Biophysical Journal, Volume 97

Supporting Material

Characterization of photoactivated singlet oxygen damage in single-molecule optical trap experiments

Markita P. Landry, Patrick M. McCall, Zhi Qi, and Yann Chemla

SUPPLEMENTARY MATERIAL

Characterization of photoactivated singlet oxygen damage in single-molecule optical trap experiments

Markita P. Landry[†,¶], Patrick M. McCall[‡], Zhi Qi[§], Yann R. Chemla

MATERIALS AND METHODS

Optical trap

The dual trap optical tweezers has been described in detail previously (1, 2). Briefly, the instrument consisted of two optical traps generated by two orthogonally polarized beams from a single 5-W, 1064-nm diode-pumped solid-state laser (J20I-BL-106C; Spectra Physics, Mountain View, California). The position of one trap relative to the other was controlled by a piezo-actuated mirror stage (Nano-MTA-2; Mad City Labs, Madison, Wisconsin). A custom flow cell served as the experimental trap chamber, and could be displaced relative to the two traps in all directions by a three-axis translational stage (ESP300; Newport, Irvine, California). To visualize the specimen plane, Köhler illumination from a white light-emitting diode (LED) was used. Brightfield images were collected on a charge-coupled device (CCD) camera (902-C; Watec, Orangeburg, New York). The total and relative laser trap intensities were controlled by two independent motorized halfwave plates (Model 8401; New Focus, San Jose, California), as described in Bustamante *et al.* (2). Laser power at the sample plane was determined by taking the geometric mean of the light intensity before the trap-forming objective and after the condenser objective. In the text, laser powers are reported throughout as power at the sample plane for each optical trap.

Fluorescence-optical trap setup. Fluorescence excitation of SOSG was provided by a 488-nm 50 mW laser (Sapphire 488-50; Coherent, Santa Clara, California) aligned for Köhler illumination at the sample plane. The apparatus could switch from brightfield to fluorescence imaging of the specimen plane by reflecting the excitation light with a 488-nm dichroic flip-mount mirror (bandpass 450-515 nm; z488rdc, Chroma, Rockingham, Vermont). Fluorescence

images were monitored through a 525-nm bandpass filter (bandpass 500-550 nm; HQ525/50m, Chroma) by a CCD camera (902-C; Watec, Orangeburg, New York).

DNA preparation

Our dsDNA tethers were synthesized using a 5'-mono-biotinylated forward and a 5'-mono-digoxigenated reverse PCR primer (Integrated DNA Technologies, Coralville, Iowa) to amplify the desired 3.4 kb sequence of the pBR322 *E. coli* plasmid (Fermentas, Glen Burnie, Maryland). A high fidelity Phusion PCR kit (F-513S, Finnzymes, Woburn, Massachusetts) was used to carry out the PCR amplification. Subsequent DNA purification was performed with a Qiagen PCR purification kit with a 50-µl DNA elution volume. An identical protocol was followed for the synthesis of dual-biotinylated DNA tethers, replacing the reverse primer above with a 5'-mono-biotinylated PCR primer with the same sequence.

The DNA hairpin construct was synthesized adapting a protocol by Woodside *et al.* (3). Briefly, the 3131-nt construct consisted of an 89-bp DNA hairpin flanked by two ~1.5-kb dsDNA functionalized "handles". One handle was synthesized from a 1.5-kb PCR-amplified section of the pBR322 plasmid (New England Biolabs, Ipswich, Massachusetts) using a 5'-mono-digoxigeninated primer (Integrated DNA Technologies); the other handle was also PCR-amplified from a different 1.55-kb section of the same plasmid using a 5'-mono-biotinylated primer.

