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# Highly Fluorescing Solid DNA-Cationic Polyelectrolyte Complexes Prepared from a Natural DNA and a Poly(fluorenevinylene-alt-phenylene) Bearing Quaternary Ammonium Pendants

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**Abstract:** A fluorescing, copolymer (Q)-bearing, quaternary ammonium pendant was mixed with excess natural salmon sperm DNA with a molecular weight of $1.3\times10^6$ (2,000 base pairs) to afford highly fluorescing, complex mixtures. The fluorescence life-time of the polymer Q was greatly increased when mixed with DNA: for the mixture of Q:DNA=1:750 the fast and slow decay lifetimes increased from ca. 10 to 100 ps and from 20 ps to ca. 1 ns, respectively. The enhanced fluorescence of the mixtures was ascribed to efficient compartmentalization and reduced conformational relaxation of the polymer Q by complexation with excess DNA.

*Keywords*: DNA, fluorescence, complexation, polyelectrolyte, conjugated polymer.

## Introduction

The interest in the materials science on natural and synthetic DNA is rapidly increasing among scientists and engineers, although up until recent time DNA have been regarded as a class of biopolymers only relevant to life science associated with genes or genetic information. While the biological importance of DNA is still being explored intensively in different perspectives, many research groups are focusing their interests in materials aspect of DNA. Biological sensing[1,2] based on DNA's ability to form complementary base pairs has been a research subject of great interest. Such applications of DNA science mainly rely either on electrochemical method or fluorescence spectroscopy. Another popular research area is self-assembly capability of DNA,[3-8] which is utilized in the construction of one, two, or three dimensional structures, very often, of nano sizes. The new computational logic[9,10] based on DNA' recognition ability also is extremely didactic. Utilization of DNA templates in the preparation of metal nano particles[11,12] and wires[13,14] is expanding their values into nano worlds. Electrical properties[15,16] of DNA have been the research subject of many groups. But, the exact nature of their electrical conductivity is still in debate although more people appear to believe that they belong to semiconductors having wide-gaps. We[17-19] and others[20,21] recently reported magnetic properties of DNA, which are considered rather unique. In addition, optical and electro-optical properties of modified DNA have been to be very attractive in applications such as waveguides,[22] organic lasers,[23] and even in the fabrication of organic light-emitting diodes (OLEDs).[24] For those applications DNA[24,25] whose sodium ions were replaced with long alkyl chain quaternary ammonium ions were utilized because of their easy solubility in volatile organic solvents such as alcohols and tetrahydrofuran.

Since natural DNA is water-soluble polyelectrolyte, their complexes or mixtures with other water-soluble polyelectrolyte or amphiphilic compounds have been studied with regard to DNA detection or optical sensors,[26] energy transfer,[27,28] and fluorescence amplification.[23,27,29] DNA complexes

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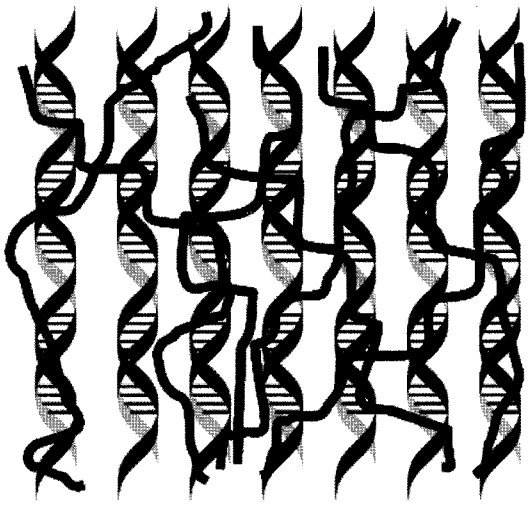

