Immobilization of heptyl viologens in DNA strands both to inhibit dimerization and to accelerate quasi-reversible electron transfer reaction

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Viologens, mono-molecularly immobilized in DNA grooves, show reversible colour changes without attenuation during potential switching after covering with ionic liquid type polymers.

N,*N*′-Dialkyl-4,4′-bipyridinium halides, commonly called viologens, are redox-active organic dyes with electrochromic properties.[1–3] They show alternating changes between deep blue and pale yellow as a result of their redox reactions.[4] However, viologen mono-cation radical is not particularly stable, and is easily dimerized in homogeneous solution and even at the modified electrode.[5–7] Since dimerization of viologens is electrochemically irreversible, their absorbance diminishes during electrochemical redox cycling.[5] For a striking colour change, the viologen concentration should be high, but this increases the chance of dimerization. There are several studies of the inhibition of viologen dimerization.[8,9] However, there is no effective way to ensure colour change that is both striking and reversible.

The colouring of dye molecules such as viologens is due to their conjugated π-electron orbital. The π-conjugated and planar heteroaromatic compounds form complexes with DNA double helix through intercalation or groove binding.[10] As a result of their strong binding with DNA to inhibit gene replication, these dyes are recognized as pathogens. From the viewpoint of mono-molecular fixing of dye molecules, complexation with DNA is an excellent way to suppress dimerization of dye molecules.

In the present study, we use DNA sodium salt (Na-DNA) as a host for viologens. The DNA–viologen complex was mixed with an ionic liquid as a supporting electrolyte matrix (Fig. 1). A transmission-type electrochromic cell was fabricated using an indium–tin oxide (ITO) glass electrode coated with this complex. Several parameters were varied so as to improve the colour change of the cell. We chose *N*,*N*′-diheptyl-4,4′-bipyridinium dibromide (HV). HV has been studied extensively as an electrochromic dye.[11] The HV was mixed with DNA sodium salt (about 500 base pairs) in a molar ratio [DNA base pair (bp)] : [HV] of 10 : 1 in aqueous solution. The concentration of DNA is expressed as base pairs. *N*-Ethylimidazolium tetrafluoroborate ($EtImBF_4$), *N*-propyl-*N*′-vinylimidazolium bromide (IL_A), ethylimidazolium vinylphosphate (IL_B), and their homopolymers (pIL_A, pIL_B) or copolymer ($CoIL_m$: Scheme 1) were synthesized as reported previously.[12] These ionic liquids were mixed with an aqueous solution of DNA–HV complex. A DNA solution (2.5×10^{-2} M) 400 μl was cast on an ITO glass electrode (4.0 cm^2), and dried gently under reduced pressure at room temperature. The film-coated ITO glass electrode was faced with another ITO glass electrode which had been pre-coated with pIL_A as a supporting electrolyte (Fig. 2). These cells were analyzed by UV-vis spectroscopy (UV-2500PC, Shimadzu) under an applied voltage.

We prepared DNA–HV–$EtImBF_4$ complex film (10 : 1 : 10 by mol). The cell composed of this complex showed a colour change when a voltage of more than ±1.4 V was applied. Fig. 3 shows visible absorption spectra after applying the voltage. The DNA–HV–$EtImBF_4$ complex film turned blue, due to the HV monomer, *i.e.* radical cations (Fig. 3(b), λ_{max} = *ca.* 600 nm) upon reduction.

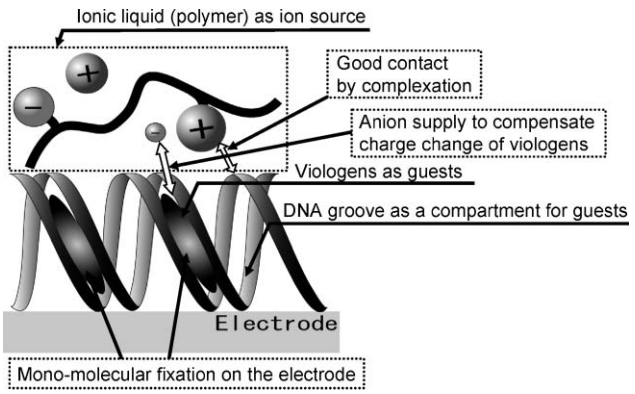
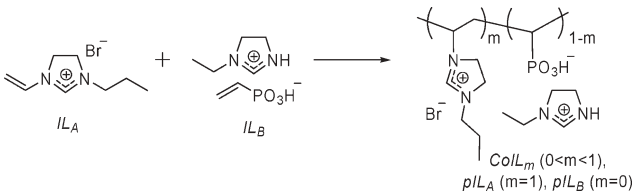


Fig. 1 DNA–viologen complex covered with ionic liquid polymer.



