Title
Conformational analysis of DNA repair intermediates by time-resolved fluorescence spectroscopy.

Permalink
https://escholarship.org/uc/item/6jd6d9zp

Journal
The journal of physical chemistry. A, 113(35)

ISSN
1089-5639

Authors
Lin, Su
Horning, David P
Szostak, Jack W
et al.

Publication Date
2009-09-01

DOI
10.1021/jp906746w

Peer reviewed
Conformational Analysis of DNA Repair Intermediates by Time-Resolved Fluorescence Spectroscopy

Su Lin, David P. Horning, Jack W. Szostak, and John C. Chaput*

Center for BioOptical Nanotechnology, The Biodesign Institute, and Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287-5201, and Howard Hughes Medical Institute and Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114

Received: July 16, 2009

DNA repair enzymes are essential for maintaining the integrity of the DNA sequence. Unfortunately, very little is known about how these enzymes recognize damaged regions along the helix. Structural analysis of cellular repair enzymes bound to DNA reveals that these enzymes are able to recognize DNA in a variety of conformations. However, the prevalence of these deformations in the absence of enzymes remains unclear, as small populations of DNA conformations are often difficult to detect by NMR and X-ray crystallography. Here, we used time-resolved fluorescence spectroscopy to examine the conformational dynamics of linear, nicked, gapped, and bulged DNA in the absence of protein enzymes. This analysis reveals that damaged DNA is polymorphic in nature and able to adopt multiple individual conformations. We show that DNA repair intermediates that contain a one-nucleotide gap and bulge have a significant propensity to adopt conformations in which the orphan base resides outside the DNA helix, while DNA structures damaged by a nick or two-nucleotide gap favor intrahelical conformations. Because changes in DNA conformation appear to guide the recognition of DNA repair enzymes, we suggest that the current approach could be used to study the mechanism of DNA repair.

Structural analyses of many DNA–enzyme complexes reveal that cellular repair enzymes recognize DNA in a variety of conformations that distort the normal helical structure of DNA. Some of the conformations regularly observed in these structures include DNA bending, major groove compression, and extrahelical base flipping. However, the prevalence of these deformations in the absence of enzymes remains unclear, as small populations of DNA conformations are often difficult to detect by NMR and X-ray crystallography. Efforts to resolve this question have led to the use of time-resolved fluorescence spectroscopy as a highly sensitive technique for examining the conformation and structure of DNA. While previous studies have focused on the conformation and dynamics of abasic lesions and single-base mismatches, no systematic analysis of other DNA repair intermediates, such as structures with one- and two-nucleotide gaps, has been undertaken. Here, we report the conformational states of linear, nicked, gapped, and bulged DNA by time-resolved fluorescence spectroscopy. Data obtained in this study provides valuable information about the distribution of conformations available to several important DNA repair intermediates.

We designed a series of DNA structures (Figure 1) to study the effect of different repair intermediates on the conformation of DNA. The structures were named according to their DNA modification. This included a nicked DNA backbone (NICK), a backbone with a gap of one and two nucleotides (GAP and 2NT, respectively), a helix with a single nucleotide bulge (BLG), and a linear helix with continuous base pairing (FBP).

Figure 1. DNA repair intermediates analyzed in this study. Structures with a gap in their sequence have discontinuous phosphodiester backbones. Dashes indicate continuous DNA strands. The 2AP residue is shown as P.

DNA repair intermediates in this study. Structures with a gap in their sequence have discontinuous phosphodiester backbones. Dashes indicate continuous DNA strands. The 2AP residue is shown as P.

