Ethidium Bromide Binding Sites in DNA Gel

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The analysis of fluorescence decays of ethidium bromide (EtBr) in DNA gels has revealed two EtBr binding sites: a DNA double helix site and a cross-linking site. EtBr preferentially binds to the latter site.

DNA gel is a network of cross-linked DNA. Tanaka et al. have reported a volume phase transition of DNA gel induced by a change in solvent composition.[1] DNA gel is swollen in water, while is shrunken in an acetone–water mixture. A glass-like transition of DNA gel in its dehydration process,[2] following that of egg-white gel,[3] has been found. To date, it has been known that the volume phase transitions of various synthetic and natural polymer gels are induced by any change in temperature, pH, solvent composition and so on. For the synthetic gels, the mechanism of the volume phase transition as well as its application to materials has been studied extensively, but scarcely for DNA gel. DNA gel could become a noble material with controllable volume phases, since its network element DNA exhibits a persistent length change and a coil-globule transition induced by various chemicals.[4–6] Revealing microscopic properties of DNA gel based on biophysical information on DNA and examining its application are attractive subjects. Fluorescent probe techniques are known to be useful for studies on microscopic properties of synthetic gels.[7] To our knowledge, however, the techniques have not been applied to DNA gel so far. Ethidium bromide (EtBr) is well-known as a cationic stain dye for DNA, which intercalates between adjacent base pairs of double-stranded DNA to become strongly fluorescent. Due to a fluorescence lifetime as long as 30 ns in the intercalating state, EtBr has been utilized to probe the torsional and bending dynamics of DNA.[8,9] Such peculiar fluorescence properties of EtBr to DNA should also allow us to study the microscopic properties of DNA gel. At the first stage of the study, EtBr binding sites in DNA gel should be revealed.

DNA (SIGMA, Type III, sodium salt from salmon tests) in water (20% of water) was cross-linked using ethylene glycol diglycidyl ether (50% of DNA) at 55 °C, pH 11, for 3 h. After gelations in a microcapillary tube of 1.25 mm in the inner diameter, rod-like gels 3 mm long were extracted from the tube. The gels were washed with plenty of water to remove residual chemicals, and then stained in 6×10^{-5} M EtBr-buffer solution (tris 5 mM, NaCl 50 mM, pH 7). In order to obtain a swelling ratio curve, the stained gels were immersed in acetone-buffer mixtures of various compositions for 24 h, and then the volume was measured. For the measurements of the fluorescence spectrum and decay of EtBr, three types of DNA samples stained by EtBr were prepared: a DNA solution (30:70 DNA-buffer weight composition), a swollen DNA gel and a shrink DNA gel. The swollen gel contained no acetone. The shrink gel was prepared by immersing a swollen gel in a 90:10 acetone–buffer mixture. The fluorescence spectra were measured by a fluorescence spectrometer (Hitachi 850) whose the resolution was 5 nm and the excitation wavelength 520 nm. For the fluorescence decay measurements, a time-resolved single photon counting fluorescence spectrometer (Horiba NAES-700) was employed. The excitation source was a pulsed dye laser beam, whose the wavelength was 520 nm and the pulse width 800 ps. The fluorescence decay was monitored at 600 nm with a bandwidth of 50 nm.

Figure 1 shows the swelling ratio curve of stained DNA gel. A discontinuous volume phase transition is observed at 65:35 acetone–buffer composition. The volume change is about 16 times. The swelling ratio curve of DNA gel, not stained, shows a discontinuous volume phase transition at a 63:37 acetone–water composition,[1] which is slightly lower than that for stained DNA gel. Comparing the swelling ratio curves of the two DNA gels, one can find that the discontinuity of the volume phase transition of stained DNA gel is somewhat smaller. Although the staining

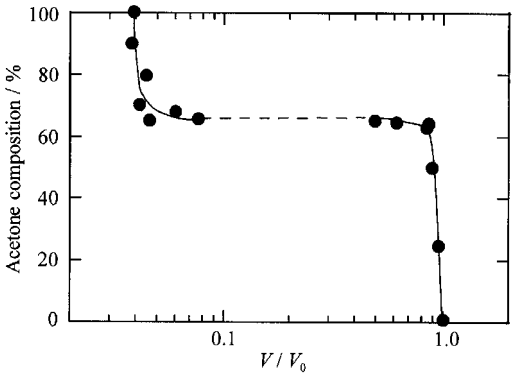

Figure 1. Swelling ratio curve of DNA gel stained by EtBr. V_0 is a volume of the DNA gel in the buffer solution and V is that after immersion in an acetone-buffer mixture. The line is a guide for eyes.

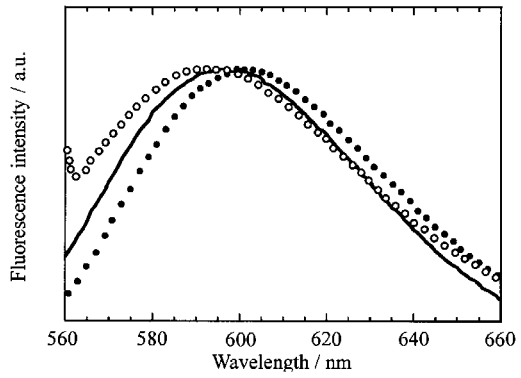

Figure 2. Fluorescence spectra of EtBr in three different environments as follows: the DNA solution (closed circles), the swelling DNA gel (solid line) and the shrink DNA gel (open circles).