Scheme 1 Synthesis of copolymerized ionic liquids.

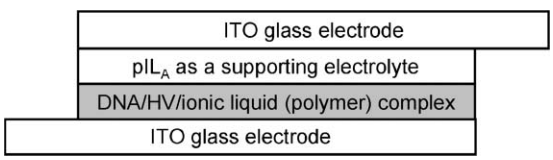


Fig. 2 Scheme of an electrochromic cell composed of DNA–viologen–ionic liquid polymer film.

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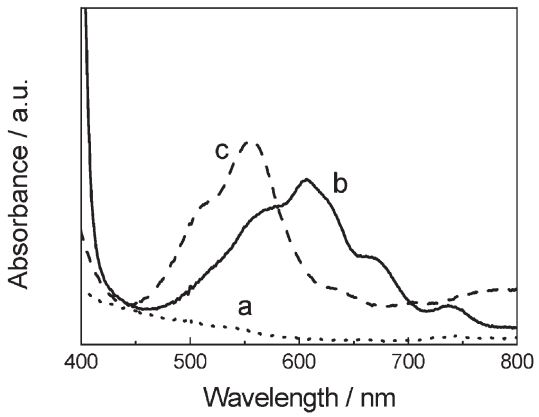

Fig. 3 Visible absorption spectra of the measurement cell of DNA–HV–$EtImBF_4$ complex film before (a) and after (b) applying −2.0 V for 10 s. The spectrum for $EtImBF_4$ solution containing HV after applying −2.0 V for 10 s is shown as a reference (c).

In the case of a homogeneous solution, dimer formation usually occurred in the solution. The formation of radical cation dimer can be detected spectroscopically as seen in Fig. 3(c). No shift of the maximum absorption of HV was found for this cell. A shoulder attributable to the dimer is visible in Fig. 3(b). These results clearly indicate that most HVs were monomolecularly immobilized in DNA grooves, and that HV radical cation was individually stabilized with much less chance of dimerization.

To achieve deeper colouring, more $EtImBF_4$ was added to the complex to improve the ionic conductivity. The absorbance of the complex films increased with increasing amounts of $EtImBF_4$. This increase is due to the increase in the concentration of anions required for the redox reaction of viologens. The decrease in the glass transition temperature (T_g) was a further effect that improves the ionic conductivity. However, absorbance related to the dimer was found to increase with increasing $EtImBF_4$. The HV was soluble in $EtImBF_4$. HVs incorporated into the minor grooves of DNA dissolved and then dimerized in the liquid $EtImBF_4$. Furthermore, at a molar ratio of $EtImBF_4$ to DNA of 5.5, the resulting DNA–HV–$EtImBF_4$ was a very soft gel. The DNA matrix cannot retain more $EtImBF_4$ than 5.5 (by mol). It is therefore difficult to improve the ionic conductivity simply by increasing the ionic liquid content. At the same time, an ion conductive liquid is not adequate to suppress dissolution of viologens while retaining sufficient compatibility with viologens.

