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Adsorption Kinetics of Carcinogenic Agent to DNA Gel Beads

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ABSTRACT
Adsorption of a carcinogenic agent, acridine orange, to DNA liquid crystalline gel (LCG) and DNA-combined poly(dimethylsiloxane) (DNA-PDMS) have been measured as a function of time. The adsorption process is represented by the stretched exponential function for both the DNA LCG and the DNA-PDMS. The experimental data of the adsorption process are compared with a simple model based on the diffusion process of acridine orange. For the DNA LCG, the adsorption rates are affected by preparation conditions. The DNA LCG with higher cross-linking density shows slower adsorption of acridine orange. The DNA-PDMS is a new DNA-based material with high elastic modulus, which is expected for use under high stress.
Key words: liquid crystalline gel, DNA, adsorption, carcinogenic agent, poly(dimethylsiloxane)

1. INTRODUCTION

DNA has attracted interest as a functional biomaterial not only as the genetic material. Carcinogenic agents having planar aromatic groups intercalate into DNA double helix and result in inhibition of interpretation of heredity code.[1,2] Because of this characteristic property, DNA can be used for adsorbents selective to carcinogens. A lot of methods for the preparation of water-insoluble DNA materials have been developed intended for application as DNA-based adsorbents.[3-5] Recently, it has been reported that liquid crystalline gel (LCG) of DNA is obtained by a dialysis of concentrated DNA solutions into solutions of metal ion.[6,7] The LCG beads of DNA are shown to adsorb a carcinogenic agent, acridine orange. For efficient material design, it is essential to understand the adsorption process quantitatively. In this study, the adsorption kinetics of acridine orange to the DNA beads is investigated. The DNA beads prepared at different concentrations of metal ion were used to study the effect of cross-linking density on the adsorption process.

In the previous study, it was shown that the DNA LCG can be also obtained from the DNA solution sandwiched between two slide glasses after the immersion to the metal ion solution.[6] In this case, DNA molecules near the interface of the solution are cross-linked just after the immersion and the gel film at the interface plays the same role as the dialysis tubing. In the same way, drops of the DNA solution immersed in the metal ion solution form spherical LCG beads.[6,7] The spherical shape would be suitable for developing a theoretical model to be compared with experimental data of the adsorption process.

In practical application, it is often necessary to be stable at high shear stress. In this paper, we also describe newly developed silicone-based DNA beads with high elastic modulus.

The outline of the paper is as follows: We describe the results for DNA LCG in Section 2-4 and DNA-combined poly(dimethylsiloxane)(PDMS) in Section 5. In Section 2, preparation of LCG beads of DNA and details of the measurement of the adsorption process are described. The experimental results of the adsorption process are shown in Section 3. In Section 4, the experimental data are discussed with a simple model based on the diffusion process of acridine orange. The practical use of DNA-based adsorbents has been limited because of poor mechanical properties. In Section 5, we will briefly present a method for preparation of DNA-combined PDMS with high elastic modulus. Finally, our main results are summarized in Section 6.

2. EXPERIMENT

Double-helical DNA sodium salt with 10 kbp from salmon milt was provided by Nippon Chemical Feed Co. Ltd.. Cobalt chloride was purchased from Wako Pure Chemicals and used without further purification. De-ionized ultra-pure water was used as a solvent. DNA was dissolved in an aqueous solution of 20mM mM sodium borate at the concentration of 1 wt%. The DNA solution was dripped into a copious solution of cobalt chloride by a syringe. The concentration C_{Co} of cobalt chloride was varied in the range of 50-1000 mM. The drops of the DNA solution were cross-linked by cobalt ions to form LCG beads. The formation of LCG beads of DNA was described in detail in Ref. 7. The diameter of LCG beads was in the range between 2-3 mm.

For the experiments of adsorption process, an aqueous solution of acridine orange at the concentration of about 10 $\mu g/cm^3$ was used as a carcinogen solution. The optical density at a wavelength λ = 495 nm was measured by UV-visible spectrometer for the solution

before each experiment. To start the experiment, 0.3 g of DNA beads were immersed in 10 cm^3 of the acridine orange solution in a glass container thermostated at 25.0 °C. The solution was occasionally transferred to the cell of the spectrometer for the measurement of optical density at λ = 495 nm, and back to the container. The measurements were repeated at appropriate time intervals for about 2 hours. The concentration $c(t)$ ($\mu g/cm^3$) of acridine orange in the solution at time t (min) was determined from the relation $A = 0.0727 \times c$ where A is absorbance of the solution.

