ISSN 0006-2979, Biochemistry (Moscow), 2012, Vol. 77, No. 5, pp. 524-531. © Pleiades Publishing, Ltd., 2012.
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Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM12-041, April 22, 2012.

Interaction of Nucleotide Excision Repair Proteins with DNA Containing Bulky Lesion and Apurinic/Apyrimidinic Site

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Received February 24, 2012
Revision received March 13, 2012

Abstract—The interaction of nucleotide excision repair (NER) proteins (XPC–HR23b, RPA, and XPA) with 48-mer DNA duplexes containing the bulky lesion-mimicking fluorescein-substituted derivative of dUMP (5-{3-[6-(carboxyamidofluoresceinyl)amidocapromoyl]allyl}-2′-deoxyuridine-5′-monophosphate) in a cluster with a lesion of another type (apurinic/apyrimidinic (AP) site) has been studied. It is shown that XPC–HR23b is modified to a greater extent by the DNA duplex containing an AP site opposite nucleotide adjacent to the fluorescein residue than by DNA containing an AP site shifted to the 3′- or 5′-end of the DNA strand. The efficiency of XPA modification by DNA duplexes containing both AP site and fluorescein residue is higher than that by DNA lacking the bulky lesion; the modification pattern in this case depends on the AP site position. In accordance with its major function, RPA interacts more efficiently with single-stranded DNA than with DNA duplexes, including those bearing bulky lesions. The observed interaction between the proteins involved in nucleotide excision repair and DNA structures containing a bulky lesion processed by NER and the AP site repaired via base excision repair may be significant for both these repair pathways in cells and requires the specific sequence of repair of clustered DNA lesions.

DOI: 10.1134/S0006297912050136

Key words: protein factors of nucleotide excision repair, bulky lesion, apurinic/apyrimidinic site, affinity labeling, DNA–protein complexes

The action of negative factors on the human organism, such as the high levels of environmental pollution, ultraviolet and ionizing radiations, and toxic compounds, results in damage to genomic DNA. To prevent irreversible mutations, numerous lesions are eliminated by different DNA repair systems. Bulky chemical adducts formed with carcinogenic substances or some chemotherapeutic agents, as well as pyrimidine dimers arising under UV radiation, are eliminated by the pathway known as nucleotide excision repair (NER). Lesions of encoding bases arising upon DNA oxidation, alkylation, and deamination as well as DNA single-strand breaks and apurinic/apyrimidinic (AP) sites are repaired by the pathway of base excision repair (BER). Both processes have many stages and require coordinated actions of a great number of enzymes and protein factors [1, 2].

Clustered damages, when oxidized bases, AP sites, bulky lesions, and strand breaks are grouped within one-two DNA helical turns and can be located in both DNA strands, are most difficult for repairing. The AP sites formed as a result of spontaneous hydrolysis of the N-glycoside bond, as well as elimination of an incorrect or damaged base by DNA glycosylases, are among the most widespread DNA lesions: about 10,000 AP sites are formed daily under physiological conditions in mammalian cells [1, 3]. The presence of AP sites is very dangerous for cells, especially during DNA replication, because the loss of the coding base in the template strand can result in mutations, including deletions and reading frame shift [4]. In addition, AP sites are reactive and can form crosslinks (Schiff bases) with proteins [5, 6]. It was

Abbreviations: AP site, apurinic/apyrimidinic site; BER, base excision repair; Flu-dUMP, 5-{3-[6-(carboxyamido-fluoresceinyl)amidocapromoyl]allyl}-2′-deoxyuridine-5′-monophosphate; NER, nucleotide excision repair; RB, reaction buffer; RPA, replication protein A; XPA, xeroderma pigmentosum factor of complementation group A; XPC–HR23b, xeroderma pigmentosum factor of complementation group C in complex with homolog of yeast Rad23 protein.

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recently shown that nucleosomal AP sites are a source of DNA–protein crosslinks, DNA strand crosslinks, and DNA double-strand breaks [7]. It is known that bulky lesions aggravate the thermodynamic instability of DNA and cause distortions of the double helix [8, 9], which may facilitate the access of oxidizing agents to the damaged region or intensify spontaneous hydrolysis of the glycoside bond [10], thereby enhancing the probability of formation of AP sites close to the bulky lesion. The repair of such DNA requires precise regulation of the succession of repairing of separate lesions to avoid the formation of double-strand breaks, which are more toxic for cells than single-strand DNA breaks [11]. Thus, the repair of different types of lesions requires cooperation between different DNA repair systems in the course of recognition and elimination of such lesions.

