Supporting On-line Materials

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

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Materials and Methods.

Oligonucleotide Synthesis and Purification. DNA oligonucleotides were obtained in deprotected form from the Keck Facility (Yale University). All oligonucleotides were purified by polyacrylamide gel electrophoresis and reverse-phase HPLC.

Steady State Fluorescence. Steady-state fluorescence measurements were performed both in high-salt (1 M NaCl, 100 mM $MgCl_2$, and 20 mM Tris-HCl pH 8.0) and low-salt conditions (150 mM NaCl, 10 mM $MgCl_2$, and 50 mM Tris-HCl pH 8.0). All measurements were performed at 20 μM oligonucleotide concentration. Samples were heated to 80 °C for 5 minutes, cooled on ice for 10 minutes, and allowed to equilibrate to 24 °C before collecting spectra. Steady-state spectra were collected on a Cary Eclipse Fluorescence Spectrometer; emission at 370 nm was monitored for excitation spectra, and samples were excited at 310 nm for emission spectra. Fluorescent intensities of the complex relative to that of the free 2AP riboside are listed in Table 1.

Fluorescence Lifetime Measurements Using Time-Correlated Single Photon Counting. All fluorescence intensity decay kinetics were performed in 620 mM NaCl, 20 mM $MgCl_2$, and 20 mM Tris-HCl pH 8.0 and 250 μM oligonucleotide concentration. Fluorescence intensity decay kinetics were measured by a time-correlated single-photon counting method (TCSPC) (1). Samples were excited by 10 ps (full-width at half maximum) laser pulses provided by an Nd:YAG laser (Coherent Antares 76) pumped cavity dumped dye laser (Coherent 700 Series Dye Laser), which was tuned to 620 nm and 7.6 MHz. The light was then frequency doubled to 310 nm using a KDP crystal for excitation. Fluorescence emission was detected at 390 nm using a double monochromator (Jobin Yvon HD-10) with a band-pass filter of 20 nm, and photons were collected by a microchannel plate photomultiplier tube (Hamamatsu R2809U-06). The instrument response function was detected at 310 nm using light scattered by Non-Dairy Coffee Mate. Decay curves were collected with a time resolution of 6.28 ps/channel.

Ultrafast fluorescence spectroscopy. The time vs. wavelength fluorescence intensity surfaces were recorded on a system consisting of an ultrafast laser and a streak camera[(2)]. The 130 fs light pulses at 800 nm were generated by a mode-locked Ti:Sapphire laser (Mira 900, Coherent Laser) pumped by a frequency-doubled Nd:YVO_4 laser (Verdi, Coherent Laser). The output was used to seed a regenerative amplifier, which pumped an OPA (RegA and Vis OPA, Coherent Laser). The system provided excitation pulses at 310 nm and repetition rate of 250 KHz. The excitation light was focused onto a sample cuvette with a 3 mm path length. Fluorescence was collected at a right angle to the excitation beam and focused on the entrance slit of a Chromex 250IS spectrograph which was coupled to a Hamamatsu C5680 streak camera with a M5675 synchroscan sweep unit. The polarization of the fluorescence light was measured at 54.7° (magic angle) relative to that of the excitation. The streak images were recorded on a Hamamatsu C4742 CCD camera. Measurements were performed on 2 ns timescale with 1024 pixels of time resolution and spectral resolution was ~ 8 nm.

To reveal any possible emission spectral variations associated with the conformation change, fluorescence intensity of 2AP in each DNA complex was recorded as a function of time and wavelength from 330-420 nm with a wavelength resolution of 8 nm. The instrument response of the streak camera system is 10 – 15 ps, instead of 100 ps in TCSPC. The fluorescence emission

spectra recorded at various time delays follow the laser pulse excitation show no significant systematic spectral evolution over the first 2 ns measured. Therefore kinetic traces of fluorescence decay obtained from streak camera data were integrated from 360 to 410 nm and analyzed as a single kinetic curve. The kinetics from 330 – 360 nm were not included in the integration to avoid the stimulated Raman signal at around 350 nm at early times. The data obtained from TCSPC and streak camera measurements were normalized in the overlapped time region between 1 and 2 ns and fit simultaneously using a multi-exponential decay model.

Kinetic Data Analysis. Time-resolved fluorescence decay curves were analyzed by locally-developed software ASUFIT (http://www.public.asu.edu/~laserweb/asufit/) in MatLab, using a standard deconvolution procedure and nonlinear regression. The fluorescence intensity decay was fit to a sum of exponentials:

$$f(t) = \sum_i A_i e^{-t/\tau_i} \qquad (1)$$

where the preexponential factors A_i are the amplitudes of each component, and τ_i are fluorescence lifetimes. The numerically averaged decay lifetime, τ_{num}, was calculated according to equation (2).

$$\tau_{num} = \sum_i A_i \tau_i / \sum_i A_i \qquad (2)$$

Kinetics for the 2AP containing DNA complexes were fit with up to 4 exponentials according to equation (1). The numerically averaged decay lifetime, τ_{num}, was calculated according to equation (2) and normalized to that of 2AP. The fitting results are summarized in Table II. As can be seen from Table 2 the amount of fluorescence quenching observed from the steady state measurement correlates directly with the average lifetime decrease in the kinetics measurement.

