycosylases in cells, including OGG1 and SMUG1 [27, 28]. Recently it has been shown that XPC accumulates at sites of locally induced oxidative DNA damage in live cells yet does not establish a GG-NER response [50]. Stimulation of BER by XPC could possibly be the result of two phenomena. First, recruitment of DNA glycosylases to potential DNA damage sites by direct interaction with XPC could significantly reduce the amount of scanning required for glycosylases to identify isolated lesions [51]. Second, binding of XPC to the DNA substrate leads to extrusion of the damaged nucleotide out of the helix and could facilitate the base-flipping step in the BER damage recognition process (reviewed in [52]).

5. Conclusions

Based on the available biochemical and cellular data, we propose XPC may serve as a general sensor of DNA duplex instability and as an adapter molecule capable of initiating multiple repair pathways. XPC-HR23B constantly scans the genome for aberrations utilizing its strong dsDNA binding affinity to transiently associate with chromatin. In the current model based on the structure of Rad4-Rad23B, upon encountering a site containing a destabilized base pair(s), XPC inserts a γ -hairpin between the strands forming a complex that is longer lived than that formed in the scanning mode. Formation of this complex serves as a signal for recruitment of downstream repair proteins such as TFIIH and XPA (GG-NER) and/or DNA glycosylases (BER) via protein-protein interactions with XPC. The

potential damage site is then further interrogated to validate it as a target for a specific repair pathway. If lesion validation fails, the nascent repair complex dissociates and the process begins anew.

We previously reported the XPC transglutaminase-like domain (TGD) physically interacts with XPA [29]. Interestingly, the XP-associated mutation P334H in the TGD has similar effects on co-localization of XPC with OGG1 and XPA in cells [27], suggesting that these two proteins may share a common binding interface on the TGD. Although the initial recruitment of repair proteins by XPC is most likely stochastic, the use of the TGD as a common protein interaction platform by both XPA and the DNA glycosylases suggests that competitive crosstalk between NER and BER could occur at the lesion validation step. It should be noted that this model does not preclude XPC activity in other DNA repair processes, such as mismatch repair or repair of DNA strand breaks. However, although XPC binds tightly to DNA mismatches and ssDNA overhangs *in vitro* it has yet to be demonstrated that XPC participates in the repair of these types of DNA damage in cells. Future studies to fully elucidate the physical and functional interactions of XPC should clarify its role(s) in initiating and possibly integrating the repair of damaged DNA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- High-throughput fluorescence anisotropy assay used to measure affinity of human XPC for damaged DNA
- Binding affinity tested for eight DNA lesions contained in a common DNA sequence
- XPC binds all damaged DNA with essentially the same affinity
- XPC efficiently recognizes oxidative lesions repaired by BER pathway
- XPC may coordinate recognition of damaged DNA for multiple repair pathways

Figure 1. XPC DNA Binding Substrates

Chemically modified oligonucleotide sequence indicated by underline, triangles indicate the modified base in damaged DNA substrates.

Figure 2. Purification of full-length XPC-HR23B

(A) Full-length XPC-HR23B complex purified from Sf9 insect cells. (B) Stoichiometric titration of 3' fluorescein-labeled 36 bp bubble substrate (25 nM) with XPC-HR23B. The equivalence point is derived from the crossing point of the two solid linear regression fit lines (30.7 nM XPC-HR23B, dashed line). (C) Plots of fluorescence anisotropy versus added protein for 36 bp DNA substrates. Fluorescence intensity remained constant throughout the titration. Each data point represents the mean of three titrations, error bars represent the standard deviation.

Figure 3. Effects of DNA length on XPC binding affinities

Plots of normalized fluorescence anisotropy versus protein added for XPC binding to duplex (A) or bubble (B) substrates. Bubbles were generated by nucleotide mismatches of either 2 nucleotides (60 bp) or 6 nucleotides (36 bp, 42 bp). Fluorescence intensity remained constant throughout the titration. Each data point represents the mean of three titrations, error bars represent the standard deviation.

Figure 4. Systematic analysis of XPC DNA substrate binding affinities

Plots of fluorescence anisotropy versus added protein for 60 bp (A) and 42 bp (B) duplexes containing single nucleotide chemical modifications. Lesions represent different excision repair profiles, BER (8-oxo-dG), NER (-OH-PdG), and TC-NER only (DMBA).

Fluorescence intensity remained constant throughout the titration. Each data point represents the mean of three titrations, error bars represent the standard deviation.

Table 1
Dissociation constants for XPC-HR23B binding to DNA substrates

Dissociation constants (K_d) were determined by fitting fluorescence anisotropy data (e.g. Figure 3) to a simple two-state binding model.

Substrate	Repair	$K_d(\text{nM})^1$		
		60 bp ²	42 bp ²	36 bp
Duplex	-	48 ± 5.8	56 ± 8.7	51 ± 1.7
Bubble ³	-	13 ± 2.0	9.3 ± 1.6	5.6 ± 1.3
7-(2-oxoheptyl)- dG	NER	25 ± 4.3	31 ± 5.4	
1, N ² - dG	NER	38 ± 7.9	41 ± 4.0	
Propano-dG	NER	30 ± 7.0	33 ± 5.5	
-OH-Propano-dG	NER	27 ± 4.7	23 ± 2.5	
8-oxo-dG	BER	28 ± 5.1	27 ± 5.2	
THF (Abasic)	BER	11 ± 2.1	13 ± 1.0	
DMBA	NER ⁴	47 ± 2.8	40 ± 3.2	
M ₁ -dG	NER	26 ± 5.7		
Methyl-FAPY	BER	25 ± 4.3		

¹ K_d values represent the mean of at least six titrations per substrate.

² 59 bp and 41 bp for DMBA substrates.

³ Bubbles formed by either a 2 nucleotide (60 bp) or 6 nucleotide (36 bp, 42 bp) mismatch.

⁴ Repaired by transcription-coupled NER only.