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Local diffusive dynamics in DNA A time-resolved fluorescence and molecular-dynamics study of dinucleotides with 2-aminopurine

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Abstract

Fluorescent base analogues such as 2-aminopurine report DNA dynamics on the scale of single bases. We find that the time-dependent fluorescence of various 2-aminopurine-containing dinucleotides can be described by only two components: a fast (~20 ps) exponential decay and a much slower (~1 ns) stretched exponential. This is much simpler than previously proposed models. The fast component reflects quenching in the stacked equilibrium conformation. The slow stretched exponential indicates diffusive dynamics towards the equilibrium conformation. Depending on the dinucleotide, this migration effectively takes place in a one- or two-dimensional manifold. Molecular-dynamics simulations indicate that it involves twisting and sliding with parallel base planes. Our very simple representation of the data provides a powerful tool to study DNA fluorescence quenching and diffusive dynamics independently.

Keywords: DNA; Local dynamics; 2-Aminopurine; Stretched exponential; Molecular dynamics

1. Introduction

2-Aminopurine (2AP) is a fluorescent analog of adenine, obtained by shifting the amino group from the 6- to the 2-carbon position. 2AP is a sensitive probe for local dynamics in DNA. It can replace adenine in a DNA fragment without disrupting the structure [1] or function [2]. Its fluorescence yield is high (67% [3]) compared to that of the natural bases (<0.01% [4]). The absorption of 2AP is red-shifted with respect to that of the natural bases. Thus, it can be selectively excited, even in large DNA fragments.

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When 2AP is inserted in a DNA fragment, its fluorescence is strongly quenched due to interaction with the neighboring bases. The fluorescence decay is multi-exponential [5]. It has been proposed that this is due to structural heterogeneity [6,7], but the nature of the conformations is not known in any detail. The quenching process itself is also under investigation. A part of it has been attributed to electron transfer [6,8], but the involvement of a dark state has also been proposed [5,9,10].

Here, we use stretched exponentials to analyze the fluorescence decay of dinucleotides with 2AP. In dinucleotides, the interaction is limited to a single neighboring base [11], while the fluorescence decay is similar to that in larger DNA fragments [5]. Stretched exponentials have been applied to many disordered processes [12], including dynamics of polymers [13] and DNA [14] and protein folding [15]. The successful description of the kinetic data with a stretched exponential implies a similar physical mechanism for the dynamical fluorescence quenching in dinucleotides.

2. Experimental

Dinucleotides 5′ 2AP–X 3′ with X = adenine, cytosine, guanine, thymine or inosine (a base analog of guanine) have been synthesized by Biolegio (Malden, Netherlands). Free 2AP was purchased from Sigma-Aldrich. In both cases, the specified purity was >99%. All samples were dissolved in 20 mM phosphate buffer (pH = 7.0) with 100 mM NaCl, to OD ≈ 0.1 at the excitation wavelength. In all experiments, $10 \times 4\,mm^2$ quartz fluorescence cuvettes (Hellma) were used. Steady-state absorption and fluorescence spectra were recorded on Cary 5E and Spex Fluorolog spectrophotometers, respectively.

Time-resolved fluorescence spectra were recorded with single photon counting as described elsewhere [5,16]. Briefly, short laser pulses (0.2 ps, 290 nm, 3.8 MHz) were produced with a mode-locked laser setup. Fluorescence was detected through a filter (376.4 nm, FWHM = 7.8 nm) and rotatable sheet polarizer. The arrival times of single photons were determined with time-to-amplitude conversion and accumulated in a multi-channel analyzer to obtain a decay profile. The channel spacing was 7.56 ps. The count-rate was kept below 30 kHz. The instrument response was obtained from para-ter-phenyl in a 1:1 (volume) mixture of cyclohexane and CCl_4 and was typically 50 ps (FWHM).

3. Results

Fig. 1 shows a typical fluorescence-decay profile for 2AP-guanine with the results of an exponential fit. Depending on the dinucleotide, the steady-state fluorescence yield is 7–27%, relative to monomeric 2AP [5]. While the fluorescence decay of monomeric 2AP (not shown) can be fitted with a mono-exponential decay of 9.3 ns, the dinucleotides require a fit with at least four exponentials. Even a four-exponential fit shows some correlations of the same magnitude as the noise in the residual plot. The fastest decay component is in the range of 20–30 ps while the slowest is around 8 ns.