**Figure 1.** Mixture of DNA and Polymer Q.

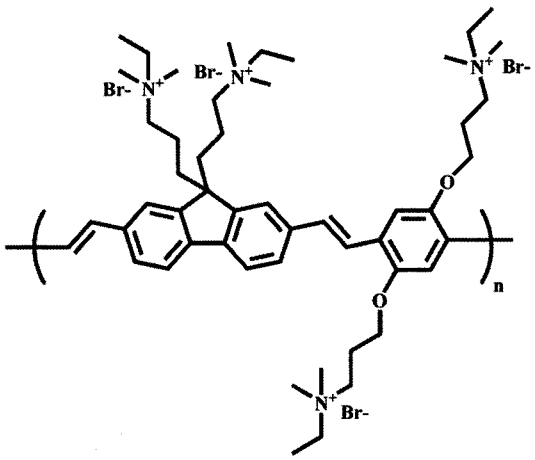

**Figure 2.** Polymer Q.

with soluble polyconjugated polyelectrolyte, in particular, are closely related with those described in this report.[27-29] In this report, we would like to discuss the fluorescence properties of a series of solid mixtures of a natural DNA partially complexed with a water-soluble fluorescing polyconjugated polymer (polymer Q[30] whose structure is shown below in Figure 2) that bears quaternary ammonium pendant groups (Figure 1). Applications of polyelectrolyte polymers are very diverse, which can be found elsewhere.[31-33]

## Experimental

The salmon sperm DNA sample used in the present investigation was purchased from Aldrich Chemical (U.S.A.) and had molar mass of $1.3\times10^6$ (2,000 base pairs). The average molar mass of the repeating nucleotide unit is estimated to be 325 g/mol. This DNA sample is the same as the one described in one of our recent papers.[17-19] The fluorescing polyelectrolyte polymer Q is the same as described earlier by us[30] and the molar mass of the repeating unit is 1096 g/mol. Aqueous solutions of DNA ($5.94\times10^{-2}$ M in the repeating unit) and polymer Q ($9.12\times10^{-5}$ in the repeating unit) were mixed at room temperature in varying proportions so that we could attain a series of solutions of Q: DNA in the mole ratio of the repeating units of 1: 100-1,000. In other words, all the solutions contained far excess DNA. The solutions were dialyzed against triply distilled water using dialysis tubes (Aldrich Chemical, molecular weight cut-off: 12,500) to remove sodium bromide formed from the polymer Q and DNA and other low molar mass impurities. The dialyzed solutions were cast on quartz plate to prepare uniform films of ca. 1 μm thick. The films were finally dried at 70 °C for 1 h under vacuum (ca. $1\times10^{-4}$ torr). UV-Vis absorption spectra of the films were recorded on a Hewlett Packard (U.S.A.) 8452 Diode Array spectrophotometer. Their fluorescence spectra were obtained on an AMINCO-Bowman Series 2 (U.S.A.) fluorometer.

**Fluorescence Lifetime Measurement.** For time-resolved fluorescence studies, all samples were excited with 315 nm picosecond pulses generated from a Raman shifter (18 atm, $CH_4$) which was pumped by the fourth harmonic pulse (266 nm, fwhm 20 ps, 10 Hz) of a mode-locked Nd:YAG laser (Continuum, Leopard D10, U.S.A.). Time-resolved fluorescence spectra[34] and decay data were detected with a picosecond streak camera (Optronics, SCMU-ST-S20, U.S.A.) coupled to a spectrometer (CVI, DKSP240,U.S.A.) and a CCD system (Optronics, SCRU-SE-S, U.S.A.). The typical accumulation number for a fluorescence spectrum was 100 laser shots. Fittings of the fluorescence decays were carried out by a non-linear least squares iterative deconvolution method.