Microsphere preparation

The following microspheres were used in our experiments: 0.79-µm streptavidin (SA) polystyrene particles, 1.0% w/v (SVP-08-10, Spherotech, Lake Forest, Illinois); 0.86-µm protein-G polystyrene particles, 1.0% w/v (PGP-08-5, Spherotech); 2.1-µm SA polystyrene particles, 0.5% w/v (SVP-20-5, Spherotech); 2.1-µm protein-G polystyrene particles, 0.5% w/v (PGP-20-5, Spherotech); 0.78-µm silica microspheres, 10% solids (SS03N, Bangs Laboratories, Fishers, IN), 0.97-µm SA silica microspheres, 1% solids (CS01N, Bangs Laboratories). All microsphere samples were prepared as follows: a 30-µl aliquot of microspheres was washed in 1X phosphate buffered saline (PBS) twice to exchange the microsphere storage buffer by alternating centrifugation and resuspension. To prepare AD microspheres, 10 µl of anti-digoxigenin polyclonal antibody (11 333 089 001, Roche, Indianapolis, Indiana) was added to

the protein-G microsphere aliquot after the final wash. These microspheres were shaken with the antibody for 30 min at low vortex speed and washed again three times in PBS. In all cases the final pellet was resuspended and stored in 200 µl PBS.

Tethering protocol

To attach DNA onto microspheres, 5 µl SA or AD microspheres were vortexed for 5 seconds, and sonicated for 20 seconds to dissemble microsphere aggregates. Subsequently, varying amounts of biotin- and digoxigenin-labeled DNA (typically 5 ng) was incubated with the microsphere aliquot, for one hour at room temperature unless otherwise noted. DNA-microsphere particles were then resuspended in 5 ml TS buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.6). TS buffer was used for all assays unless otherwise noted. The salt concentration was chosen due to previous studies that determined 150 mM to be the ideal ionic condition for the formation of DNA tethers (4).

Tethers were formed and tested in our custom flow cells. These consisted of three separate chambers cut from Nescofilm sealing film (N-1040; Karlan, Cottonwood, Arizona) one to contain AD microspheres, another to contain SA microspheres, the third to contain pure buffer for tether formation. Individual chambers were shunted together by glass capillaries (P0147447; Garner Glass, Claremont, California). First, an AD microsphere was trapped in the stationary optical trap. A SA microsphere was subsequently trapped in the second (movable) optical trap. The microspheres were brought into contact until a tether formed between them, as determined by the observation of force on the microspheres as they were moved apart from each other. Only tethers formed with a single DNA molecule were considered as determined by the observation of tether breakage in a single step. Tethers with two biotin-streptavidin linkages were formed as described by Wuite *et al.* (5).

Quantification of DNA on microspheres

To determine the exact number of DNA molecules on the DNA-microsphere aliquots used in the assay described above, DNA quantization by agarose gel electrophoresis was employed. DNA was incubated with SA and AD microspheres in ratios of 0, 1, 10, 20, 40, 70, 100, 500, and 1000 ng per µl microspheres in a total volume of 47.6 µl. Each aliquot was incubated for 2.5 hours at room temperature. The aliquots were then spun down at 13,000 rpm for 5 min, and a 15-µl

aliquot of the supernatant was run on a 1% agarose gel with ethidium bromide staining, along with a 15-µl control sample corresponding to the amount of DNA incubated with the microspheres. Band intensities were analyzed on a Kodak image station gel scanner (864 2985; Carestream Health, Rochester, NY) to determine the amount of DNA on the microspheres.

The number of DNA molecules per microsphere was calculated using the expression $DNA_{micro} = \left(\left(I_{cont} - I_{micro} \right) / I_{cont} \right) \times DNA_{in}$, where I_{micro} is the band intensity in lanes containing the supernatant of DNA-incubated microspheres, I_{cont} is the band intensity in the corresponding control lanes containing only DNA, DNA_{micro} is the amount of DNA per microspheres in ng/µl, and DNA_{in} is the concentration of DNA in ng/µl in the control lanes. To convert DNA_{micro} into number of DNA molecules per microsphere, we used the molecular weight of our 3.4-kb construct, 2.1×10^6 g/mol / 6.022×10^{23} molecules/mol, and the microsphere number density 3.7×10^7 or 3.0×10^7 microspheres/µl (corresponding to 1% w/v SA and AD microspheres, respectively).