A four-exponential fit provides a good representation of the data. Also, the χ^2 values are excellent [5]. We use this deconvoluted data for

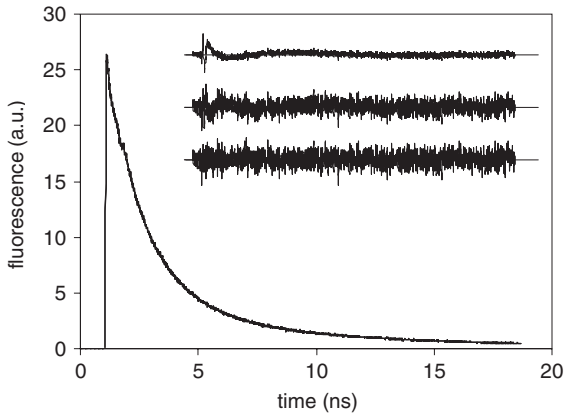

Fig. 1. Fluorescence decay profile for 2AP-guanine (raw data, i.e. before deconvolution) with a four-exponential fit. Inset: Normalized residuals of a fit to 2AP-guanine with (top to bottom) three, four and five exponential decay components on the same timescale.

further analysis with stretched exponentials. Initial attempts to fit the fluorescence decay profiles with a single stretched exponential were not successful. This is because a significant part of the dinucleotides is in a stacked conformation. This fraction is quenched without base dynamics and does not show the characteristics of a stretched exponential. Therefore, we fit the decay profiles with the sum of a fast exponential component and a stretched exponential:

$$F(t) = a_s e^{-(t/\tau_s)^d} + a_f e^{-t/\tau_f}. \quad (1)$$

Here, a_s and a_f are, respectively, the amplitudes of the stretched and fast exponential, while τ_s and τ_f are the corresponding time constants. The power d characterizes the nature of the stretched component.

The result of a typical fit is shown in Fig. 2. As can be seen from the residual plot, the accuracy of the fit to our model is comparable to that of a three-exponential fit. However, it has less degrees of freedom (5 vs. 6) and should thus be regarded as a simpler representation of the data. More importantly, the fit curve follows the data accurately, with a deviation below 1%, so that the fit parameters will provide a good representation of a tentative stretched-exponential component in the fluorescence decay.

Table 1 shows the fit parameters for all dinucleotides in our study. The results show a good consistency. Firstly, a fast exponential component is indeed found in all dinucleotides. The decay time of this component is comparable to the fastest component obtained in four-exponential fits [5,17,18]. This component has been assigned to quenching in a stacked conformation. The biggest and fastest component is found for the guanine dinucleotide. This is consistent with steady-state experiments [5].

The fitted value of the power in the exponential is 0.5 for the purine bases and 0.7 for the pyrimidines. The exception is inosine, which also behaves like a pyrimidine. The distinction between the two cases is significant. If the power is changed from 0.5 to 0.7 or vice versa, a significantly worse fit is obtained. The power is considerably less than one, indicating a low dimensionality for conformational motions.

Table 1
Fit parameters for the fluorescence decay profile of five 2AP-containing dinucleotides

Sample	a_s (a.u.)	τ_s (ns)	d ( )	a_f (a.u.)	τ_f (ps)
2AP-G	34	0.96	0.55	66	21
2AP-A	92	0.63	0.55	11	25
2AP-C	54	1.68	0.74	46	30
2AP-T	45	1.85	0.80	56	32
2AP-I	75	2.20	0.73	25	23

The amplitude (a_s), time-constant (τ_s) and power (d) of the stretched-exponential component and the amplitude (a_f) and time constant (τ_f) of the fast exponential component (see Eq. (1) are shown).

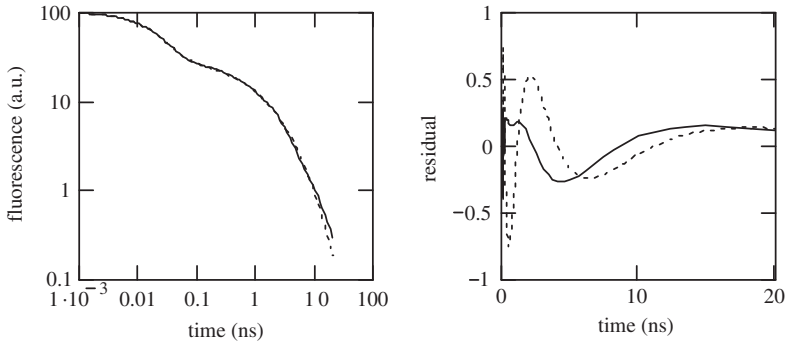

Fig. 2. Comparison of two fits to the fluorescence decay profile of 2AP-guanine. Left: double logarithmic plot of the deconvoluted (see text) data (solid) together with the results of a stretched-exponential fit as in Eq. (1) (dotted). Right: Residual for the stretched-exponential fit (solid) and a three-exponential fit (dotted).

