Reversible Optical Recording by a Dinitrophenol Antibody-Catalyzed Ring Opening of 6,8-Dinitro Spiropyran

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Photochemical reversible switching of the biological functions and activities of proteins is of substantial interest in the development of bioelectronic devices.[1-3] Design of biological computers, optical recording devices,[4,5] light-signal amplification units, and reversible biosensors could be assembled by photoswitchable biomaterials.[6] Light-stimulated reversible activation and deactivation of catalytic functions of enzymes[7-9] and binding properties of proteins were achieved by chemical modification of proteins with photoisomerizable components.[10] Reversible photocontrolled association and dissociation of an antigen–antibody complex was achieved by applying an azobenzene-derivatized antigen.[11] Here we report on a catalyzed ring opening of 6,8-dinitro-1′,3′,3′-trimethylspiro[2*H*-1]-benzopyran-2,2′-indoline[12] (**1a**) by the anti-dinitrophenol monoclonal antibody, Ab-DNP.[13] We reveal that the reaction exhibits enzyme-like kinetics, and we apply the catalytic properties of the antibody to tailor a reversible optical recording and erasing system. Characterization of enzyme-like catalytic properties of the native Ab-DNP toward ring opening of **1a** complements the extensive activities of development of catalytic antibodies.[14-16]

Figure 1 shows the rates of the thermal ring opening of **1a** to **1c** in the presence and absence of Ab-DNP. At a **1a** concentration corresponding to 2.3×10^{-6} M and an Ab-DNP concentration corresponding to 1×10^{-6} M, the rate of ring opening is a ca. 15-fold faster compared to the rate in the system that lacks the antibody ($k_{un} = 1.6 \times 10^{-5}$ s^{-1}). The rate of the reaction, eq 1, at a constant Ab-DNP concentration of 8.3×10^{-9} M levels off as the concentration of **1a** increases. The process can be analyzed according to the Michaelis–Menten kinetic model, eq 2 and Figure 2. The derived K_m and V_{max} values correspond to 170 mM and 8.7 μM·s^{-1}, respectively. Thus, the monoclonal Ab-DNP recognizes the 2,4-dinitro derivative **1a**. The binding site itself or a very close location includes a catalytic site that

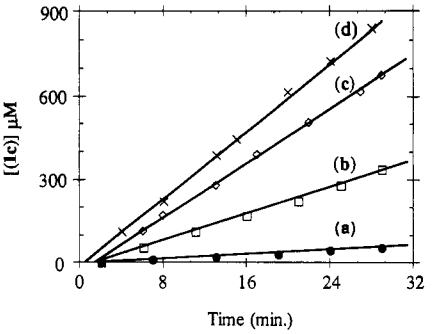


Figure 1. Rates of **1a** ring opening to **1c** in the presence and absence of Ab-DNP: (a) without Ab-DNP; (b) with 0.1 μM Ab-DNP; (c) with 0.25 μM Ab-DNP; (d) with 1 μM Ab-DNP. For all experiments: [**1a**] = 2.3 μM; phosphate buffer 1×10^{-2} M (pH = 7.4); 0.9% NaCl.

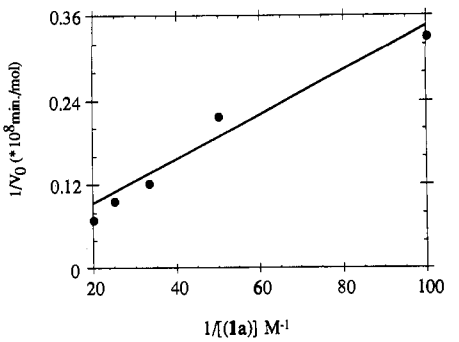
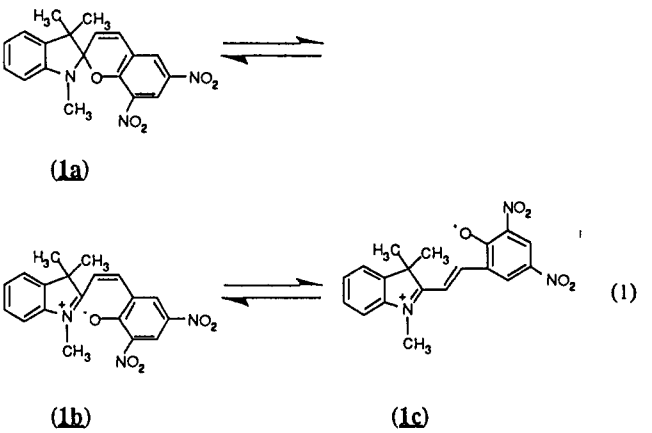


Figure 2. Kinetic analysis of the Ab-DNP catalyzed isomerization of **1a** to **1c** according to the Michaelis–Menten model. Initial rates of isomerization were determined at a constant Ab-DNP concentration of 0.1 μM, in phosphate buffer, pH = 7.4, and 0.9% NaCl solution, using different concentrations of **1a**. All experiments were conducted at 23 ± 1 °C.



enhances the chemical transformation of **1a** to **1c**.

