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COMMUNICATIONS

Published on Web 02/06/2002

The Effect of Varied Ion Distributions on Long-Range DNA Charge Transport

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Received September 24, 2001

Oxidative damage to DNA from a distance has been demonstrated in a variety of systems using a range of photooxidants.[1−4] These studies have been useful not only in delineating new routes to biochemical damage but also in exploring mechanisms for DNA charge transport (CT). Our laboratory has employed metallointercalators to demonstrate oxidative damage over a distance of 200 Å,[5] to explore the effects on CT of intervening DNA sequence,[6] of DNA structure,[7,8] and of protein binding to DNA[9] and to examine DNA CT within the cell nucleus.[10] Typically, DNA assemblies are constructed containing the tethered metallointercalator $Rh(phi)_2bpy'^{3+}$ as the photooxidant, which is spatially separated from two 5′-GG-3′ sites. The extent of charge transport is assessed through measurements of the ratio of yields of damage at the guanine doublet distal versus that proximal to the metal binding site. Theoretical[11] and experimental studies[1−4] have shown that the 5′-G of 5′-GG-3′ sequences in DNA are preferentially oxidized, and this 5′-G reactivity has become a hallmark for electron-transfer damage to DNA. Oxidative damage in these studies is quantitated by measuring strand breaks after piperidine treatment of 5′-^{32}P-end-labeled DNA and gel electrophoresis.[12]

Since CT through well-stacked DNA duplexes appears to be much faster than trapping of the resultant guanine radical by O_2 and H_2O,[13] one might expect that the ratio of the damage at the distal versus proximal guanine doublets would be ≤ 1, assuming that the thermodynamic potentials and the trapping rates at the two sites are equal. Yet, with metallointercalators, distal/proximal damage ratios are significantly >1.[14,15] One explanation that we considered was that the cationic charge on the complex bound near the duplex terminus might be sufficient to increase the oxidation potential of the proximal GG doublet versus the distal site.[19]

To examine how the charge distribution on the DNA helix affects charge transport, we simply compared distal/proximal damage ratios after photooxidation of otherwise identical Rh-tethered assemblies, except for ^{32}P-labeling either at the 5′- or 3′-end (Figure 1). Since the unlabeled end of the oligonucleotide is a hydroxyl moiety, while the labeled end is a phosphate, this labeling difference corresponds, in the absence of charge neutralization by condensed counterions, to an increase in one negative charge on the proximal side of the oligomer and a decrease in two negative charges on the distal side of the oligomer. Table 1 summarizes the results. The highest distal/proximal damage ratio we observed was 5.2 with the 5′-^{32}P-end-labeled assembly containing an intervening A_6 tract (AA (5′-OPO_3^{2-}, 3′-OH)). 3′-end-labeling resulted in a ratio of 0.4 (AA (5′-OH, 3′-OPO_2^--OR)). Thus, moving the negative charge to the proximal end of the duplex dramatically decreased hole transport to the distal end.

Assemblies containing intermediate charge distributions were also examined. In assembly AA (5′-OPO_3^{2-}, 3′-OPO_2^--OR), we added an unlabeled phosphate to the 5′- end but maintained the 3′-^{32}P-end-label. In this case, where some negative charge was now returned to the distal side of the oligomer, the ratio increased to the intermediate value of 0.8. We also introduced a single-base overhang, effectively adding one negative charge to the 3′-end of the Rh-tethered strand. With 3′-^{32}P-end-labeling of the complementary strand, and no phosphate on the 5′-end, the damage ratio was also 0.8 (AA* (5′-OH, 3′-OPO_2^--OR)); an added phosphate on the distal side of the oligomer, through 5′-labeling, increased the ratio to 3.6 (AA* (5′-OPO_3^{2-}, 3′-OH)).