## Results and Discussion

**UV-Vis Absorption and Fluorescence.** Figure 3 compares the UV-Vis absorption spectra of the thin films (1.0 μm

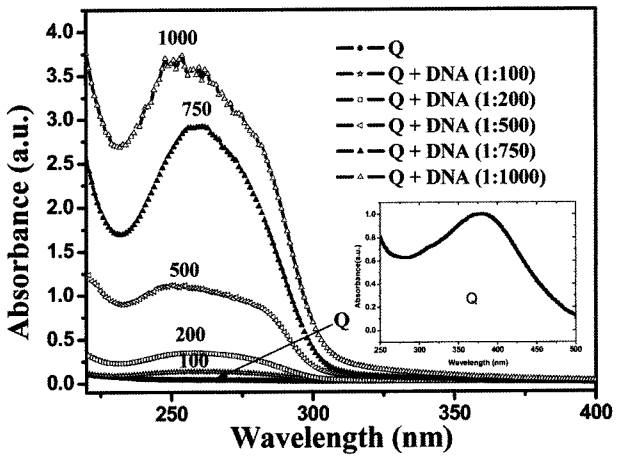


**Figure 3.** UV-Vis spectra of DNA-Q complexes (molar ratio of the repeating unit).

thick) of the polymer Q and DNA mixtures in varying mole ratio of the repeating units of the two polyelectrolytes. DNA's maximum absorption position is located at 260 nm whereas $\lambda_{max}$ of Q is at 380 nm (refer to the inset in Figure 3). This absorption arises due to the presence of $\pi$-electron systems. The absorption by DNA dominates the spectra of the mixtures due to DNA's higher concentration and stronger absorbance. No special absorption spectral feature is observed for the mixtures.

The situation for their fluorescence spectra (Figure 4(a)), however, is drastically changed. Although the fluorescence properties of DNA are rather complex and the fluorescence spectrum at room temperature is very weak and broad (ca. 300-450 nm), Daniels[35] first succeeded in obtaining salmon sperm DNA's fluorescence spectrum at room temperature and found that the integrated quantum yield relative to adenine was $\phi_f = 2\times10^{-5}$ at the excitation wavelength of 250 nm. The maximum fluorescence was observed at 357 nm, which is not far from the position of the maximum absorption position of the polymer Q (380 nm).[30] The fluorescence from DNA is so weak that there were even last instances[36] where DNA was considered non-fluorescence. The spectra shown in Figure 4 were obtained at room temperature and at the excitation wavelength of 315 nm. We can extract two most important observations from this figure: 1) the fluorescence intensity integrated over 425-700 nm, that is originated from the polymer Q, increases 10.4 times when the polymer Q was mixed with DNA in the ratio of 1:750 and 2) the fluorescence emission maximum shows a slight red-shift from 500 nm for the polymer Q to 516 nm for the mixture of Q:DNA=1:500 and 510 nm for the Q/DNA=1/750 mixture.

When the polymer Q was further diluted with DNA, the fluorescence intensity was diminished due to too much lowered concentration of the light-emitting polymer Q in the mixture and also possibly to the presence of too much scattering centers of DNA aggregates. The fluorescence intensity, as estimated from the peak area over 425-700 nm in Figure 4(b), reveals a steady increase as the polymer Q is diluted with DNA up to Q:DNA=1:750: the fluorescence intensity of the composition of Q/DNA=1/100 is 3.0 times, Q/DNA=1/200 4.2 times, Q/DNA=1/500 8.0 times and Q/DNA=1/750 10.4 times of the polymer Q. But, further dilution to Q/DNA=1/1,000 reduces the fluorescence amplification to 5.4 times the fluorescence of the polymer Q. This is an impressive elevation of emission intensity with dilution, which is far much more than that expected from simple dilution effect, a reverse phenomenon to so-called concentration quenching. It was earlier reported[30] that the quantum efficiency of the polymer Q in ethanol is 0.32. Its quantum efficiency in the solid state, however, is not yet available, although it is expected to be far lower than in solution. We believe that the polymer Q molecules are efficiently compartmentalized in the continuous phase of DNA through complexation. Compartmentalization or reduction of intermolecular contacts among fluorescing molecules is known to increase fluorescence efficiency by reducing the radiationless transition pathways of the excited states of chromophores. Moreover, it is expected that strong electrostatic attraction between the neighboring Q and DNA chains reduces vibronic energy loss of the excited states of the Q chains. Such fluorescence amplification has been reported and discussed for many other DNA-polyelectrolytes[29] or fluorescent intercalator mixtures.[22,23]

In addition, there is a possibility for Föster resonance energy transfer or fluorescence resonance energy transfer (FRET)[28] between the surrounding DNA molecules and embedded Q chains. DNA emission although its intensity may be insignificant, overlaps well with the absorption range (300-475 nm) of the polymer Q, which favors the FRET process. We, however, believe that this makes only minor contribution in the total fluorescence of the mixtures due to the extremely poor emitting capability of DNA molecules as mentioned earlier.[35] As implied by Liu and Bazan,[27] we observed a red-shift for the fluorescence of the polymer Q in the Q/DNA mixtures, although we did not observe any peculiar or conspicuous changes in the UV absorptions of the composites.