In this work, we have studied the interaction of NER factors (XPC–HR23b, XPA, and RPA) with DNA structures containing bulky lesions introduced into the DNA opposite the lesions of another type, namely AP sites repaired by the BER system. The XPC–HR23B heterodimer is currently considered as the main contender for the role of the factor performing primary lesion recognition in the course of NER [12-14]. RPA and XPA are also the factors that are absolutely required for this repair process [15-17].

MATERIALS AND METHODS

The following materials and reagents were used in the work: [γ-^{32}P]ATP (5000 Ci/mmol) produced at the Laboratory of Biochemistry, Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences; phage T4 polynucleotide kinase (Biosan, Russia); stained molecular weight markers (Fermentas, Lithuania); reagents for electrophoresis and the main components of buffers from Sigma (USA) or domestic high purity buffers.

Oligonucleotides containing the fluorescein derivative of dUMP were synthesized by V. N. Silnikov (Nanotech-C, Russia). The structures of oligonucleotides and the nucleotide analog are shown in Fig. 1.

Protein preparations. Recombinant human RPA was isolated as described [18]. Recombinant human XPA containing a polyhistidine fragment at the N-end was isolated as described [19]. Recombinant heterodimer XPC–HR23b (Flag-XPC and 6HisTag-HR23B) was obtained by the method described in [20] with minor modifications. Recombinant DNA encoding heterodimer subunits were kindly provided by K. Sugasawa (Kobe University, Japan).

Generation of 5′-^{32}P-labeled DNA duplexes. Radioactive label was introduced into the 5′-end of dUMP-containing oligonucleotides using phage T4 polynucleotide kinase by a method described previously [21]. The labeled oligonucleotides were purified in MicroSpinTM G-25 columns (Amersham Pharmacia Biotech, USA) according to the manufacturer's protocol. For generation of DNA duplexes, 5′-^{32}P-labeled oligonucleotides were annealed with complementary oligonucleotides at a ratio of 1 : 1.2, incubated for 5 min at 95°C, slowly cooled to 75°C, left to stand for 15 min at that temperature, and then slowly cooled to room temperature. The efficiency of DNA duplex formation was evaluated by electrophoresis in 10% polyacrylamide gel (acrylamide/*bis*-acrylamide = 40 : 1) in TBE buffer (50 mM Tris-HCl, 50 mM H_3BO_3, 1 mM EDTA, pH 8.3).

Protein–DNA binding was assayed by gel retardation. DNA structures containing dUMP residues were preincubated with uracil-DNA glycosylase (UDG, 0.5 activity units/μl) at 37°C for 15 min. The complexes were formed in 10 μl of the reaction mixture containing RB buffer (50 mM Tris-HCl, pH 7.5, 100 mM KCl, 1 mM DTT), 0.6 mg/ml BSA, 10 nM 5′-^{32}P-labeled DNA, and different concentrations of tested proteins at 37°C for 20 min. Then the samples were supplemented with application buffer (1/5 volume) containing 20% glycerol, 0.015% bromophenol blue, and RB buffer (the temperature of the application buffer was also 37°C). Reaction mixtures were applied onto precooled gels (4°C). Electrophoresis was performed in nondenaturing 5% polyacrylamide gel (acrylamide/*bis*-acrylamide = 60 : 1) in TBE buffer at a fixed voltage of 17 V/cm at 4°C. The positions of radiolabeled oligonucleotide and protein/nuclein complexes were determined by autoradiography using a Molecular imager FX Pro+ (BioRad, USA). Quantitative data processing was performed using Quantity One software (BioRad).

Affinity modification of proteins by DNA structures containing an AP site was performed in reaction mixture (10 μl) containing RB buffer, 0.6 g/liter BSA, 10 nM 5′-^{32}P-labeled DNA pretreated with UDG, and the respective protein at test concentrations. The mixture was incubated for 20 min at 37°C followed by addition of $NaBH_4$ up to 20 mM and incubation for 15 min at 37°C. The reaction was stopped by adding 1/5 volume of stop buffer (5% SDS, 5% 2-mercaptoethanol, 0.3 M Tris-HCl, pH 7.8, 50% glycerol, 0.005% bromophenol blue). Modification products were separated by the Laemmli electrophoresis technique [22] in 10% SDS-polyacrylamide gel (acrylamide/*bis*-acrylamide = 40 : 1) followed by autoradiography.