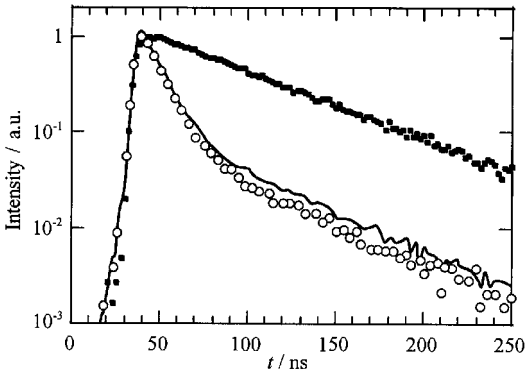

Figure 3. Fluorescence decay curves of EtBr in the DNA solution (closed squares), in the swelling DNA gel (open circles) and in the shrink DNA gel (solid line).

slightly affects the features of the volume phase transition, it never changes the macroscopic nature.

The normalized fluorescence spectra of EtBr in the DNA solution and the swollen and shrink DNA gels are shown in Figure 2. The spectral shapes for the three DNA samples are similar, though the spectral peaks for the DNA gels are blue-shifted by more than 4 nm with respect to the DNA solution. Unlike the fluorescence spectra, the fluorescence decays of the three DNA samples, whose the curves are shown in Figure 3, provide definitive information on EtBr binding sites in DNA gel. Obviously, the decay curves of the DNA gels and the DNA solution are considerably different. The decay curves for the three DNA samples $I(t)$ were analyzed by means of a non-linear least square fitting method using a trial function $Q(t)$ so as to minimize χ^2,

$$Q(t) = \int_0^t P(t')L(t-t')dt', \qquad (1)$$

$$\chi^2 = \frac{1}{n-1}\sum_{t=1}^{n}\frac{[I(t)-Q(t)]^2}{I(t)}, \qquad (2)$$

where $P(t)$ is a multi-exponential function, $L(t)$ an instrumental function including a temporal profile of the excitation laser and the time response of the detection system and n the number of the sample points. As a result of the analysis, it was found that all the curves could be best-fitted by using $P(t)$ which consists of only two exponentials as follows:

$$P(t) = A[f\exp(-t/\tau_f) + (1-f)\exp(-t/\tau_s)], \qquad (3)$$

where A is an amplitude, τ_f and τ_s the lifetimes of the fast and slow components and f the fraction of the fast component. Best-fit results for τ_f , τ_s and f are listed in Table 1 along with χ^2. Miller et al. have found two fluorescence decay components of EtBr in calf thymus DNA, plasmid DNA and synthetic polynucleotides: the fast component with a lifetime of 2–3 ns originates from non-intercalated EtBr, which is not free but somewhat perturbed by DNA, and the slow one with a lifetime of 20–28 ns from intercalated EtBr.[8] Similarly the origins of the fast and slow decay components for all the DNA samples prepared in the present work can be assigned to non-intercalated EtBr and intercalated one, respectively. As can be seen from Table 1, the considerable difference between the fluorescence decay curves of the DNA gels and the DNA solution is ascribed to the fractions of non-intercalated EtBr, that are larger in the DNA gels. There are two distinct sites in DNA gel: a covalent cross-linking site and a DNA double helix site that is the intercalative binding site of EtBr. A plausible explanation for the larger fractions of non-intercalated EtBr in the DNA gels is that EtBr preferentially binds to the cross-linking site. Because DNA has no cross-linking site, the microenvironment of non-intercalated EtBr binding to the site in DNA gel should be different from that of EtBr perturbed by DNA. This is supported by a difference between the observed fluorescence lifetimes of non-intercalated EtBr in the swollen DNA gel and in the DNA solution. Moreover, since the cross-linking site is rigid, the microenvironment of non-intercalated EtBr binding to the site, as well as the microstructure of the site, before and after the volume phase transitions is expected not to change significantly. This is supported by a quite small difference between the fluorescence lifetimes of non-intercalated EtBr in the swollen and shrink gels. On the other hand, there is an appreciable difference between the fluorescence lifetimes of intercalated EtBr in the swollen and shrink gels. This could be ascribed to a difference between the microstructures of the flexible DNA double helix site before and after the volume phase transition.

Table 1. Best-fit results obtained from fluorescence decay analyses of EtBr in the three DNA samples

Sample	τ_f / ns	τ_s / ns	f / %	χ^2
DNA solution	2.20	24.8	11.8	1.26
Swelling DNA gel	1.07	21.0	87.5	1.26
Shrink DNA gel	1.20	24.5	85.1	1.18

Since no structural information on DNA gel has been obtained so far, it will be meaningful to speculate the structure of the cross-linking site by taking into account the cross-linking process. Ethylene glycol diglycidyl ether (EGDE) used in the present work interstrand-cross-links single-stranded DNAs in epoxy polymerization; epoxy groups at both the ends of EGDE can react with active amine groups of the DNA bases and a phosphate group on the DNA backbone. The condition of pH 11 stimulates the separation of double-stranded DNA into a pair of single-stranded one and, as a result, the cross-linking. The separation is partial, however, because the intercalated EtBr is observed in the stained DNA gels. If many parts of single-stranded DNA undergo multiple interstrand cross-links, the cross-linking site could have a rigid and tight bundle structure to trap EtBr for a long time. Since the site involves many negatively charged phosphate groups, it is likely that the cationic form of EtBr electrostatically binds to the site.

In the present work, EtBr binding sites in DNA gel are revealed by the time-resolved fluorescence measurements. The site-specific information on DNA gel will be useful for further studies so as to reveal microscopic properties of DNA gel, such as the inhomogeneous microstructures and the structural fluctuations before and after the volume phase transition.

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