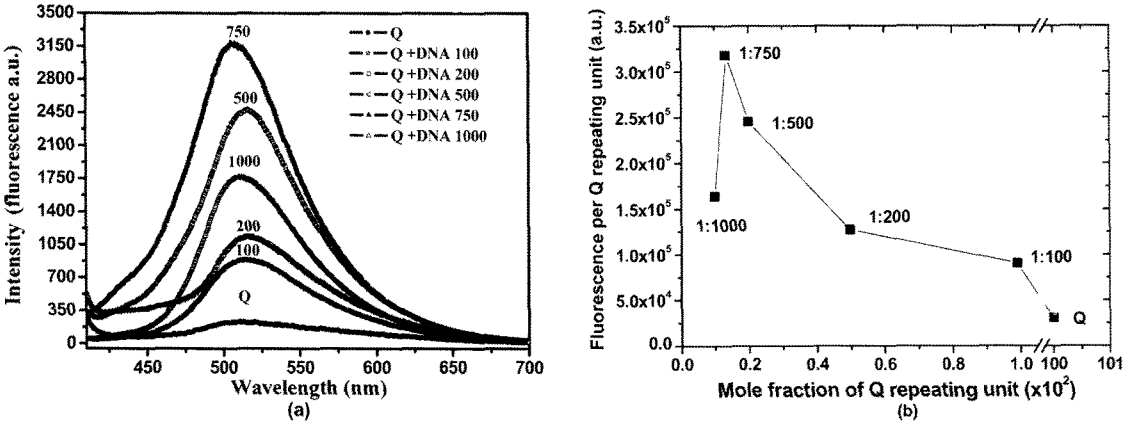


**Figure 4.** Fluorescence spectra (a) of DNA-Q complexes (molar ratio of the repeating unit) and (b) dependence of fluorescence intensity per Q repeating unit on its mole fraction in the mixtures.

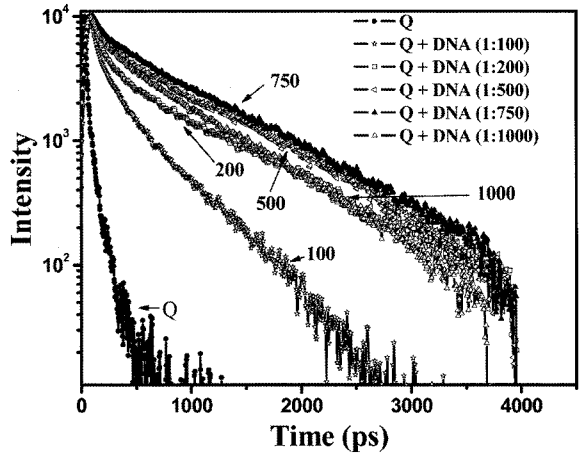


**Figure 5.** Time-resolved fluorescence decay profiles of DNA/Polymer Q blend films (ca. 1.0 μm thick) ($\lambda_{em}$=510 nm).

For example, the compositions of Q/DNA=1/100~1/50 exhibit fluorescence maximum at 516 nm and Q/DNA=1/750 and 1/1,000 at 510 nm, all of which are red-shifted from the original emission maximum at 500 nm for the polymer Q's fluorescence. This is taken as an indication that electrostatic complexation between Q and DNA gives rise to an increase of the coplanarity and/or less bent backbone structures. Liu and Bazan[27] earlier observed even a stronger red-shift for a poly(fluorine-*co*-phenylene) bearing quaternary ammonium pendants when it was complexed with a single-strand DNA.