RESULTS AND DISCUSSION

The 48-mer DNA duplexes containing a bulky lesion in one strand and a dUMP residue in the opposite strand (Fig. 1a) with an AP site generated instead of the latter after UDG treatment were used as model DNA substrates for studying the interaction with NER protein factors. The dUMP analog containing the fluorescein residue

attached to the base (Flu-dUMP; Fig. 1b) was used as a bulky lesion. This analog, when introduced into DNA, is recognized as a lesion repaired by the NER system [23]. Oligonucleotides containing an AP site in different positions of the strand were used as single- or double-stranded, with the opposite strand containing no lesion or bearing the Flu-dUMP residue. The constructed DNA duplexes contained AP sites facing nucleotide 3′-adjacent

a

DNAss 5′-p*-CTATGGCGAGGCGATTAAGTTGGGCAACGTCAGGGTCTTCCGAACGAC-3′

DNA-F 5′-p*-CTATGGCGAGGCGATTAAGTTGGGCAACGTCAGGGTCTTCCGAACGAC-3′
3′-GATACCGCTCCGCTAATTCAACCCGFTGCAGTCCCAGAAGGCTTGCTG-5′

DNA-Nd 5′-p*-CTATGGCGAGGCGATTAAGTTGGGCAACGTCAGGGTCTTCCGAACGAC-3′
3′-GATACCGCTCCGCTAATTCAACCCGTTGCAGTCCCAGAAGGCTTGCTG-5′

AP_{-1} 5′-p*-CTATGGCGAGGCGATTAAGTTGGGUAACGTCAGGGTCTTCCGAACGAC-3′

AP_{-1}-F 5′-p*-CTATGGCGAGGCGATTAAGTTGGGUAACGTCAGGGTCTTCCGAACGAC-3′
3′-GATACCGCTCCGCTAATTCAACCCGFTGCAGTCCCAGAAGGCTTGCTG-5′

AP_{-1}-Nd 5′-p*-CTATGGCGAGGCGATTAAGTTGGGUAACGTCAGGGTCTTCCGAACGAC-3′
3′-GATACCGCTCCGCTAATTCAACCCGTTGCAGTCCCAGAAGGCTTGCTG-5′

AP_{-11} 5′-p*-CTATGGCGAGGCGAUTAAGTTGGGCAACGTCAGGGTCTTCCGAACGAC-3′

AP_{-11}-F 5′-p*-CTATGGCGAGGCGAUTAAGTTGGGCAACGTCAGGGTCTTCCGAACGAC-3′
3′-GATACCGCTCCGCTAATTCAACCCGFTGCAGTCCCAGAAGGCTTGCTG-5′

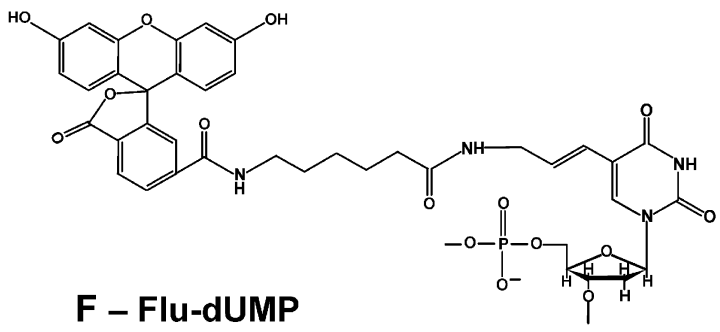
AP_{-11}-Nd 5′-p*-CTATGGCGAGGCGAUTAAGTTGGGCAACGTCAGGGTCTTCCGAACGAC-3′
3′-GATACCGCTCCGCTAATTCAACCCGTTGCAGTCCCAGAAGGCTTGCTG-5′