3. EXPERIMENTAL RESULTS

The adsorption kinetics of acridine orange could be expressed in terms of $\Gamma(t)=(c_0-c(t))V/(W_{beads}\ w_{DNA})$ as a function of time t, where c_0 is the initial concentration of acridine orange, V is the volume of the solution, W_{beads} is the weight of the DNA beads and w_{DNA} is the weight fraction of of DNA in the DNA bead. Γ represents the grams of acridine orange adsorbed to 1 g of DNA. After the immersion of DNA beads into the acridine orange solution, Γ increased rapidly at first and gradually leveled off. The equilibrium value of Γ was not obtained in the experimental time period about 2 hours. The rate of the adsorption process was faster for the DNA beads prepared at higher concentrations C_{Co} of cobalt chloride.

The adsorption process for the DNA beads was represented by neither the exponential function nor the power law function, but well represented by the stretched exponential function as

$$\Gamma = \Gamma_e \{1 - \exp[-(t/\tau)^{\beta}]\} \qquad (1)$$

where Γ_e, τ and β are constants independent of time t. Γ_e represents the maximum weight of acridine orange adsorbed to DNA. The values of Γ_e, τ and β could be obtained by the fitting of eq. 1 to the experimental data. Figure 1 shows Γ_e, τ and β plotted against C_{Co}. The maximum adsorption weight Γ_e seems to decrease slightly with increasing C_{Co}. The characteristic time τ increases with C_{Co} for the conditions of C_{Co} lower than 200 mM and is almost constant for those higher than 200 mM. The exponent β seems to have a constant value ~ 0.7 irrespective of C_{Co}, except for the condition of C_{Co} = 50 mM where β is considerably small ~ 0.5.

According to eq 1, the almost constant value of β indicates that the plot of Γ/Γ_e versus t/τ for various conditions of C_{Co} can be superposed to a single curve. Figure 2 shows the adsorption processes by the plot of Γ/Γ_e as a function of t/τ. The different symbols are used for different conditions of C_{Co}. The solid curves are depicted by eq 1 for corresponding values of Γ_e, τ and β shown in Figure 1. All the processes are well represented by a single curve except for the later process for the condition of C_{Co} = 50 mM. The deviation would be attributed to a small difference in the value of β.

4. DISCUSSION

In Figure 1, it is seen that Γ_e gradually decreases with C_{Co} and τ tends to increase with C_{Co}. The DNA LCG beads prepared at higher C_{Co}s would have higher cross-linking densities. The high cross-linking density would decelerate the diffusion process of acridine

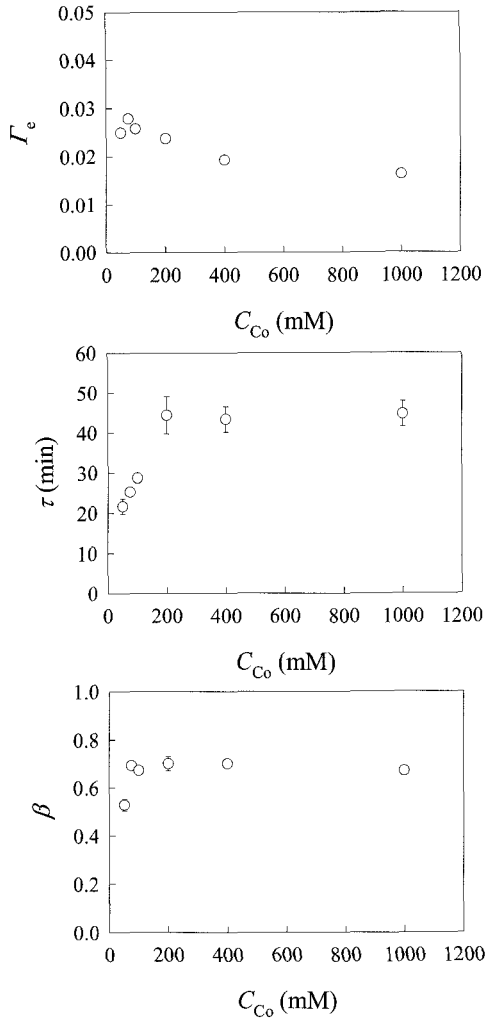

Figure 1: Values of Γ_e, τ and β used for the fitting by eq. 1 as a function of the concentration C_{Co} of cobalt chloride for DNA LCG.