5′-GCTGCCAGTGTTGAACCTCTAC 3′-CGACGGTCACPCCTTGAGATG FBP
5′-GCTGCCAGTGGAACTCTAC 3′-CGACGGTCACP-CCCTTGAGATG NICK
5′-GCTGCCAGTGGAACTCTAC 3′-CGACGGTCACPCCTTGAGATG GAP
5′-GCTGCCAGTGTTGAACCTCTAC 3′-CGACGGTCACPCCTTGAGATG 2NT
5′-GCTGCCAGTGTTGAACCTCTAC 3′-CGACGGTCAC-P-CCCTTGAGATG BLG

The Journal of Physical Chemistry A

09/2009, 113, 9585–9587
Published on Web 08/12/2009

9585
Under equilibrium conditions, these DNA structures were predicted to exist in an ensemble of conformations (Figure 2) in which the environment of the 2AP reporter exchanges between multiple distinct states. Because 2AP is sensitive to static and dynamic quenching by neighboring bases, it is possible to distinguish distinct DNA conformations by monitoring the decay kinetics of fluorescence quenching. Kinetic decay profiles that exhibit rapid quenching similar to undamaged DNA are consistent with 2AP residing in a stacked helical state, while slow decay kinetics similar to free 2AP are consistent with 2AP residing in an extrahelical state that isolates the reporter from neighboring bases. The ability to distinguish different DNA populations based on the local environment of the fluorescent reporter makes it possible to examine the conformational flexibility of different DNA repair intermediates in the absence of protein enzymes.

We began by examining the steady-state fluorescence emission of each DNA complex at ambient temperature. The fluorescence emission intensity was measured at 370 nm with excitation at 310 nm (Supporting Information). As expected from earlier time-resolved fluorescence studies, the FBP structure is strongly quenched, while fluorescence in the BLG structure remains high. This is consistent with the prediction that higher steady-state fluorescence correlates with increased isolation of 2AP from neighboring bases. The remaining structures vary between these extremes: NICK is quenched nearly as strongly as FBP, the GAP structure is comparable to the BLG structure, and the 2NT structure is intermediate but more quenched than GAP. In general, greater disruption to the DNA helix near the 2AP reporter leads to higher fluorescence. A similar trend was observed under both low and high salt conditions, although the BLG and GAP structures exhibit 2-fold less fluorescence under simulated physiological conditions when compared to the higher salt conditions. Taken together, this data supports the notion that certain repair intermediates are more disruptive to the helical structure of DNA than others. However, this data does not describe the distribution of conformational states adopted by the different DNA structures.

Time-correlated single-photon counting (TCSPC) was used to study the distribution of individual DNA conformations. Fluorescence decay curves were recorded at 390 nm for each DNA structure as well as the free 2AP nucleoside (Figure 3A). We used the higher salt conditions for these measurements to stabilize base pairing of the duplex and prevent fraying of the ends. Because the instrument response time of TCSPC is 100 ps, streak camera imaging, which has a resolution limit of 10 ps, was required to capture the fast component for the NICK and FBP structures. The fast fluorescence decay component, which is embedded in the instrument response time of the TCSPC, was clearly resolved in the streak camera measurements. The fast decay kinetics of the FBP and NICK structures are plotted in Figure 3A (inset). The trend in the fluorescence decay curves is consistent with data acquired under steady-state conditions. Because the DNA sequence is the same in each case, we reasoned that the change in the rate of fluorescence quenching must be due to differences in the conformation of the DNA structure near the 2AP reporter.

To examine the distribution of conformational states, the kinetic decay profiles were fit to a sum of exponentials (Supporting Information). With the exception of 2AP, which exhibits free fluorescent decay, and FBP, which has very rapid fluorescence quenching, four exponential terms were required to fit the fluorescence decay curves. The kinetic components fall into the following categories: (i) a fast component with a lifetime of ~30 ps that corresponds to 2AP stacked within the DNA helix; (ii) two intermediate components with lifetimes of 90–200 ps and ~1 ns which result from less efficient quenching due to dynamic disorder caused by motion of the DNA bases; and (iii) a slow component with a lifetime of 4.5–11 ns that corresponds to 2AP residing in the extrahelical environment. This conformation can have lifetimes approaching that of free 2AP, but rapid exchange reduces the lifetime by kinetic averaging.