AP_{+10} 5′-p*-CTATGGCGAGGCGATTAAGTTGGGCAACGTCAGGGUCTTCCGAACGAC-3′

AP_{+10}-F 5′-p*-CTATGGCGAGGCGATTAAGTTGGGCAACGTCAGGGUCTTCCGAACGAC-3′
3′-GATACCGCTCCGCTAATTCAACCCGFTGCAGTCCCAGAAGGCTTGCTG-5′

AP_{+10}-Nd 5′-p*-CTATGGCGAGGCGATTAAGTTGGGCAACGTCAGGGUCTTCCGAACGAC-3′
3′-GATACCGCTCCGCTAATTCAACCCGTTGCAGTCCCAGAAGGCTTGCTG-5′

b



F – Flu-dUMP

5-{3-[6-(carboxyamido-fluoresceinyl)amidocapromoyl]allyl}-2′-deoxyuridine-5′-monophosphate

Fig. 1. Sequences of DNA structures used (a) and structure of bulky lesion (b).

to Flu-dUMP in the opposite strand (AP_{-1}-F), shifted to the 5′-end (AP_{-11}-F) or to the 3′-end (AP_{+10}-F) of the strand. The AP site-containing DNA duplexes with no lesion in the opposite strand (AP_{-1}-Nd, AP_{-11}-Nd, and AP_{+10}-Nd) were obtained in a similar way. The acyclic aldehyde form of deoxyribose in the AP site can form Schiff bases with the primary amino groups of proteins. Borohydride reduction of this intermediate leads to formation of stable covalent protein–DNA adducts, allowing covalent fixation of the proteins interacting with AP sites on DNA [24-26]. Since the DNA-binding domains of XPC–HR23B, XPA, and RPA contain lysine residues, these proteins are potential targets for the formation of Schiff bases with AP sites in DNA.

The XPC–HR23B heterodimer is currently considered as the main contender for the role of a primary lesion recognition factor in the course of NER. According to the literature data, XPC–HR23B has high affinity to the DNA double helix regions destabilized by lesion [27]. It is also known that XPC–HR23B recognizes not lesion as it is, but the region of unpaired bases in the DNA, which appears as a result of duplex destabilization and interacts with the region of undamaged strand opposite the lesion. The hypothesized mechanism of interaction between XPC–HR23B and damaged DNA was originally based on data showing the absence of repair of DNA containing bulky lesions in both strands in HeLa cell extracts [28]. This hypothesis was soon confirmed by X-ray structure analysis of the complex of yeast ortholog of XPC (Rad4) with Rad23 and a fragment of damaged DNA [29], as well as by the data on photoaffinity modification of XPC–HR23B by various DNA structures [19, 30]. In this context, it was interesting to study the interaction between XPC–HR23B and the DNA lacking a base in the region opposite the bulky lesion. Figure 2 shows the results of electrophoretic separation of the products of XPC–HR23B modification by single-stranded DNA (AP_{-1}), the DNA duplex containing an AP site in the region opposite the fluorescein residue (AP_{-1}-F), and the duplex with no lesion in the opposite strand (AP_{-1}-Nd). The XPC–HR23B concentration in the reaction mixture was varied in the range 100 to 700 nM on the basis of protein concentration in the stock preparation. As the figure shows, XPC–HR23B forms covalent crosslinks with the AP site within all of the studied DNA structures; however, noticeable modification levels are observed at a protein concentration of 300 nM and more (Fig. 2a, *3*, *8*, *13*). At the same time, the level of modification by the DNA duplex AP_{-1}-F (Fig. 2a, *6-10*) was ~1.5-2 times higher compared to the AP_{-1}-Nd duplex (Fig. 2a, *11-15*). Formation of covalent crosslinks between XPC–HR23B and the AP site in the region opposite the Flu-dUMP residue indicates the presence of protein contacts with this DNA region; in other words, the contacts between XPC–HR23B and the DNA region opposite the bulky lesion are maintained in the absence of the base. On the other hand, introduction of a bulky lesion increases the level of protein crosslinking to an AP site in the opposite strand. The level of XPC–HR23B modification by single-stranded DNA (Fig. 2a, *1-5*) was much lower compared to the DNA duplexes.