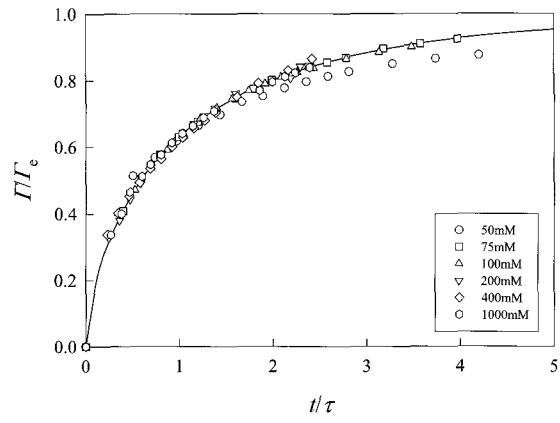

Figure 2: Adsorption process by the plot of Γ/Γ_e versus t/τ for the DNA LCG beads prepared at C_{Co} = 50 mM (circles), 75 (squares), 100 (up-triangles), 200 (down-triangles), 400 (diamonds) and 1000 (hexagons). The solid curves are depicted by eq. 1 with corresponding values of Γ_e, τ and β shown in Figure 1.

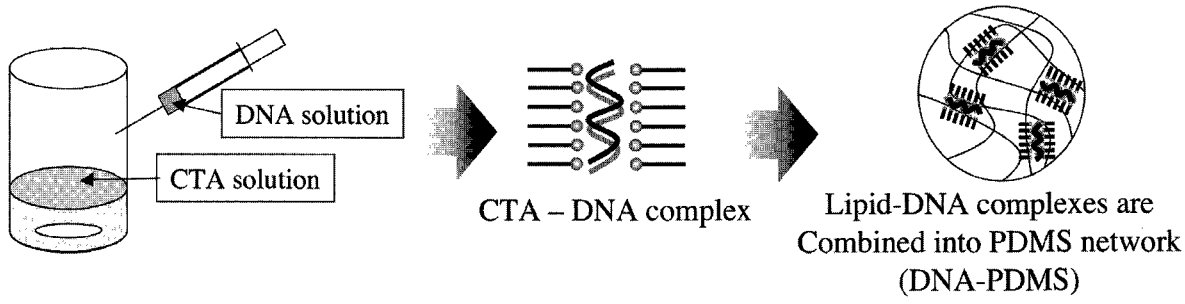

Figure 3: Schematic diagram of the preparation of DNA-PDMS.

orange, which results in a larger value of τ. The cross-linking due to an ionic bond between a phosphate group of DNA and cobalt ion reduces negative charges of DNA beads. Acridine orange molecules are trapped in the major groove of the DNA double helix or intercalated between base pairs, however, their adsorption to DNA could be affected by the negative charges of DNA because an acridine orange molecule has a positive charge. The decrease in the negative charges of the DNA beads due to the formation of the more cross-linking would result in the decrease in Γ_e. The adsorption rate of acridine orange could be dominated by the diffusion of acridine orange into the beads. A position δ of the diffusion front of acridine orange measured from the surface of a spherical gel would change as $\delta \sim t^{1/2}$. In a simple model assuming the density ρ of the adsorbed acridine orange is independent of the position of the gel, the weight W_a of adsorbed acridine orange molecules is estimated as

$$W_a = (4\pi\rho/3)\,[R^3\text{-}(R\text{-}\delta)^3] \sim 4\pi\rho R^2\delta$$

for the initial process of the adsorption, where R is the radius of the bead. Using the relation $W_a(t) = (c_0\text{–}c(t))V$, the time evolution of Γ is obtained as

$$\Gamma(t) = 4\pi\rho R^2\delta(t)/(W_{beads}\, w_{DNA}) \sim t^{1/2}. \quad (2)$$

Thus the initial process of the adsorption for the simple model is represented by a power law function with the exponent 1/2. The empirical equation (eq. 1) for the adsorption process can be approximated by a power law function at small t as

$$\Gamma(t) \sim \Gamma_e\,(t/\tau)^{\beta}. \quad (3)$$

The value of the exponent $\beta \sim 0.7$ as shown in Figure 1 is considerably larger than that of 0.5 in eq. 2. The deviation would be attributed to the approximate nature of the model. For a quantitative comparison with the experimental results, a more rigorous theoretical model would be needed.

5. DNA-BASED ADSORBENT COMBINED TO CROSS-LINKED PDMS

Although there are a lot of methods to prepare water-insoluble DNA materials as adsorbents, their practical use has been limited because they lack sufficiently high elastic modulus. In the case when DNA beads are used as media packed in a column, the efficiency in the adsorption would be reduced if the beads are collapsed by shearing force. Cross-linked PDMS could be suitable for a matrix of the DNA-based carcinogen adsorbent because of its high elasticity and biosafety. In this section, preparation of PDMS combined with a DNA-based adsorbent (DNA-PDMS) is described briefly, and its adsorption process of acridine orange is presented.

