Available online at www.sciencedirect.com
SCIENCE DIRECT
Archives of Biochemistry and Biophysics 446 (2006) 1–10
ABB
www.elsevier.com/locate/yabbi

Thermodynamic properties of damaged DNA and its recognition by xeroderma pigmentosum group A protein and replication protein A

Viktor Brabec *, Kristýna Stehlíková, Jaroslav Malina, Marie Vojtíšková, Jana Kašpárková *

Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, CZ-61265 Brno, Czech Republic

Received 31 October 2005, and in revised form 5 December 2005
Available online 22 December 2005

Abstract

The effects of the lesions induced by single, site-specific 1,2-GG or 1,3-GTG intrastrand adducts of *cis*-diamminedichloroplatinum(II) formed in oligodeoxyribonucleotide duplexes on energetics of DNA were examined by means of differential scanning calorimetry. These effects were correlated with affinity of these duplexes for damaged-DNA binding-proteins XPA and RPA; this affinity was examined by gel electrophoresis. The results confirm that rigid DNA bending is the specific determinant responsible for high-affinity interactions of XPA with damaged DNA, but that an additional important factor, which affects affinity of XPA to damaged DNA, is a change of thermodynamic stability of DNA induced by the damage. In addition, the results also confirm that RPA preferentially binds to DNA distorted so that hydrogen bonds between complementary bases are interrupted. RPA also binds to nondenaturational distortions in double-helical DNA, but affinity of RPA to these distortions is insensitive to alterations of thermodynamic stability of damaged DNA.

Keywords: Differential scanning calorimetry; Xeroderma pigmentosum group A protein; Replication protein A; Cisplatin; DNA adducts; DNA distortion; Electrophoretic mobility shift assay; Thermodynamic stability

Extension of the database of factors affecting binding affinity of damaged-DNA binding-proteins is a focus of current research. There is now clear evidence that the energetics of DNA distortion can play an important role in defining protein–DNA affinity [1–4]. Pre-distortion of DNA, such as for instance pre-bending, has been shown to increase the binding affinity of some proteins [5,6], presumably by removing the thermodynamic penalty associated with deformation of DNA double helix. These studies employ systems in which it is possible to vary the character of the DNA distortion along with the associated changes in its energetics and to measure how these changes affect binding affinity of damaged-DNA binding-proteins. In the present study, we compare affinity for damaged DNA of two proteins, which bind preferentially to different types of damaged DNA, with the energetics of two different DNA distortions. We examine the affinity for damaged DNA of xeroderma pigmentosum group A protein (XPA)[1] and replication protein A (RPA), which have been implicated in the recognition of damaged DNA in mammalian cells [7] and which exhibit distinctly different preferences for high-affinity interactions with damaged double-helical DNA [8]. For the present study, we employed DNA damaged in a different, but well defined way, namely DNA damaged by 1,2-GG or 1,3-GTG intrastrand cross-links of cisplatin [*cis*-diamminedichloroplatinum(II)].

XPA is a 32 kDa protein. One particular helical distortion, namely rigid DNA bending, was identified as the

* Corresponding authors. Fax: +420 541240499.
E-mail addresses: brabec@ibp.cz (V. Brabec), jana@ibp.cz (J. Kašpárková).

[1] *Abbreviations used:* XPA, xeroderma pigmentosum group A protein; RPA, replication protein A; cisplatin, *cis*-diamminedichloroplatinum(II); T_m, melting temperature; ΔG, free energy of duplex melting; ΔC_p, excess heat capacity; DSC, differential scanning calorimetry; ΔH_{cal}, enthalpy of duplex melting; ΔS, entropy of duplex melting; ΔG^0_{25}, free energy of duplex melting at 25 °C; ΔH_{vH}, model-dependent van't Hoff enthalpy; EMSA, electrophoretic mobility shift assay.

0003-9861/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2005.12.003

specific molecular determinant that induces high-affinity interactions of this protein with DNA [8,9]. Importantly, XPA protein does not bind to single-stranded DNA. RPA is a heterotrimeric protein consisting of 70-, 34-, and 14-kDa subunits. In contrast to XPA protein, it is the single-stranded DNA binding protein [10].

A major alteration induced in DNA by 1,2-GG intrastrand adduct is that it generates a directional rigid bend of the helix axis towards the major groove (32–34°) and a local unwinding of ~13°; however, the local base pairing remains intact around the adduct [11]. On the other hand, the 1,3-GTG intrastrand adduct generates a similar bend towards major groove (~30°) and local unwinding (19°) as 1,2 adduct, but induces a localized denaturation and flexibility of the duplex around the lesion [11]. Thus, it is reasonable to expect that these two lesions, each distorting DNA in a unique way, will also result in different changes of thermodynamic stability of the duplex DNA.