Two trends are evident from the contribution of each kinetic component (Figure 3B). First, as the undamaged DNA helix is progressively disrupted from the FBP to the NICK to the 2NT complex, a drop in the fast decay component is compensated by a rise in the intermediate decays, which suggests that these structures adopt alternative conformations other than those described by the fully exposed and fully buried states. It is
notable that the 90–200 ps decay is even more pronounced in the fluorescence decay of a single-stranded 2AP complex (Supporting Information), which suggests that the local structure near 2AP becomes more disordered as the extent of damage increases to the modified site. Comparison of the NICK, GAP, and BLG complexes indicates that the decreasing fast decay is compensated by a rise in the slow decay, which exceeds 6 ns only in the GAP and BLG structures. This indicates that these structures exchange the 2AP residue between intrahelical and extrahelical conformations, with the GAP and BLG structures able to collapse into a helix with 2AP looped out into solution. Furthermore, the calculated numerical lifetimes obtained from the fluorescence decay measurements follow the same trend as the fluorescence intensity change obtained from steady-state measurements (Supporting Information), indicating that the kinetics directly reflect the distribution of DNA conformations.

Our fluorescence data demonstrates that damaged DNA is dynamic and able to adopt multiple individual conformations. We show that DNA repair intermediates that contain a nicked DNA helix remain unaltered relative to other modifications, which is consistent with previous electrophoretic and NMR data obtained on similar DNA structures.5,6 We found that structures that contain a one-nucleotide gap or a bulge adopt a mixture of conformations in which the orphan base resides both inside and outside the DNA helix. The lifetime of the extrahelical state relative to the intermediate and intrahelical states is long when compared to other repair intermediates such as the two-nucleotide gap. This result is consistent with two previous NMR studies that describe the three-dimensional structures for a one-nucleotide gap complex.7,8 In both structures, the DNA adopts a standard B-type helix with the orphan base residing inside the helix. However, in one of the NMR studies, molecular dynamics calculations indicated the presence of two equal populations of DNA conformations—one in which the DNA adopts a standard B-form helix and a second conformation in which the helix is kinked at the gap.7 The molecular dynamics data is consistent with recent electrophoretic data that predicts an anisotropic bend on DNA containing a one-nucleotide gap.9

Data collected on the bulge structure indicates that a single unpaired base in an otherwise intact helix adopts an almost equal distribution of intra- and extrahelical states. Numerous NMR and X-ray crystallography studies have examined DNA structures that contain this modification,10,11 as unpaired bases have been implicated as a possible cause of frame-shift mutations during DNA replication. Comparison of our data to previous structural data demonstrates that we were able to capture the full ensemble of states available to a bulged complex. This data stands in contrast to the NMR structures, which shows an unpaired residue stacked into the helix, and the X-ray crystal structure, which shows a B-form helix with a single looped out residue.10,11 Lastly, analysis of the two-nucleotide gap structure indicates that this modification adopts a conformation in which the 2AP reporter is buried between adjacent bases, presumably in an extended conformation with the unpaired residues open to solvent. To our knowledge, this is the first detailed analysis performed on this type of DNA repair intermediate.

In summary, this report provides a unique opportunity to examine the distribution of structural conformations that are available to different types of DNA repair intermediates. We examined DNA structures that contained a nick, gap, and bulge in their helix, and show that the dominant conformation is not always in agreement with known three-dimensional structures. This finding highlights the importance of studying the complete ensemble of conformations rather than one or two dominant populations of molecules. Because changes in DNA conformation might guide the handoff between DNA repair enzymes, we suggest that the current approach could be used to study the mechanism of DNA repair.

Acknowledgment. This work was supported by grants from the Science Foundation of Arizona (CAA 0265-08) and National Institutes of Health (R21 CA126622-01) to J.C.C. J.W.S. is an Investigator of the Howard Hughes Medical Institute. The streak camera spectrometer was funded by NSF Grant CHE-0541835.