Oxygen scavengers and ROS quenchers

Oxygen scavengers. Two enzymatic oxygen scavenging systems were assayed: the glucose oxidase/catalase oxygen scavenging system (GODCAT), and the protocatechuic acid/protocatechate 3,4-dioxygenase oxygen scavenging system (PCA/PCD). GODCAT was prepared by dissolving 20 mg of glucose oxidase and 4 mg of catalase into 200 µl of T50 buffer (10 mM Tris, 1 mM EDTA, 50 mM NaCl, pH 7). The solution was then centrifuged twice at 13,000 rpm for 5 min, and the supernatant was subsequently centrifuge filtered twice at 11,000 rpm for one min to remove undissolved protein (6). For each GODCAT tethering assay, 1 µl of GODCAT and 0.4% glucose was added to the standard TS buffer. PCA/PCD was prepared by adding 1 mg of PCA and 71.4 µl of 100% glycerol to 71.4 µl KET buffer (100 mM KCl, 2 mM EDTA, 200 mM Tris-Cl, pH 8.0) (7). For each PCA/PCD tethering assay, a buffer composed of TS buffer and 10 nM PCD and 100 mM PCA was used. Tether longevity assays in PCA/PCD and GODCAT were performed as described above.

ROS quenchers. Three singlet oxygen quenchers were assayed: ascorbic acid, lipoic acid, and sodium azide. Each of these was assayed at the maximum quencher concentration that would allow tether formation. Ascorbic acid and lipoic acid both abolish tether formation at

concentrations higher than 12.5 mM and 3.1 mM, respectively. Tether longevity assays in the presence of these three singlet oxygen quenchers were thus performed by making 12.5-mM, 3.1-mM, and 100-mM solutions of ascorbic acid, lipoic acid, and sodium azide (concentration chosen arbitrarily) in TS buffer, respectively. Two hydroxyl radical quenchers were assayed: Tris and manitol. 200-mM solutions of each of these quenchers were made in TS buffer. Tether longevity assays were performed in these solutions as described previously.

Singlet oxygen sensors

Anthracene assay. A 13.6-μM solution of 3-(10-(2-carboxy-ethyl)-anthracen-9-yl)-propinoic acid (CEAPA) in methanol was prepared. Both optical traps were set such that the total power at the sample plane was 1.6 W. The CEAPA/MeOH solution was flowed through a sample chamber in the absence of microspheres at a rate of 10 μl/hr, using a remote-controlled syringe pump (70-2100 PHD 2000; Harvard Apparatus, Holliston, Massachusetts). The laser-exposed and non-exposed control solutions of CEAPA were analyzed by electrospray ionization mass spectrometry (ESI-MS).

SOSG Assay. Singlet oxygen sensor green (S36002, Invitrogen) was prepared by dissolving the contents of one 100-μg vial in 33 μl methanol (~5 mM). 1 μl of this solution was dissolved into 100 μl water for all SOSG assays.

Supplementary References

1. Moffitt, J. R., Y. R. Chemla, D. Izhaky, and C. Bustamante. 2006. Differential detection of dual traps improves the spatial resolution of optical tweezers. Proc Natl Acad Sci U S A 103:9006-9011.
2. Bustamante, C., Y. R. Chemla, and J. R. Moffitt. 2008. Single-Molecule Techniques: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harb. Lab.
3. Woodside, M. T., W. M. Behnke-Parks, K. Larizadeh, K. Travers, D. Herschlag, and S. M. Block. 2006. Nanomechanical measurements of the sequence-dependent folding landscapes of single nucleic acid hairpins. Proceedings of the National Academy of Sciences of the United States of America 103:6190-6195.
4. Fuller, D. N., G. J. Gemmen, J. P. Rickgauer, A. Dupont, R. Millin, P. Recouvreux, and D. E. Smith. 2006. A general method for manipulating DNA sequences from any organism with optical tweezers. Nucleic Acids Res 34:e15.
5. Wuite, G. J., R. J. Davenport, A. Rappaport, and C. Bustamante. 2000. An integrated laser trap/flow control video microscope for the study of single biomolecules. Biophys J 79:1155-1167.
6. Selvin, P. R., T. Lougheed, M. Tonks Hoffman, H. Park, H. Balci, B. Blehm, and E. Toprak. 2008. In Vitro and In Vivo FIONA and Other Acronyms for Watching Molecular Motors Walk. Cold Spring Harbor Press, Cold Spring Harbor.
7. Aitken, C. E., R. A. Marshall, and J. D. Puglisi. 2008. An oxygen scavenging system for improvement of dye stability in single-molecule fluorescence experiments. Biophys J 94:1826-1835.