4. Discussion

The stretched exponential law has been derived to describe polarization decay in polymeric and glass materials [19]. The underlying model assumes that frozen dipoles are vacated by the flow of diffusing defects. This suggests a similar mechanism for the slow component in our present analysis. The ground-state equilibrium conformation of the bases is most favorable for the fluorescence quenching. The fast mono-exponential component represents quenching in the central part of the equilibrium distribution. The other conformations are out of equilibrium and fluorescence quenching is achieved after their relaxation to the equilibrium conformation.

The most striking characteristic of our results is that two distinct values are obtained for the power in the exponential (≈ 0.5 and ≈ 0.7). This indicates that the space for the conformational motion of the dinucleotide is restricted in the excited state. A value of 0.5 indicates motion in a one-dimensional space, while a value of 1 indicates motion in three dimensions [19]. The purines move with essentially one degree of freedom (the value is slightly larger than 0.5). The conformational motion of the pyrimidine dinucleotides has a higher degree of freedom but is certainly restricted to a less than three-dimensional space. Preliminary measurements at higher temperatures show that the power parameters are mildly dependent on temperature.

Molecular dynamics simulations have been performed on an adenine–guanine dinucleotide using the Amber99 force field. As shown in Fig. 3, a single conformation dominates while distinct conformations are occurring. The lifetime of these conformations is short with respect to the stretched-exponential decay. The main motions

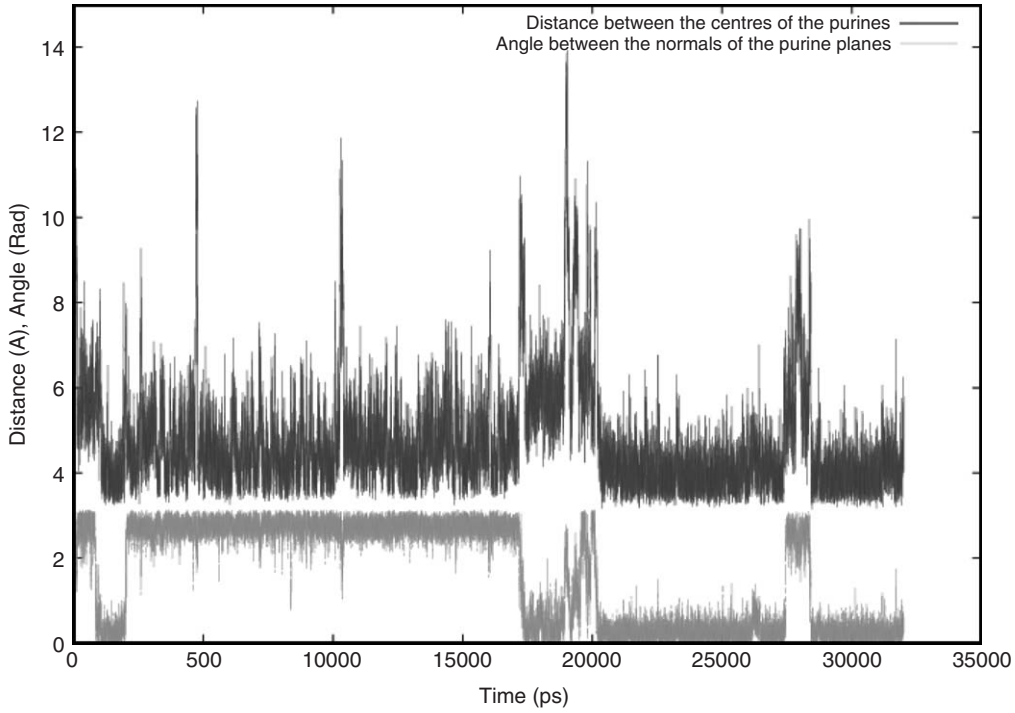

Fig. 3. A trajectory of the distance between the centers of the purines (dark gray) and the angles between their normals (light gray) as obtained by MD simulation of an adenine–guanine dinucleotide using the Amber 99 force field and the Amber8 molecular-dynamics package [20]. A short-range cut-off of 1.0 nm was used in combination with the PME (Particle Mesh Ewald) method for handling the long-range cut-off. The time step was 2 fs. The fluctuations around an equilibrium combined with excursions to a second state are visible.

that can be distinguished involve twisting and sliding of the bases with parallel base planes.

On the basis of power values in our stretched exponentials (see Table 1), we suggest that 2AP dinucleotides with adenine and guanine have effectively only one conformational degree of freedom that involves either the twisting or sliding, while in dinucleotides with cytosine, thymine and inosine, both types of motion lead to the quenching conformation.

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