Supporting Information Available: Materials and Methods section, tables showing complexes incorporating 2AP and their steady-state fluorescent intensity under high salt and low salt conditions and fluorescence decay lifetimes of 2AP in DNA, and figure showing a comparison of the fluorescence decay of 2AP, NICK, and FBP with SS. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) For a comprehensive review, see: (a) Lukin, M.; de los Santos, C. Chem. Rev. 2006, 106, 607–686. (b) Patel, D. S.; Shapira, L.; Hare, D. Q. Rev. Biophys. 1987, 20, 35–112.

(2) (a) Nordlund, T. M.; Andersson, S.; Nisson, L.; Rigler, R.; Graeslund, A.; McLaughlin, L. W. Biochemistry 1989, 28, 9095–9103. (b) Rachofsky, E. L.; Osman, R.; Ross, J. B. A. Biochemistry 2001, 40, 946–956. (c) Wan, C.; Rebig, T.; Schleman, O.; Barton, J. K.; Zewail, A. H. Proc. Natl. Acad. Sci. U.S.A. 2000, 97, 14052–14055. (d) Larsen, O. F. A.; van Stokkum, I. H. M.; Gobets, B.; van Grondelle, R.; van Amerongen, H. Biophys. J. 2001, 81, 1115–1126. (e) Jean, J. M.; Hall, K. B Biochemistry 2004, 43, 10277–10284.

(3) (a) Rachofsky, E. L.; Seibert, E.; Stivers, J. T.; Osman, R.; Ross, J. B. A. Biochemistry 2001, 40, 957–967. (b) Guest, C. R.; Hochstrasser, R. A.; Sowers, L. C.; Millar, D. P. Biochemistry 1991, 30, 3271–3279.

(4) (a) Ward, D. C.; Reich, E.; Stryer, L. J. Biol. Chem. 1969, 244, 1228–1237. (b) Eritja, R.; Kaplan, B. E.; Khodakar, D.; Sowers, L. C.; Petruska, J.; Goodman, M. F. Nucleic Acids Res. 1986, 14, 5869–5884.

(5) Nakamura, J.; Walker, V. E.; Upton, P. B.; Chiang, S. Y.; Kow, Y. W.; Swenberg, J. A. Cancer Res. 1998, 62, 6382–6386.

(6) Pieters, J. M. L.; Manst, R. M. W.; van den Elst, H.; van der Marel, G. A.; van Boom, J. H.; Altman, C. Nucleic Acids Res. 1989, 17, 4551–4556.

(7) Roll, C.; Ketterle, C.; Fabris, V.; Fazakerley, G. V.; Bouy, L. Biochemistry 1998, 37, 4059–4070.

(8) Junker, H.-D.; Hoehn, S. T.; Bunt, R. C.; Marathius, V.; Chen, J.; Turner, C. J.; Stubbie, J. Nucleic Acids Res. 2002, 30, 5497–5506.

(9) Guo, H.; Tullius, T. D. Proc. Natl. Acad. Sci. U.S.A. 2003, 100, 3743–3747.

(10) (a) Joshua-Tor, L.; Rabinovich, D.; Hope, H.; Frolow, F.; Apella, E.; Sussman, J. L. Nature 1988, 334, 82–84. (b) Miller, M.; Harrison, R. W.; Wlodawer, A.; Apella, E.; Sussman, J. L. Nature 1988, 334, 85–86.

(11) (a) Patel, D. J.; Kozlowski, S. A.; Marky, L. A.; Rice, J. A.; Broka, C.; Itakura, K.; Breslauer, K. J. Biochemistry 1982, 21, 445–451. (b) Hare, D.; Shapira, L.; Patel, D. J. Biochemistry 1985, 25, 7456–7464.

JP906746W