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Structural basis for the recognition and processing of DNA containing bulky lesions by the mammalian nucleotide excision repair system

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ABSTRACT

Mammalian nucleotide excision repair (NER) eliminates the broadest diversity of bulky lesions from DNA with wide specificity. However, the double incision efficiency for structurally different adducts can vary over several orders of magnitude. Therefore, great attention is drawn to the question of the relationship among structural properties of bulky DNA lesions and the rate of damage elimination. This paper studies the properties of several structurally diverse synthetic (model) DNAs containing bulky modifications. Model DNAs have been designed using modified nucleosides (exo-*N*-{2-*N*-[*N*-(4-azido-2,5-difluoro-3-chloropyridin-6-yl)-3-aminopropionyl]aminoethyl}-2′-deoxycytidine (Fap-dC) and 5-{1-[6-(5[6]-fluoresceinylcarbomoyl)hexanoyl]-3-aminoallyl}-2′-deoxyuridine (Flu-dU)) and the nonnucleosidic reagent *N*-[6-(9-antracenylcarbomoyl)hexanoyl]-3-amino-1,2-propandiol (nAnt). The impact of these lesions on spatial organization and stability of the model DNA was evaluated. Their affinity for the damage sensor XPC was also studied. It was expected, that the values of melting temperature decrease, bending angles and K_D values clearly define the row of model DNA substrate properties such as Flu-dU-DNA > > nAnt ≈ Fap-dC-DNA. Unexpectedly the experimentally estimated levels of the substrate properties were actually in the row: nAnt-DNA > > Flu-dU-DNA > > Fap-dC-DNA. Molecular dynamics simulations have revealed structural and energetic bases for the discrepancies observed. DNA destabilization patterns plotted explain these results on a structural basis in terms of differences in dynamic perturbations of stacking interactions.

1. Introduction

The DNA nucleotide excision repair (NER) system recognizes and removes from DNA a wide variety of structurally diverse helix-distorting bulky adducts, mostly modified nitrogen bases. NER substrates include UV- or ionizing radiation-induced lesions and other bulky base-adducts that can be induced by numerous external chemical reagents like environmental pollutants, antineoplastic drugs and several chemically active endogenous compounds. Mammalian NER eliminates from the DNA 24–32 nucleotide-long fragments containing damage and reconstitutes the nucleotide sequence using an undamaged DNA chain as a template for the reparative synthesis via the action of about 30 core protein factors and enzymes [1–3]. There are two NER pathways, transcription-coupled repair (TCR) and global genomic repair (GGR), which differ in the initial damage sensor. In TCR, the damage sensor function executed by transcriptionally active RNA polymerase stalled at the lesion [4–6]. It is believed that the rate-limiting step in NER is the lesion recognition [5–7].

In the global genome NER subpathway the DNA-binding subunit XPC forms a stable complex with structurally distorted and destabilized DNA. XPC binds to a region containing the damage and causes DNA bending and local separation of DNA strands. XPC does not form contacts with the adduct but interacts with both the intact and damaged DNA strands around a lesion [8,9], which results in additional DNA bending and destabilization [10]. The efficiency of XPC-HR23B interaction with DNA is also influenced by the damage-flanking context of

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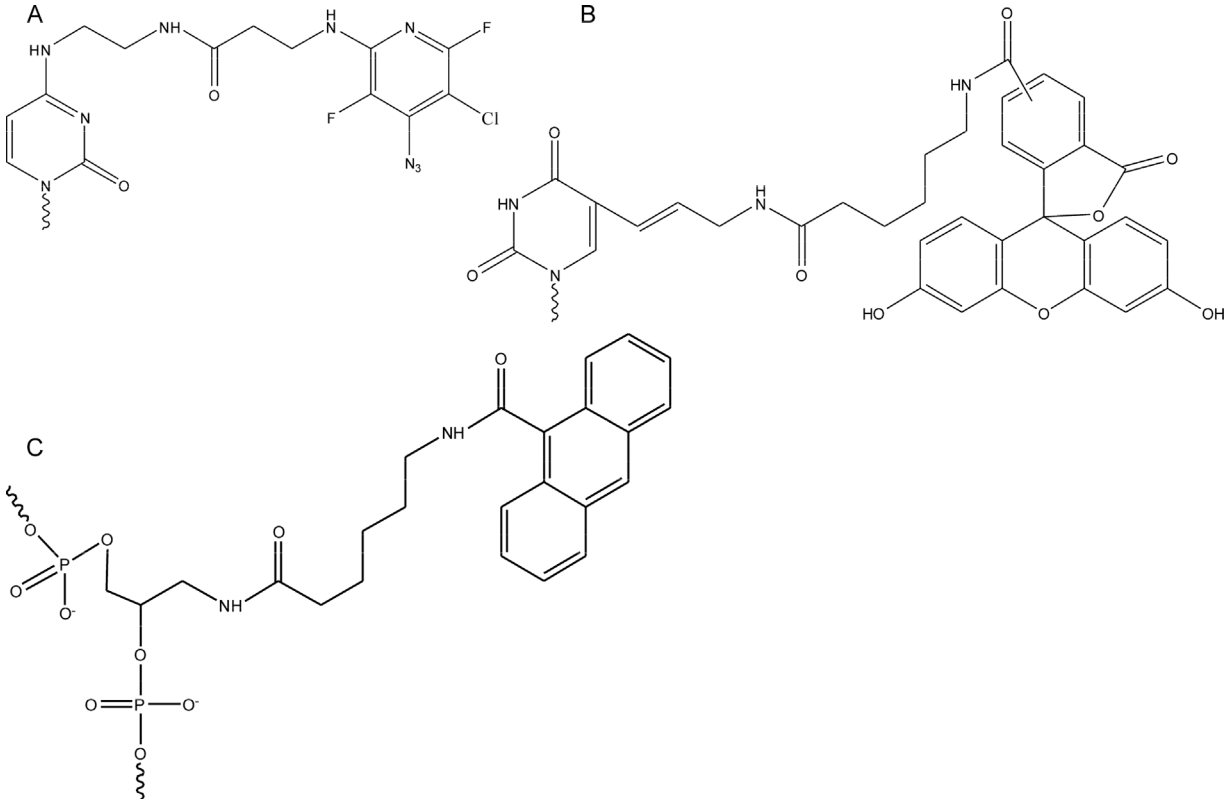