We accordingly focused on polymerized ionic liquids. These are expected to have adequate compatibility with viologens but less solubilizing ability. For the supporting electrolyte matrix of the electrochemical reaction of viologens, mobile anions should be included as component ions. Mobile anions are needed for efficient colour change of viologens. We prepared *N*-propyl-*N*′-vinylimidazolium bromide (IL_A) and its homopolymer (pIL_A). An aqueous solution of pIL_A was then added dropwise to the DNA–HV complex solution. However, the DNA–HV complex formed insoluble precipitates immediately after adding the pIL_A solution. This precipitation is the result of cooperative (and therefore strong) electrostatic interaction forces between the polycation (pIL_A) and polyanion (DNA–HV). These polymer complexes are brittle and hard to treat after complex drying. We then prepared ethylimidazolium vinylphosphate (IL_B) and its homopolymers (pIL_B). The DNA–HV–pIL_B complex was obtained as a homogeneous solution without precipitate after mixing with pIL_B, since both DNA and pIL_B are polyanions. However, pIL_B exhibited high affinity with HV, and HVs were solubilized from the minor groove of DNA to a polymer phase, probably due to electrostatic attractive forces. They were dimerized after redox cycling in this system. As a result of the trade-off, we prepared the ionic liquid type copolymer ($CoIL_m$) with a different composition. HVs were confirmed to be scarcely miscible in the resulting copolymers. To prepare the DNA–HV–$CoIL_m$ complex films, $CoIL_m$ was mixed with a solution of DNA–HV (10 : 1). The absorption at around 600 nm attributed to HV monomers was found in all the DNA–HV–$CoIL_m$ complex films. Fig. 4(a) shows the copolymer composition dependence of the absorption at 600 nm in DNA–HV–$CoIL_m$ complex films after applying a voltage of −2.0 V for 3 s. With increasing IL_A fraction, the absorbance reached a constant value. Since IL_A supplies mobile bromide anions after copolymerization, the relation seen in Fig. 4(a) shows corresponding saturating behavior. We chose $CoIL_{0.5}$ (pIL_A : pIL_B = 1 : 1), which forms no precipitate and contains an adequate amount of mobile anions.

We next added $CoIL_{0.5}$ to DNA in different compositions. As shown in Fig. 4(b), a greater amount of $CoIL_{0.5}$ gives greater colour changes in this system. This result is attributed to both the decrease of T_g and the increase in the concentration of bromide anions. The ionic conductivity of $CoIL_{0.5}$ is around 1.0×10^{-6} S cm^{-1}, which is not so bad for supplying anions to viologens during redox cycling. In the film in which the ratio of $CoIL_{0.5}$ to DNA was 5.0, we estimated that about 25% of HVs in the feed contribute to the redox reactions. This system displayed an

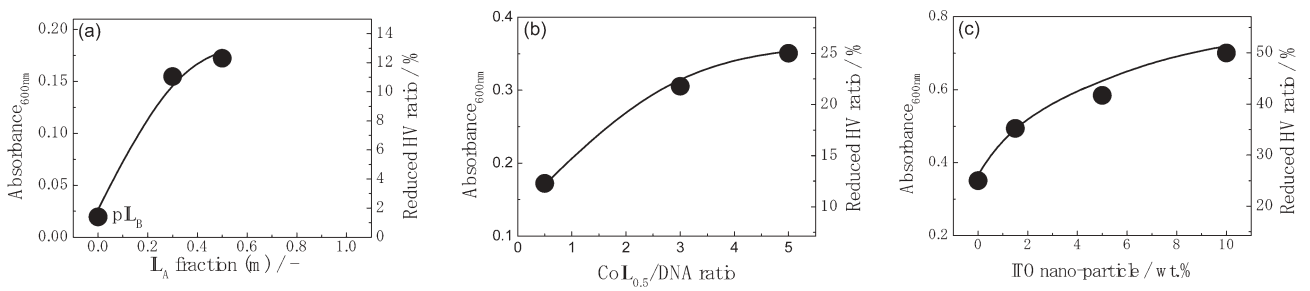

Fig. 4 Absorbance at 600 nm for DNA–HV ([HV]/[DNA bp] = 1/10) films as a function of monomer fraction in the copolymer $CoIL_m$ (a), $CoIL_{0.5}$ concentration (b), and amount of ITO nanoparticles (c). Reduction of HV was carried out by applying −2.0 V for 3 s.

absorbance about 5 times larger than that of the initial $EtImBF_4$ containing system.

Although there were enough anions to compensate the charge change of HVs, the electric conduction through the DNA strands is still inadequate. A deeper colour change would take place by introducing electroconductive pathways. Improvement of the electrical conductivity for double-strand DNA complexes can be made by several methods, including intercalating π-conjugate compounds. Introduction of other dye molecules is not favourable toward a deeper colour change of the present system. We therefore chose ITO nanoparticles. Addition of ITO nanoparticles should significantly increase the electroconductive pathways for electron transport between HVs and the electrode. Addition of these ITO nanoparticles corresponds directly to an increase in the effective surface area of the working electrode. In the present study, we used ITO nanoparticles with an average diameter of 100–140 nm. Dispersions of ITO nanoparticles were donated by Sumitomo Metal Mining Co. and were used as received. Large particles induce scattering of light and lowering transparency as well as decreasing the total surface area of conductive solid surface. The ITO particles used for these experiments are the smallest ones among commercially available particles. Fig. 4(c) shows the effect of ITO nanoparticles on the absorbance at 600 nm after applying −2.0 V for 3 s with DNA–HV–$CoIL_{0.5}$ (1 : 0.1 : 5 by mol) complex films. Addition of the ITO nanoparticles has a positive effect on the blue colouring of the complexes. Although films that contained more than 10 wt% ITO nanoparticles displayed greater colour change, they also exhibited considerable light scattering that hindered spectroscopic analysis. This might be attributed to the formation of aggregates of ITO nanoparticles in the matrix. According to these data, the DNA–HV–$CoIL_{0.5}$ film containing 10 wt% ITO nanoparticles was chosen for further experiments. Through the same experiments, the absorption of this cell was amplified by about 50 times relative to the starting cells.