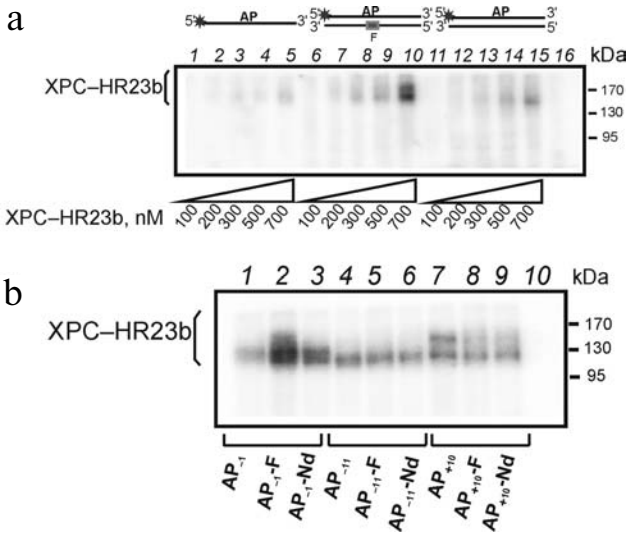


Fig. 2. Modification of XPC–HR23b by DNA structures containing an AP site. a) Dependence of efficiency of XPC–HR23b modification on protein concentration. Reaction mixtures (10 μl) contained standard buffer components indicated in "Materials and Methods", 0.6 g/liter of BSA, 10 nM 5′-^{32}P-labeled DNA structure, and different concentrations of XPC–HR23b. Lanes: *1-5*) single-stranded DNA structure containing an AP site in the middle of the strand (AP_{-1}); *6-10*) DNA duplex with a bulky lesion opposite the AP site (AP_{-1}-F); *11-15*) DNA duplex with an AP site in the middle of the strand (AP_{-1}-Nd); *16*) control without the protein. b) Efficiency of XPC–HR23b modification by DNA structures containing an AP site in different positions relative to the bulky lesion. Reaction mixtures (10 μl) contained the standard buffer components indicated in "Materials and Methods", 0.6 g/liter of BSA, 10 nM 5′-^{32}P-labeled DNA structure, and 700 mM XPC–HR23b. Lane *10*, control without the protein.

The data on the structure of the Rad4–damaged DNA complex show that the protein is located on the DNA asymmetrically relative to the lesion, so that the major part of the protein is localized on the undamaged strand in the 5′-direction [29]. Analogous localization of XPC–HR23B follows from the data on photoaffinity modification of this protein by DNA structures containing 5I-dUMP residues in different positions of the undamaged strand [19]. Figure 2b presents the data on XPC–HR23B modification by DNA structures containing an AP site in different positions of the strand. The figure shows that the DNA duplex AP_{-1}-F gives the highest yield of crosslinking to XPC–HR23B (Fig. 2b, *2*). When the AP site is shifted to the 5′- or 3′-end of the strand, modification efficiency decreases both for the duplexes with bulky lesion in the opposite strand (Fig. 2b, *2* cf. *5* and *8*) and for "native" duplexes (*3* cf. *6* and *9*). However,

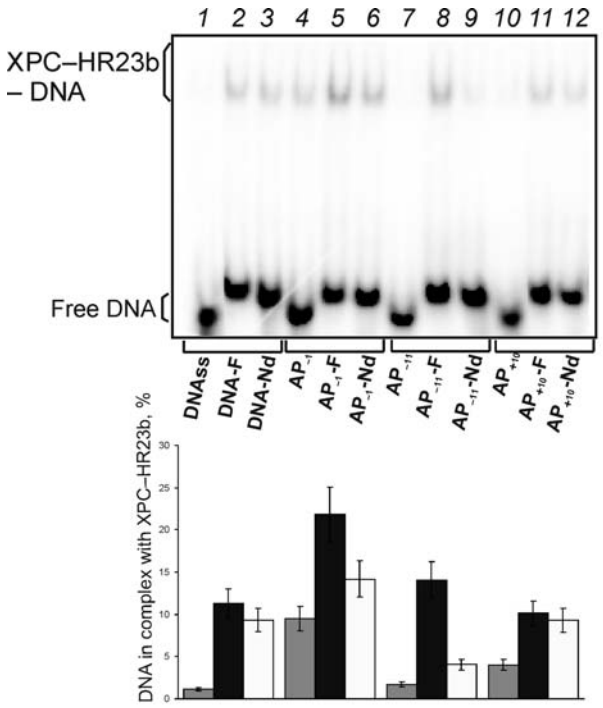


Fig. 3. Binding of XPC–HR23b to different DNA structures. Reaction mixtures (10 μl) contained the standard buffer components indicated in "Materials and Methods", 0.6 g/liter of BSA, 700 nM XPC–HR23b, and 10 nM 5′-^{32}P-labeled DNA structures. The histogram was plotted from the results of three experiments and reflects the percentage of XPC–HR23b binding to the DNA structures indicated under the autoradiograph.

