Multistep Coloring and Bleaching of Viologens Monomolecularly Incorporated in DNA

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Two different viologens have been incorporated into double-strand DNA on ITO glass electrodes. Thus prepared DNA/viologen complex film showed reversible two-step color change by potential control.

DNA, a well-known biopolymer, is an excellent functional polymer material having features not provided by synthetic polymers. One unique property is the fixation of many different molecules. DNA binds organic compounds especially aromatic dye molecules, to spaces in the helical structure by intercalation or groove binding. This leads to serious toxicity of dye molecules, but DNA is one of the best hosts to fix such molecules. Dimerization of aromatic dye molecules lowers reliability as chromic compounds during electrochemical redox reactions. Such planar compounds can be dispersed and fixed monomolecularly in the DNAs to inhibit dimerization. We have reported that DNA/dye complexes are excellent chromic systems to drive electrochemical redox reactions.[1]

DNA sodium salt with average molar mass of 300000, isolated from salmon milt, was donated by Daiwa Kasei Co. Heptyl viologen (V**1**) is one of typical redox active compounds,[2,3] but easily forms a dimer in the reduced state. We have already reported a complex of V**1** and DNA by groove binding.[1] A water-insoluble DNA complex film was prepared on an ITO electrode, and then the DNA film-coated electrode was soaked in a V**1** aqueous solution. Thus prepared DNA/V**1** complex film showed efficient coloration.[4] To improve the response we have also prepared a cast film containing ionic liquid copolymer [poly(1-propyl-3-vinylimidazolium bromide-*co*-1-ethylimidazolium vinylphosphate): P**1**],[1,4] ITO nanoparticles (ITO-NP: Sumitomo Metal Mining Co.), and *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD: Aldrich)[5] as a supporting electrolyte layer, electroconductive additives, and redox couple at the counter electrode, respectively.

It is possible to design chromic cells showing multicoloration with DNA films containing different viologens. Both the maximum absorption wavelength and redox potential varied by changing side chain structure of the viologens. Among some viologen derivatives examined, we selected *p*-cyanophenyl viologen (V**2**) to prepare multistep redox system. Figure 1 shows the structure of viologen derivatives examined in this study.[6,7]

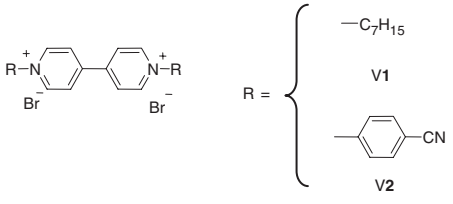

Figure 1. Structure of two viologen derivatives examined here.

To incorporate viologens from aqueous phase, DNA containing 1-dodecyl-3-methylimidazolium counter cations (C_{12}MIDNA) which is insoluble in water, was prepared owing to sufficient stability during dipping in an aqueous solution.[8] Viologens were introduced into the C_{12}MIDNA complex film by soaking.[4]

V**1** showed color change between pale yellow and blue by the potential switching. Introduction of aromatic groups onto viologens were carried out to synthesize viologens with other color change. To archive a multistage color change, redox potentials of a partner viologen should be different from those of V**1**. In the present study, we selected V**2** that has lower redox potential than V**1** and high enough molar extinction coefficient at the reduced state. The first and second redox potential of V**1** was -0.72 and -1.0 V vs. Ag/Ag^+ in acetonitrile. Also, that of V**2** was -0.40 and -0.66 V under the same condition. Both V**1** and V**2** were then introduced into C_{12}MIDNA/P**1**/ITO-NP complex film. In the present experiment, we used ITO-NP with an average diameter of 100–140 nm. Addition of electro-active molecules onto the counter electrode is known to suppress charge-up and allow a lower operating voltage.[9] We therefore added TMPD[5] as a counter active material. TMPD is an anodic material that shows redox reaction at -0.12 V vs. Ag/Ag^+, in acetonitrile. Uniform complex films were obtained by mixing bimolar TPMD relative to the DNA base-pairs and C_{12}MIDNA/P**1**/ITO-NP complex. Hereafter, host polymer film of C_{12}MIDNA/P**1**/ITO-NP/TMPD complex should be abbreviated simply as C_{12}MIDNA film. UV–vis spectral measurement (UV-2500PC, Shimadzu) was then used to determine the amount of the viologen dissolved in the solution, and this was used to determine the degree of incorporation of viologens into C_{12}MIDNA thin film. The electrochromic response of the cells prepared was analyzed also by UV–vis spectroscopy. The reduction efficiency of viologens was calculated from both the maximum absorption of the radical cation and the amount of the viologens incorporated.