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \frac{1}{[\mathbf{1a}]} + \frac{1}{V_{max}} \qquad (2)$$

The availability of an active site in the Ab-DNP for isomerization of **1a** is further emphasized by inhibition experiments. Addition of 2,4-dinitrophenol inhibits the ring opening of **1a**. The Ab-DNP acts as cyclic catalyst for isomerization of **1a** and ca. 200 cycles were observed without loss of the Ab activity. The binding properties of **1a** to Ab-DNP and the kinetics of ring opening were characterized according to Scheme 1 and eq 3. By following the rate of **1a** isomerization at different Ab-DNP concentrations and applying eq 3, the derived values of K_1 and

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(13) The Ab-DNP was prepared against DNP linked to BSA by ε-aminocaproic acid as linker. The molar ratio of DNP to BSA was 22. The epitope does not include any part of the carrier protein. ELISA and PCA analyses revealed that Ab-DNP does not recognize BSA or RSA but interacts with DNP-RSA.
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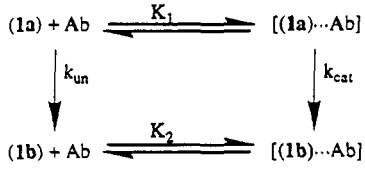
Scheme 1



k_{cat} correspond to $K_1 = 426\,000\ M^{-1}$ and $k_{cat} = 0.30\ s^{-1}$, and hence $k_{cat}/k_{un} = 1.9 \times 10^4$.

$$\frac{[Ab]^\circ}{k_{obs} - k_{un}} = \frac{1}{K_1(k_{cat} - k_{un})} + \frac{([Ab]^\circ[\mathbf{1a}]^\circ[\mathbf{1a}\cdots Ab])}{k_{cat} - k_{un}} \quad (3)$$

The catalytic properties of the Ab-DNP to isomerize **1a** to **1c** and the feasibility of stimulating by light the reverse ring closure of **1c** to **1a**, $\lambda = 532$ nm, allows us to organize a cyclic optical recording system, Figure 3. The system consisting of **1a** and Ab-DNP results in isomerization to **1c**. At the time marked with the arrow the system is exposed to the light signal, $\lambda = 532$ nm, optical recording is evident by a decrease in the characteristic absorbance of **1c** at $\lambda = 480$ nm, and ring closure to **1a** takes place. In the dark, the Ab-DNP catalyzed reaction proceeds and **1a** is recovered. This allows the cyclic recording of optical signals, as shown in Figure 3.

Thus, the Ab-DNP catalyzed transformation acts as a "readout" signal of the recorded information as the rate of the process is controlled by the concentration of **1c**, or as an "erase" mechanism for the recorded optical signal. This latter process proceeds on a time scale of ca. 80 s.

The catalytic properties of Ab-DNP toward isomerization of **1a** are of specific interest. In contrast to catalytic antibodies cultured for a transition-state analog of the reacting substrate, Ab-DNP was cultured for a foreign substrate. We speculated that within a DNP binding site a zwitterion of two amino acid residues is present. Such a zwitterion could interact with the primary intermediate **1b** formed by isomerization of **1a** by complementary electrostatic interactions. Such interactions could enhance the transition of **1b** to **1c** and effect the overall catalyzed

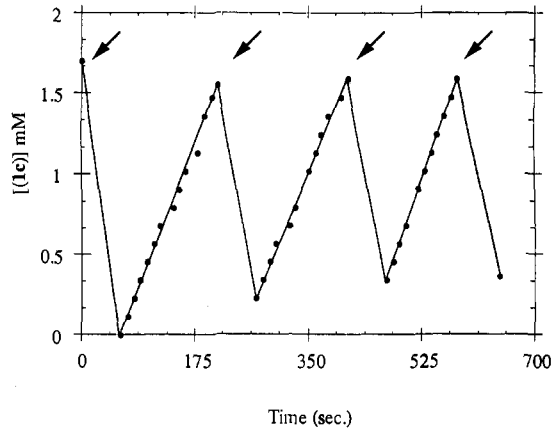


Figure 3. Cyclic optical recording and erasing using the **1c**–Ab-DNP assembly. The experiment follows the absorbance changes of the system at $\lambda = 480$ nm. The system in configuration **1c** is exposed to a laser flash ($\lambda = 532$ nm) at each time indicated by an arrow. The dotted lines represent the Ab-DNP catalyzed isomerization of **1a** to **1c**.

isomerization. This explanation is further supported by the observation that isomerization of **1a** to **1c** is catalyzed in the presence of γ-aminobutyric acid (GABA). Nonetheless, the catalytic effect of GABA (lacking a binding site) requires a 25000-fold-higher concentration of the catalyst as compared to the Ab-DNP system. In fact, the isomerization of spiropyran by BSA was recently reported[17] yet the catalysis rate $k_{cat}/k_{un} = 180$ is substantially lower than in the present system. This process might be similarly enhanced by a zwitterion site in the protein backbone. We thus conclude that culturing of Ab to zwitterionic amino acid modified dinitrophenol could yield an improved Ab for isomerization of **1a**. In addition, synthesis of rigid zwitterionic amino acids capable of binding **1a** could generate artificial optical recording devices based on the catalyzed isomerization of **1a**.

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