Figure S1 shows fluorescence decay kinetics of single stranded DNA (SS) compared with those from free 2AP, FBP and nick. The lifetimes and amplitudes from 4-component fitting are listed in Table II.

Table S1. Complexes incorporating 2-Aminopurine and their steady-state fluorescent intensity under high-salt (1 M NaCl, 100 mM $MgCl_2$, and 20 mM Tris-HCl pH 8.0) and low-salt (150 mM NaCl, 10 mM $MgCl_2$, and 50 mM Tris-HCl pH 8.0) conditions.

Name	Complex (Fluorophore 2AP = P)	Relative Intensity (High Salt)	Relative Intensity (Low Salt)
r2AP	5′-r-P-3′	1	1
BLG	5'-d-GCTGCCAGTG---GGAACTCTAC 3'-d-CGACGGTCAC-P-CCTTGAGATG	0.4115	0.2159
GAP	5'-d-GCTGCCAGTG GGAACTCTAC 3'-d-CGACGGTCACPCCTTGAGATG	0.1174	0.0547
2NT	5'-d-GCTGCCAGT GGAACTCTAC 3'-d-CGACGGTCACPCCTTGAGATG	0.0272	0.0204
nick	5'-d-GCTGCCAGTGT GGAACTCTAC 3'-d-CGACGGTCACP-CCTTGAGATG	0.0044	0.0045
FBP	5'-d-GCTGCCAGTGTGGAACTCTAC 3'-d-CGACGGTCACPCCTTGAGATG	0.0015	0.0056
SS	3'-d-CGACGGTCACPCCTTGAGATG	0.0357	0.0294

Table S2. Fluorescence decay lifetimes of 2AP in DNA. Data were fit with up to 4 exponential decay components according to equation (1). The relative value of τ_{num} was calculated according to equation (2) and normalized to the value for the 2AP riboside in solution.

	τ_1 (ps)	A_1 (%)	τ_2 (ps)	A_2 (%)	τ_3 (ns)	A_3 (%)	τ_4 (ns)	A_4 (%)	Relative τ_{num}
r2AP	--	--	--	--	--	--	11.0*	100	1
BLG	36.8	44	185.1	13	0.87	4	9.55	39	0.3453
GAP	34.3	60	183.6	16	1.29	4	6.87	20	0.1341
2NT	43.2	49	199.1	24	0.92	17	5.93	10	0.0743
nick	25.0	67	92.1	12	0.93	15	4.52	6	0.0399
FBP	22.1**	96	138.2	2	1.41	2	--	--	0.0047
SS	61.7	60	214.8	33	1.64	5	8.56	2	0.0330

* The fluorescence decay kinetics of 2AP in solution obtained from TCSPC could be fit adequately with a single exponential decay. The early time fluorescence kinetics obtained from streak camera measurement did not show any additional fast decay component (see inset of Figure 3A). Therefore the single exponential fitting result is used. This result is in excellent agreement with the published data[3].

** Analysis using 4 exponential fitting for FBP returns 5 ps (17%), 26 ps (79%), 138.4 (2%) and 1.4 ns (2%), with less than 1% of improvement of the χ^2 value. The first 2 fast components are likely due to the mathematical description when forced to fit with an extra lifetime. No lifetime fell within the category of τ_4, i.e. in the range of 4.5 – 11 ns. Therefore only 3 decay components were presented in Table II with all 3 lifetimes nicely fit in each lifetime group.

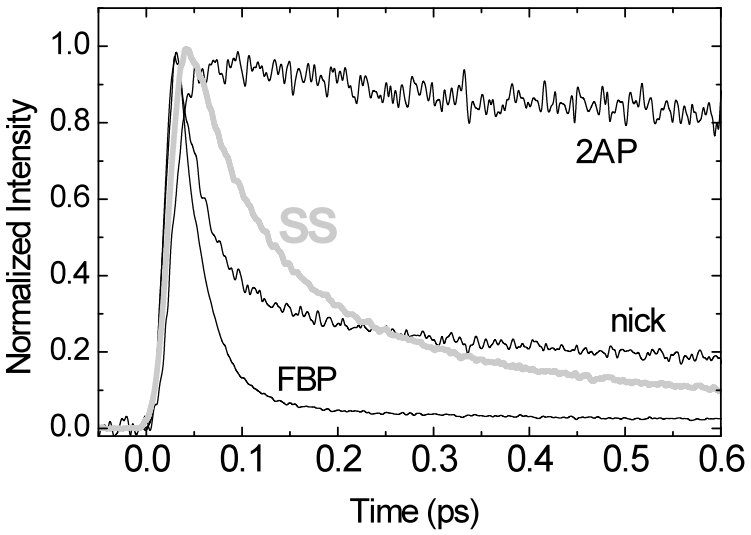

Figure S1. Comparison of fluorescence decay of 2AP, nick and FBP with SS. Data were recorded on the streak camera system.

References

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