In spite of a considerable increase in studies designed to correlate damaged-DNA binding-affinity of proteins with energetics of lesion-containing DNA duplexes, a detailed study of this kind focused on damaged-DNA binding-affinity of XPA and RPA proteins is missing. DNA-binding affinity of RPA has been already shown to correlate with the ability of RPA to denature DNA substrates and with the degree of thermal (not thermodynamic) instability of duplex DNA [12]. However, changes in melting temperature (T_m) are not necessarily good predictors of changes in thermodynamic stability (free energy of duplex melting, ΔG) because there is no simple correspondence between changes in T_m and ΔG due to the presence of a lesion in DNA [13,14]. The reason for the failure of changes of T_m values to reflect reliably the lesion-induced changes in thermodynamic stability (changes of ΔG values) is neglect of the temperature dependence of the duplex stability. T_m values reflect the behavior of the duplex at high temperature whereas the free-energy changes are evaluated for a low-temperature standard state, typically 25 or 37 °C; these low-temperature standard states correspond to the temperature domain of the processes of biological significance.

In the present work, we compare the effects of the lesions induced by single, site-specific 1,2- or 1,3-intrastrand adducts of cisplatin and contained in short oligodeoxyribonucleotide duplexes on DNA stability with emphasis on the thermodynamic origins of that stability. The lesion-induced alterations of duplex energetics are correlated with the affinity of these duplexes to XPA or RPA.

Materials and methods

Chemicals

Cisplatin was purchased from Sigma (Prague, Czech Republic). The synthetic oligodeoxyribonucleotides (Fig. 1) were purchased from IDT (Coralville, IA) and purified as described previously [15]. In the present work, the molar concentrations of the single-stranded oligonucleotides are related to the oligomers (not to the monomer content). Molar extinction coefficients for the single-stranded oligonucleotides (related to the 15–36-mer strands) were determined by phosphate analysis [16]. The formation of 1:1 complexes between the top strands unmodified or containing the intrastrand adduct and bottom strands of the duplexes (Fig. 1) was verified by recording isothermal UV absorbance mixing curves at 25 °C [3]. In the present work, the molar concentrations of the duplexes are related to the double-stranded molecules. The N-terminal His_6-tagged XPA protein was obtained by expressing the plasmid DNA pET15b/XPA template [17] in RTS 500 *Escherichia coli* HY (Roche) and purified on Ni^{2+}–NTA agarose and by hydroxyapatite chromatography [8]. The plasmid DNA pET15b/XPA was kindly provided by Richard D. Wood. Human recombinant RPA purified from *E. coli* [18] were a kind gift of John J. Turchi. T4 DNA ligase and T4 polynucleotide kinase were purchased from New England Biolabs (Beverly, MA). Acrylamide, bis(acrylamide), urea, and NaCN were from Merck KgaA (Darmstadt, Germany). Dimethyl sulfate (DMS) was from Sigma (Prague, Czech Republic). [γ-^{32}P]ATP was from Amersham (Arlington Heights, IL).

dsTGTGT(15): 5′-CTTCTT**G**T**G**TTCTTC
GAAGAACACAAGAAG-5′

dsTGGT(15): 5′-CTTCTT**GG**TTTCTTC
GAAGAACCAAAGAAG-5′

dsTGTGT(20): 5′-TCTCTTCTT**G**T**G**TTCTTCTC
AGAGAAGAACACAAGAAGAG-5′

dsTGGT(20): 5′-TCTCTTCTT**GG**TTTCTTCTC
AGAGAAGAACCAAAGAAGAG-5′

dsAGGA(20): 5′-TCTCTTCTA**GG**ATTCTTCTC
AGAGAAGATCCTAAGAAGAG-5′

dsCGGC(20): 5′-TCTCTTCTC**GG**CTTCTTCTC
AGAGAAGAGCCGAAGAAGAG-5′

Fig. 1. The sequences of the synthetic oligodeoxyribonucleotide duplexes used in this study with their abbreviations. The top and bottom strands in the pair of oligonucleotides are designated top and bottom, respectively, in the text. Central G-residues depicted in bold in the top strand of each duplex indicate the location of the intrastrand adduct after modification of the oligonucleotide by cisplatin in the way described in Materials and methods.

Platination of oligonucleotides

The single-stranded oligodeoxyribonucleotides (the top strands of the duplexes shown in Fig. 1) at the concentration of 90 μM were reacted in stoichiometric amounts with cisplatin for 24 h at 37 °C in 10 mM $NaClO_4$ [15]. The platinated oligonucleotides were repurified by ion-exchange fast protein liquid chromatography (FPLC). It was verified by platinum flameless atomic absorption spectrophotometry (FAAS) and by the measurements of the optical density that the modified oligonucleotides contained one platinum atom. It was also verified using DMS footprinting of platinum on DNA [19,20] that in the platinated top strands of the duplexes the N7 position of both G residues was not accessible for reaction with DMS. The nonmodified or