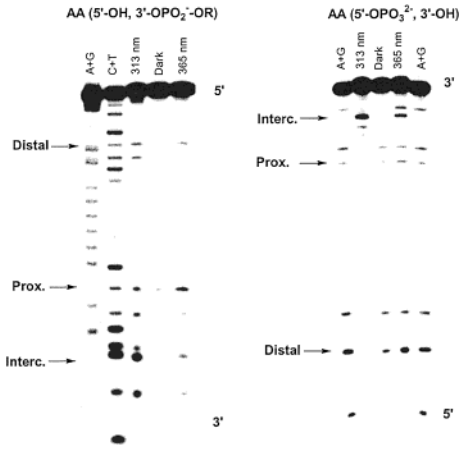

Figure 1. Phosphorimagery of a denaturing 20% polyacrylamide gel that delineates the effect of different labeling on long-range charge transport for assemblies AA (5′-OPO_3^{2-}, 3′-OH) and AA (5′-OH, 3′-OPO_2^--OR), using tethered Δ-$Rh(phi)_2bpy'^{3+}$. The sequence designations are shown in Table 1, where the strand containing the guanine doublets are either 5′- or 3′-^{32}P end-labeled. For each assembly, the lanes are as follows: A+G, C+T show Maxam−Gilbert sequencing reactions; 313 nm shows the DNA fragment after direct photocleavage by the metallointercalator at 313 nm for 10 min without piperidine treatment; 365 nm shows the DNA fragment after irradiation at 365 nm for 20 min at ambient temperature followed by piperidine treatment; Dark shows samples not irradiated but treated with piperidine. All samples contained 4 μM metal complex-tethered duplex, 20 mM Tris-Cl, pH 8, 10 mM NaCl. Sites of distal and proximal 5′-GG-3′ damage as well as the intercalation site are indicated.

It is important to note that these oxidation experiments were conducted under single-hit conditions (at most, one strand break per labeled strand).[21] Thus, the differences seen in ratios with the different labeling cannot be the result of multiple breaks on a given strand, counted differently depending upon the position of the label. Our results must instead reflect how the different charge distributions affect DNA hole transport.

Increasing the ionic strength did not alter the observed oxidative damage ratios. This result is consistent with models for condensed counterion atmosphere distributions, which do not appear to vary appreciably with ionic strength.[22] We also found that changing the associated counterion to Mg^{2+} had no significant effect on the damage ratios.[23]

The possibility that the difference in the amount of guanine oxidative damage observed with changes in ion distributions was a consequence of a conformational change in the A_6-tract[25] was

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10.1021/ja012217e CCC: $22.00

Table 1. The Long-Range Oxidative Damage Obtained in the Presence of Various Charge Distributions, Utilizing the Tethered Photooxidant, $Rh(phi)_2bpy'^{3+}$

Sequence[a]	Charge Distribution[b,c]	Distal/Proximal Guanine[d] Oxidation Ratio
Rh AC GAGCCGTTTTTTGCCGTAT-3' 3'-TG CTCGGCAAAAAACGGCATA-5'		
AA (5'-OPO_3^{2-},3'-OH)	$^{+3}$Rh—OH HO—P 2-*	5.2 (±0.4)
AA (5'-OPO_3^{2-}, 3'-OPO_2^--OR)	$^{+3}$Rh—OH *-1 P 2-	0.8 (±0.1)
AA (5'-OH, 3'-OPO_2^--OR)	$^{+3}$Rh—OH *-1 P—OH	0.4 (±0.1)
Rh AC GAGCCGTTTTTTGCCGTATT-3' 3'-TG CTCGGCAAAAAACGGCATA-5'		
AA* (5'-OPO_3^{2-},3'-OH)	$^{+3}$Rh—OH HO—P 2-*	3.6 (±0.2)
AA* (5'-OPO_3^{2-},3'-OPO_2^--OR)	$^{+3}$Rh—OH *-1 P 2-	0.9 (±0.3)
AA* (5'-OH, 3'-OPO_2^--OR)	$^{+3}$Rh—OH *-1 P—OH	0.8 (±0.5)
Rh AC GAGCCGTAGTCAGCCGTAT-3' 3'-TG CTCGGCATCAGTCGGCATA-5'		
Mixed (5'-OPO_3^{2-},3'-OH)	$^{+3}$Rh—OH HO—P 2-*	1.1 (±0.3)
Mixed (5'-OPO_3^{2-}, 3'-OPO_2^--OR)	$^{+3}$Rh—OH *-1 P 2-	0.5 (±0.02)[e]
Mixed (5'-OH, 3'-OPO_2^--OR)	$^{+3}$Rh—OH *-1 P—OH	0.6 (±0.2)
Rh AC GAGCCGTAGTCATGAGCCG-3' 3'-TG CTCGGCATCAGTACTCGGC-5'		
Mixed-2 (5'-OH, 3'-OPO_2^--OR)	$^{+3}$Rh—OH *-1 P—OH	0.1 (±0.04)