**Time-resolved Fluorescence Decay.** We made a time-resolved fluorescence study of the mixture films and the decay profiles are shown in Figure 5. It is rather impressive to note that fluorescence decay of the polymer Q emission at 510 nm slows down significantly, but gradually, as we increased the amount of DNA complexed with it. The reduced fluorescence decay was observed up to Q:DNA =1:750 and then the decay became a little faster for Q:DNA =1:1,000, which is in complete parallelism with what we described above in the discussion of fluorescence intensity changes of the mixtures.

We could satisfactorily fit the decays curves to the simple two time constants equation of $I(t)=A_1\exp(-t/\tau_1)+A_2\exp(-t/\tau_2)$, where $I(t)$, $A$'s and $\tau$'s are the time-dependant fluorescence intensity, relative amplitude, and time constants, respectively. $\tau_1$ and $\tau_2$ respectively correspond to fast and slow time constants. The results are summarized in Table I.

Very often $\tau_1$ is related to intrachain decays, whereas $\tau_2$ to interchain decays. According to the estimated parameters given in Table I, $\tau_1$ and $\tau_2$ reveal significant gains as the content of DNA in the mixtures increase, whereas $A_1$ and $A_2$ steadily diminish. We observe a consistent exception with Q:DNA=1:1,000, the reason of which is not yet quite understood clearly. It is very possible that dilution of the polymer Q with DNA diminishes the intramolecular events in fluorescence decay, which matches very well the enhanced $A_1$ and

**Table I. Fluorescence Decay Parameters**

| $\lambda_{em}$=510 nm | $A_1$ | $\tau_1$(ps) | $A_2$ | $\tau_2$(ps) |
|---|---|---|---|---|
| Q | 0.03 | 12 | 0.97 | 20 |
| Q:DNA (1:100) | 0.83 | 61 | 0.17 | 423 |
| Q:DNA (1:200) | 0.79 | 95 | 0.21 | 912 |
| Q:DNA (1:500) | 0.64 | 96 | 0.36 | 931 |
| Q:DNA (1:750) | 0.52 | 106 | 0.48 | 992 |
| Q:DNA (1:1000) | 0.58 | 110 | 0.42 | 789 |

$I(t)=A_1\exp(-t/\tau_1)+A_2\exp(-t/\tau_2)$, where $I(t)$, $A$, and $\tau$ are the time-dependent fluorescence intensity, relative amplitude, and lifetime, respectively.

$\tau_1$ values. At the same time, the dilution will hinder the intermolecular fluorescence processes, which are reflected on the decreased $A_2$ values and much lengthened $\tau_2$ as shown in the last two columns in Table I. In other words, $\tau_1$ and $\tau_2$ both significantly increase as we increase the dilution of the polymer Q with DNA. The $\tau_1$ value increased about 10 times from 12 to 110 ps, and $\tau_2$ about 50 times from 20 to 1,000 ps or 1 ns. Meanwhile, the ratio between $A_1$:$A_2$ changed from 0.03:0.97 to 0.43: 0.57. It also is very possible that $\tau_1$ and $\tau_2$ for Q arise from the spectral inhomogeneity of the sample. If this is the case, our observation can be interpreted by a single fast (~20 ps) relaxation of Q that slows down as DNA is added. But the fact that the vast difference between the magnitudes of $\tau_1$ and $\tau_2$ values for the mixtures leads to the conclusion that this conjecture is less likely to be true.

## Conclusions

We have conducted a study on the fluorescence of blends comprising a natural DNA and a water-soluble fluorescing polyconjugated polyelectrolyte bearing quaternary ammonium pendants. This investigation clearly demonstrates that replacement of sodium ions with the cationic polyelectrolyte polymers is a very efficient in isolating or compartmentalizing the latter in the mixtures. This leads to an impressive increase in the fluorescence intensity of the polymer even on high dilution with DNA. The time-resolved fluorescence study of the mixtures teaches us that dilution results in a significant enhancement in fluorescence lifetime, $\tau_1$ as well as $\tau_2$, and effectively control the inter- and intramolecular decay processes. Complexation between the cationic polymer and the anionic DNA molecules appears to stiffen the backbones of the former leading to a red-shift in its fluorescence emission. The observed enhancement of fluorescence may be utilized in sensing DNA.

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