single-stranded DNA shows an adverse effect: the crosslinks to the AP site increase when the latter is shifted from the center (*1* cf. *4* and *7*), especially in the 3′-direction (AP_{+10}); this structure shows also two products of protein modification (*7*), like in the case of DNA AP_{-1}-F (*2*). Modification products with lower electrophoretic mobility are observed also for DNA duplexes AP_{+10}-F and AP_{+10}-Nd (*8* and *9*); however, the intensity of this product is much lower compared to single-stranded DNA. The presence of two and more modification products is usually associated with DNA crosslinking to different amino acid residues in the DNA-binding site of a protein [31]. The levels of XPC–HR23B modification by DNA duplexes AP_{-11}-F and AP_{+10}-F were actually the same, whereas with 5I-dUMP-substituted DNA a higher level of protein crosslinking was observed at position –11 [19]. Such difference in the character of protein modification by various reactive groups may be due both to the difference in the spectrum of target amino acids for 5I-dUMP and the aldehyde group of the AP site and to the difference in XPC–HR23B binding to DNA as the position of the AP site changes.

It is known that an AP site disturbs DNA structure by bending it [32], which in turn may influence the binding to the DNA of protein XPC–HR23B sensitive to disturbances within the DNA double helix [27]. Though there are still no data in the literature on the involvement of the NER system of higher eukaryotes in AP site repair, the processing of AP sites has been observed in the NER systems of bacteria and yeasts [33]. To determine how much the presence of an AP site and its position on a strand influence the affinity of XPC–HR23B to DNA, we evaluated the efficiency of protein binding to the studied DNA structures by the method of gel retardation (Fig. 3). As can be seen from the figure, the maximum level of XPC–HR23B binding, just as the level of crosslinking, was observed for the DNA AP_{-1}-F (Fig. 3, *5*). The protein demonstrates somewhat lesser affinity towards DNA duplexes AP_{-1}-Nd and AP_{-11}-F (Fig. 3, *6* and *8*). It is interesting that the DNA duplex with an AP site in the middle of the strand (AP_{-1}-Nd) is bound to XPC–HR23B a little more effectively than the duplex with the bulky substituent (DNA-F). This observation suggests that the AP site introduces even heavier distortion into the DNA duplex structure than the fluorescein residue; the presence of both lesions in the adjacent pairs exerts an additive effect. It was shown previously that XPC–HR23B can effectively bind double-stranded oligonucleotides containing 3-hydroxy-2-(hydroxymethyl)-tetrahydrofuran (THF), a synthetic analog of an AP site [34]. The level of complex formation on single-stranded structures was much lower than on duplexes and depended on the presence and position of the AP site. The affinity of XPC–HR23B to single-stranded DNA, according to different estimates, varies within an order of magnitude depending on the oligonucleotide structure and the method of analysis and may be either higher or lower than the affinity to double-stranded DNA [35-37]. Though previously it was shown that XPC–HR23B has a greater affinity to undamaged than to UV-damaged oligonucleotide [38], in our case the undamaged oligonucleotide ssDNA was bound less efficiently than its AP-containing analogs AP_{-1} and AP_{+10} (*1* cf. *4* and *10*) and with actually the same efficiency as AP_{-11}. No positive correlation was altogether observed between the efficiency of binding and the level of crosslinking to the AP site, except for the duplex AP_{-1}-F. These data once again demonstrate that the level of covalent DNA–protein crosslinks depends not so much on the protein affinity to DNA as on the presence of an accessible target in the protein for the given reactive DNA group and on their mutual orientation.