[a] The Δ diastereomer of the $Rh(phi)_2bpy^{3+}$ (phi = 9,10 phenanthrenequinone diimine; bpy′ = 4′-methylbipyridine-4-butyric acid) was utilized in these studies. [b] A pictorial representation of the charge distribution around the oligomers. * denotes the ^{32}P-end labeling. [c] The 5′-labeling procedures were performed using γ-^{32}P ATP and polynucleotide kinase; 3′-labeling procedures were performed using α-^{32}P ATP and terminal transferase. [d] Ratio of the oxidative DNA damage observed at the 5′-G of guanine doublets that were located proximal and distal to the rhodium complex. This damage was measured after photooxidation, utilizing the conditions described in Figure 1. The ratios represent an average of two to four trials. [e] The mixed sequence studies did not always represent single hit conditions, given the lower levels of damage, but the correction would be <10%.

also considered. Results with mixed sequences parallel those using the AA sequences. Interestingly, with these mixed sequences, the distal/proximal ratio was at most only 1.1 compared to 5.2 for the AA-sequences. Thus, the effect of changing the charges at the termini was smaller; 3′-labeling of the mixed sequence yielded a distal/proximal damage ratio of 0.6. With these sequences, we also tested the effect of moving the end-label away from the distal site; in this case, a further decrease in oxidative yield is obtained (Mixed-2 (5′-OH, 3′-OPO_2^--OR)).

We considered that the primary effects might be the result of changes in rates of charge injection into the helix, rather than an effect primarily on thermodynamic potential. Analogous fluorescence measurements of base−base electron transfer,[26] however, showed no significant modulations in fluorescence with changes in the charge distributions at the termini.[27] Therefore, we propose that these results reflect a change in oxidation potential at the distal site relative to the proximal site due to the change in charges at the termini. Changes in the thermodynamic potential of a metalloprotein as a function of pendant charges have been seen.[28] Since DNA has an atmosphere of condensed counterions surrounding it, one might have expected only a minor perturbation in the net charge distribution around the oligomer, but our results indicate that this is not the case.

If the results reflect a change in thermodynamic potential at the guanine doublets, then, on the basis of these data, one can make a coarse calculation of the internal longitudinal dielectric constant of DNA.[29,30] Particularly high values of 10^2 for the dielectric constant, ϵ_r, are obtained using this model, assuming no screening of the pendant charges by counterions; partial screening yielded somewhat lower values (30−300). On the basis of structural modeling, the high values of the dielectric constant may in part also reflect the solvation of the terminal phosphate groups. Importantly, a high longitudinal polarizability has been proposed[31] as a factor in DNA conductivity in electrochemical measurements on DNA films.[32] The high dielectric values obtained here are consistent with such a proposal. Certainly these results suggest that further consideration be given to the longitudinal polarizability of DNA as a factor in mechanisms for charge transport.

Acknowledgment. We are grateful to the NIH for their financial support (GM49216) and to the NSF for a predoctoral fellowship (T.T.W.) We also thank D.M. Crothers for his suggestions.

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JA012217E