Fig. 1. Structures of model lesion used. Exo-*N*-{2-[*N*-(4-azido-2,5-difluoro-3-chloropyridin-6-yl)-3-aminopropionyl]aminoethyl}-deoxycytidinine (Fap-dC, A), fluorescein-5[6]-carboxyamidokaproil-[5-(3-aminoallil)-deoxyuridine (Flu-dU, B) and the non-nucleoside fragment of the modified DNA strand, containing N-[6-(9-antracenylcarbomoyl)hexanoyl]-3-amino-1,2-propandiol (nAnt, C)].(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

nucleobases [11]. The second stage of lesion recognition [12] is executed by the ATP-dependent 5′-3′-helicase XPD, the subunit of multi subunit transcription factor IIH (TFIIH). TFIIH efficiently recognizes the specific complex DNA-XPC. XPD stalled at bulky modification is the ultimate identifier of the lesion that attracts the downstream NER factors in the DNA repair complex [13].

The double incision efficiency for structurally different adducts can vary over several orders of magnitude. Special attention is paid to the interplay among structural properties of bulky DNA lesions and the rate of damage elimination [3,7,14]. The systematic study of widespread bulky DNA damages resulting from UV, platinum derivatives, adducts formed by aromatic amines and active metabolites of polycyclic aromatic compounds considerably helped to elucidate this inter-relation [1,15–20].

The synthetic DNA structures (model DNA) which imitate NER intermediates and substrates, e.g. double-stranded DNA bearing an appropriate modification, are also widely used for NER investigations *in vitro* [21–23]. To imitate bulky DNA lesions the 5(6)-fluorescein (Flu) and photoreactive arylazide 4-azido-2,5-difluoro-3-chloropyridine (Fap) were used in our previous studies [24–26] (Fig. 1A, B). The model DNA for the studies of damaged strand excision in NER has been very recently designed using the novel non-nucleoside phosphoramidite reagents which contain *N*-[6-(9-antracenylcarbamoyl)hexanoyl]-3-amino-1,2-propandiol (nAnt) moieties [27] (Fig. 1C). The extended linear nAnt-DNA proved to be the effective NER substrate suitable for estimation of specific excision activity catalyzed by mammalian whole cell extracts. Meanwhile the structural properties of the adducts are intriguing and have not yet been analyzed. A comprehensive study of the new DNA lesions is essential for further progress of their application in NER research.

The main goal of the present investigation was to gain insight into the structural basis of nAnt-, Flu-dU and Fap-dC-DNA recognition with NER system proteins. The specific excision assay, XPC-probing (quantitative equilibrium titrations), differential melting and gel retardation techniques have been used for this investigation. For the first time the correlation between characteristics of model DNA with significant differences in lesion structure and the efficiency of the specific excision of such lesions has been investigated. An additional option, Molecular Dynamics (MD) simulations, was used to obtain information about the effects of the artificial damages on the structure of the model DNA duplexes and to explain the observed experimental data.

2. Materials and methods

2.1. Reagents and protein preparations

α-[^{32}P]-dCTP and γ-[^{32}P]-ATP(3000 Ci/mmol) were produced at ICBFM SB RAS. DMEM and DMEM/F12 media for CHO cell cultivation were produced by Invitrogen. The components for polyacrylamide gel preparation and the main components of buffer systems were from Sigma.

Taq DNA polymerase, T4 DNA ligase and T4 polynucleotide kinase were from Biosan (Novosibirsk). Exonuclease λ for non-modified single-stranded DNA synthesis was kindly provided by Dr. A. Zakabunin (ICBFM SB RAS). Proteinase K was from Sigma. Recombinant XPC-HR23B was expressed and purified mainly as described previously [28].

The FAM-containing oligonucleotide (ONT 11) and the regular oligonucleotides used for the creation of model DNA duplexes and for NER-assays (ONT 1–5, 7–10, 12–16 and 17) were produced in the laboratory of medical chemistry of ICBFM SB RAS (all the sequences are presented in Table 1). Synthesis of modified ONT 1 and 6 containing the nAnt insertion was as in [27].