The XPA protein was the first NER participant for which preferential binding with damaged DNA was demonstrated [39, 40]. Later this protein was shown to give preference to certain DNA structures, such as criss-crossed DNA duplexes and DNA structures containing a junction between the duplex and single-stranded parts [41-43]. In the present work, the interaction between XPA and AP site-containing DNA structures was ana-

lyzed for the first time (Fig. 4). Figure 4a shows the gel autoradiograph after separation of XPA modification products by DNA structures containing the AP site in different positions of the strand. As the figure shows, the efficiency of XPA modification by DNA duplexes depends more on the presence of bulky lesion in the opposite strand than on the position of the AP site. In all cases, a higher level of protein modification was observed for DNA with the Flu-dUMP residue (Fig. 4a, *2*, *5* and *8* cf. *3*, *6* and *9*), while the set of modification products depended on the position of the AP site. The interaction between XPA and single-stranded DNA (AP_{-1}, AP_{-11} and AP_{+10}) revealed more substantial differences both in the levels of protein modification and in the set of modification products (Fig. 4a, *1*, *4* and *7*), and the oligonucleotide with the central position of the AP site gave the lowest yield of crosslinking to XPA as in the case of XPC–HR23B (Fig. 2b, *1*). The presence of several modification products with different electrophoretic mobility suggests that Schiff bases can be formed with ε-amino groups of different lysine residues in the DNA-binding center of XPA, which in turn may reflect the flexibility of the protein structure [44]. The literature presents conflicting data about the affinity of XPA to single- and double-stranded DNA. According to [40], the affinity of XPA to single-stranded DNA is comparable with the affinity of this protein to a lesion-containing DNA duplex. Other researchers revealed that the affinity of XPA to single-stranded DNA was less by several orders of magnitude than its affinity to damaged DNA duplex [45]. The data about XPA binding to different DNA structures presented in Fig. 4b demonstrate insignificant differences in the levels of XPA complex formation with all DNA structures, with AP_{-1}-F taking a slight advantage. These results are in agreement with the data obtained previously in our works [19, 43] and in [40].

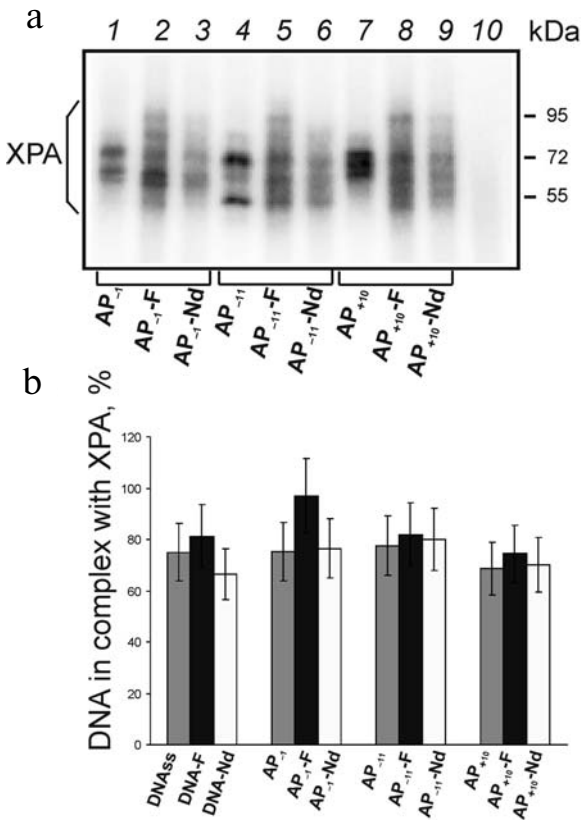


Fig. 4. Interaction of XPA with DNA structures containing an AP site. a) Efficiency of XPA modification by DNA structures containing an AP site in different positions relative to the bulky lesion. Reaction mixtures (10 μl) contained the standard buffer components, 0.6 g/liter of BSA, 700 nM XPA, and 10 nM 5′-^{32}P-labeled DNA structures. Lane *10*, control without the protein. b) XPA binding to different DNA structures. The histogram is based on the results of three experiments.