We evaluated the cycle stability between colouring and bleaching of this cell containing 10 wt% ITO nanoparticles. A square-wave between 2.0 and −2.0 V was applied to the cell, switching every 3 s. This cell switched almost reversibly between bleaching and colouring, as shown in Fig. 5. The initial transmittance varied between 80% and 35%, and settled down to oscillate between 70% and 35%. The colour change of course depends on the period for redox reactions. Longer intervals clearly improve the colouring. This shows that there is inadequate electron conduction between HVs and electrodes. Improvement of the electric conductivity of the HV–DNA complex is underway.

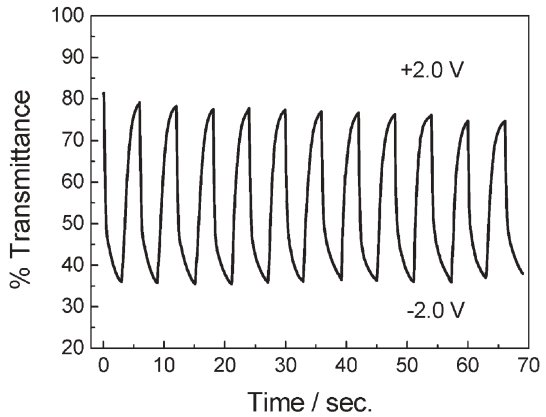

Fig. 5 Transmittance (%*T*) change at $\lambda = 600$ nm for the cell during potential switching between 2.0 V and −2.0 V for 3 s.

In summary, double-stranded DNA was used as a matrix to fix viologens mono-molecularly so as to inhibit dimerization of their cation radicals. A DNA–HV film covered with an ionic liquid type copolymer displayed reversible colour change upon voltage switching between 2.0 V and −2.0 V. After addition of 10 wt% ITO nanoparticles, the absorption change increased by a factor of approximately 50.

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Notes and references

1 C. J. Schoot, J. J. Ponjee, H. T. van Dam, R. A. van Doorn and P. T. Bolwijn, *Appl. Phys. Lett.*, 1973, **23**, 64.
2 H. C. Ko, S. Kim, H. Lee and B. Moon, *Adv. Funct. Mater.*, 2005, **15**, 905.
3 H. T. van Dam and J. J. Ponjeé, *J. Electrochem. Soc.*, 1974, 1556.
4 J. Ryu, J. Lee, S. Han and K. Suh, *Macromol. Rapid Commun.*, 2006, **27**, 1156.
5 S. H. Kim, J. S. Bae, S. H. Hwang, T. S. Gwon and M. K. Doh, *Dyes Pigm.*, 1997, **33**, 167.
6 E. E. Engelman and D. H. Evans, *Langmuir*, 1992, **8**, 1637.
7 O. Johansen, J. W. Loder, A. W.-H. Mau, J. Rabani and W. H. F. Sasse, *Langmuir*, 1992, **8**, 2577.
8 C. Lee, M. S. Moon and J. W. Park, *J. Electroanal. Chem.*, 1994, **374**, 115.
9 P. M. S. Monk, *J. Electroanal. Chem.*, 1997, **432**, 175.
10 S. Takenaka, H. Sato, T. Ihara and M. Takagi, *J. Heterocycl. Chem.*, 1997, **34**, 123.
11 K. Arihara, F. Kitamura, T. Ohsaka and K. Tokuda, *J. Electroanal. Chem.*, 2000, **488**, 117.
12 M. Yoshizawa, W. Ogihara and H. Ohno, *Polym. Adv. Technol.*, 2002, **13**, 589.