Since affinity of viologens with the DNA is not the same, preferential groove binding occurred depending on the viologen species. Both V**1** and V**2** show relatively good degree of introduction into DNA. We first expected to get DNA films containing both V**1** and V**2** by one-step dipping the DNA film into an aqueous solution of V**1** and V**2** mixture. However, in the present case, V**2** has much stronger affinity for C_{12}MIDNA than V**1** due to hydrophobic properties of V**2**. As a result, V**2** was incorporated into DNA much faster than V**1** in spite of the smaller degree of introduction after sufficient dipping time. It was difficult to introduce equal amount of both viologens by simply soaking the C_{12}MIDNA film in a mixed solution at the same time. Two kinds of viologens were then introduced stepwise into the C_{12}MIDNA film by soaking in each aqueous solution (2.5×10^{-3} M). The introduction degree of viologens in the C_{12}MIDNA film was estimated from the absorbance change of the viologen aqueous solution before and after soaking. To balance the introduction degree of V**1**, C_{12}MIDNA film was first soaked in a V**1** aqueous

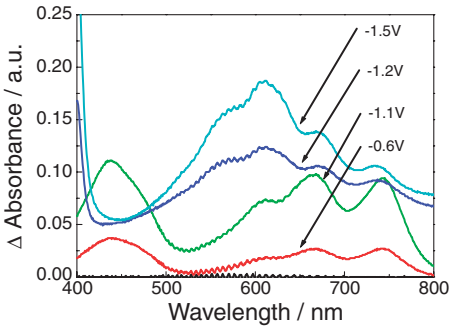

Figure 2. Differential spectra of C_{12}MIDNA film containing V**1** and V**2** at different voltage.

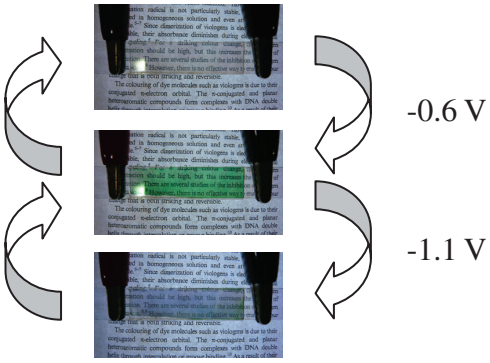

Figure 3. Photographs of stepwise color change of the C_{12}MIDNA film complexed with both V**1** and V**2**.

solution, then V**2** solution. As a result, 13% of V**1** was introduced into a C_{12}MIDNA by soaking the C_{12}MIDNA film for 30 min. Then the V**1** introduced C_{12}MIDNA film was soaked in the V**2** aqueous solution for 30 min. It was also confirmed that the introduced V**1** was not eluted again to the aqueous phase even when the C_{12}MIDNA complex film was soaked in other viologen solutions or even in pure water. C_{12}MIDNA complex film containing 13% V**1** and 7% V**2** to the base pair was prepared by two-step soaking. Under these processes, TMPD in the C_{12}MIDNA complex film eluted from the solution side of the C_{12}MIDNA complex film, and the viologens were introduced vice versa into the solution side of the film. This process is important to prepare a concentration gradient of active molecules in the DNA films. TMPD was concentrated at the film surface that would be contacted with another ITO glass electrode.

We therefore succeeded in preparing a measurement cell in which the coloring materials are incorporated into the film with higher concentration nearer the electrodes, and designed a cell wherein charge-up was suppressed. At the same time, fast and large color changes are realized. UV–vis spectral measurement was carried out applying voltage to the cell. Figure 2 shows the differential spectra of the cell prepared in this study at different voltage. The spectrum at 0 V was used as baseline. When a voltage of -0.6 V or higher was applied on the working electrode, absorption due to cation radical of V**2** (V**2**$^{\bullet+}$) was observed, and similarly, absorption at both 609 and 666 nm due to V**1**$^{\bullet+}$ monomer increased when voltage at and over -1.1 V was applied. Moreover, the absorbance at 436 nm decreased gradually at voltage over -1.0 V owing to the second reduction of V**2**$^{\bullet+}$ to generate neutral one (V**2**^{0}). Since the voltages for the first reduction step of V**1** and the second reduction step of V**2** are almost the same, these two electrochemical redox reactions occurred almost simultaneously. It is clear that spectral change of viologen monomers occurs electrochemically without dimer formation by distributing and fixing the viologens in the grooves of C_{12}MIDNA independently. These spectral changes are quasi-reversible depending solely on the voltage. Figure 3 shows typical photographs of the response of the electrochemical cell during redox reaction. Typical color change was found depending on the voltage. The cell showed reversible color change from transparent to green and blue along with the voltage. As shown in Figure 2, since redox response of viologen has not been sharp against the given voltage, switching with dramatic color change has not been found in the present cell. Figure 3 shows three different color of the cell at 0, -0.6, and -1.1 V, respectively. At -0.6 V, V**2** should be in the radical cation state to show green color. Then, V**1** should be in the radical cation state to show blue and V**2** is in the second reduction state at -1.1 V. There are a few more points to be analyzed for the detailed discussion such as complex structure of these viologens in DNA helices and diffusivity of dyes. Further experiments have been in progress. The incorporation of organic dye molecules into DNAs should improve the stable response of electrochromic cells.

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