The RPA heterotrimer is also an absolutely necessary factor for the NER process. RPA was shown to possess higher affinity to damaged compared to undamaged DNA duplexes [45-47] and to physically contact XPA [45, 48]. The main function of RPA in all processes of DNA metabolism is stabilization of the single-stranded DNA regions [49]. The most widespread model of NER assumes that RPA enters the repair complex already after partial opening of the duplex around the lesion due to helicase activity of the TFIIH complex and formation of single-stranded DNA regions [15]. The analysis of RPA interaction with AP-site-containing structures (Fig. 5) once again demonstrated preferable interaction of this protein with single-stranded DNA both by the level of crosslinking (Fig. 5a, *1*, *4*, *7*) and by the efficiency of binding (Fig. 5b). The dependence of modification efficiency on the AP site position was also more marked on single-stranded DNA than on DNA duplexes. It is known that RPA binds to single-stranded DNA in a polar manner [50, 51], which is due to a consecutive binding of its DNA-binding domains to DNA in the direction from the 5′- to 3′-end. As one can see from Fig. 5a, RPA is more effectively modified by the single-stranded DNA containing an AP site closer to the 5′-end (AP_{-11}) (Fig. 5a, *4*) than by the DNA with AP site shifted to the 3′-end (AP_{+10}) (Fig. 4a, *7*). Thus, our data confirm the polar orientation of RPA on single-stranded DNA. The efficiency and character of RPA modification by DNA duplexes, in contrast to XPC–HR23B and XPA, actually did not depend on the presence of a bulky lesion (Fig. 5a, cf. 2 and *3*, *5* and *6*, *8* and *9*), though the binding experiments showed insignificant preference to the DNA duplex containing an AP site closer to the 5′-end of the strand and fluorescein residue in the opposite strand.

Thus, the data reported in this work demonstrate the interaction of all tested proteins involved in the NER process with DNA structures containing an AP site repaired by the BER system. Such interaction may influence the course of these repair processes in cells. The binding of XPC–HR23B to the DNA duplex containing

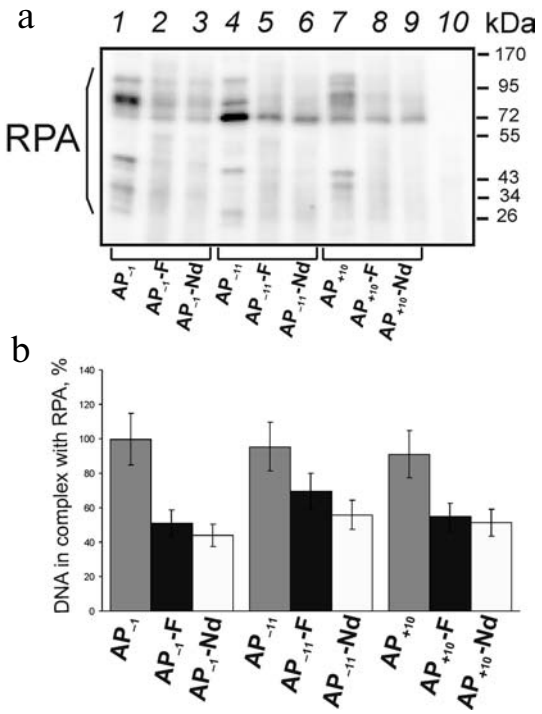


Fig. 5. Interaction of RPA with DNA structures containing an AP site. a) Efficiency of RPA modification by DNA structures containing an AP site in different positions relative to the bulky lesion. Reaction mixtures (10 μl) contained the standard buffer components, 0.6 g/liter of BSA, 300 nM RPA, and 10 nM 5′-^{32}P-labeled DNA structures. Lane *10*, control without the protein. b) RPA binding to different DNA structures. The histogram is based on the results of three experiments.

an AP site in the region opposite the bulky lesion may initiate the assembly of the NER complex and the beginning of repair by this pathway and block the access to the AP site by BER proteins. After excision of the region containing the bulky lesion, spontaneous hydrolysis of the AP site in the opposite strand will inevitably result in formation of breaks in both DNA strands, which is extremely undesirable for a cell. The most favorable scenario in the repair of such kind of lesions is pre-emptive repair of the AP site by the BER pathway. Recently, we showed the possibility of repairing an AP site in the region opposite a bulky lesion – a benz[a]pyrene derivative [52].

The authors are grateful to Kaoru Sugasawa (Biosignal Research Center, Kobe University, Kobe, Japan) for kindly providing recombinant DNA encoding XPC and HR23b and to I. O. Petruseva for protein purification.

This work was supported by the Russian Foundation for Basic Research (projects No. 10-04-00837 and No. 11-04-00559) and the Program of the Presidium of the Russian Academy of Sciences "Molecular and Cell Biology".

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