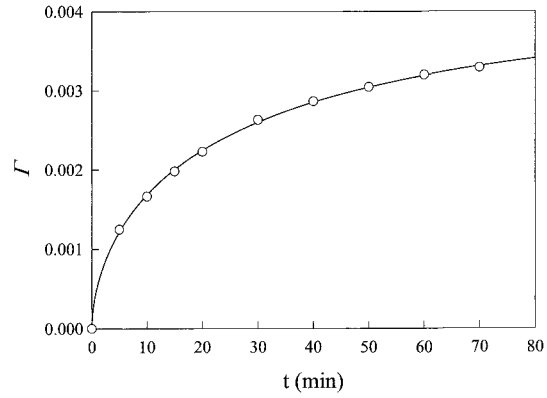

Figure 4: Plot of Γ as a function of time t for DNA-PDMS. The solid curve is depicted by eq. 1 with the values of Γ_e = 0.004, τ = 29 min, and β =

A schematic diagram of the preparation of DNA-PDMS is shown in Figure 3. To combine hydrophobic PDMS and hydrophilic DNA, DNA molecules are hydrophobically modified by complex formation with lipid molecules.[8] Aqueous solutions of DNA at 1 wt% and cetyl trimethyl ammonium bromide (CTAB) at 1 wt% were mixed and white precipitate of DNA-CTA was obtained. The precipitate was washed with water and freeze-dried. Cross-linked PDMS beads of the diameter 0.1-1 mm were obtained by an addition reaction of divinyl-terminated PDMS (Shin-Etsu Chemical Co. Ltd.) and four functional methyl hydrogen siloxane (Shin-Etsu Chemical Co. Ltd.) in an aqueous solution of sodium dodecyl sulfate at 10 wt% stirred vigorously. Platinum catalyst provied by Shin-Etsu Chemical Co. Ltd. was used for the reaction. The PDMS beads were immersed in a solution of DNA-CTA at 2 wt% in the mixed solvent of cylohexane and ethanol (20 vol%) for 24 hours. The beads swollen in the DNA-CTA solution were dried at 100 °C and DNA-CTA combined PDMS beads were obtained after the evaporation of the organic solvents.

The process of adsorption of acridine orange to the DNA-PDMS beads was measured in the same way as that to the LCG beads. For the experiment, 0.4 g of DNA-PDMS beads were used. Figure 4 shows the adsorption process for the DNA-PDMS beads. After the immersion of the beads, Γ increased rapidly at first and gradually leveled off. The adsorption process was

well represented by eq. 1. The solid curve was depicted by eq. 1 with the values of $\Gamma_e = 0.004$, $\tau = 29$ min, and $\beta = 0.6$. The value of β is smaller than that for DNA LCG beads and is larger than 0.5 for the simple model.

6. CONCLUSION

Adsorption kinetics of acridine orange to LCG beads of DNA was determined as time evolution of grams of acridine orange adsorbed to 1 g of DNA, Γ. $\Gamma(t)$ was well represented by the stretched exponential function. The adsorption process was slower for the DNA beads prepared at higher concentration C_{Co} of cobalt chloride. A simple model based on the diffusion process of acridine orange predicts that the initial adsorption process could be represented by a power law function with the exponent 0.5. Although the stretched exponential function can be approximated by the power law function at small t, the value of the exponent ~ 0.7 obtained from the experiment is considerably larger than that for the simple model. DNA-combined PDMS with high elastic modulus was prepared intended for the application as a DNA-based adsorbent used under high stress.

7. ACKNOWLEDGEMENT

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7. REFERENCES

[1] W. Saenger, "Principles of Nucleic Acid Structure", Springer-Verlag: Berlin (1987).
[2] P. Brookers and P. D. Lawley, Nature, 202, 781 (2002).
[3] K. Iwata, T. Sawadaishi, S. Nishimura, S. Tokura and N. Nishi, Int. J. Biol. Macromol., 18, 149 (1996).
[4] H. Kitamura, E. Matsuura, A. Nagata, N. Sakairi, S. Tokura and N. Nishi, Int. J. Biol. Macromol., 20, 75 (1997).
[5] H. Kitamura, C. Iwamoto, N. Sakairi, S. Tokura and N. Nishi, Int. J. Biol. Macromol. 20, 241 (1997).
[6] Y. Minamisawa, K. Furusawa, T. Yamamoto and T. Dobashi, Trans. MRSJ, 31, 739 (2006).
[7] T. Dobashi, K. Furusawa, E. Kita, Y. Minamisawa and T. Yamamoto, Langmuir, 23, 1303 (2007).
[8] K. Tanaka and Y. Okahata, J. Am. Chem. Soc., 118, 10679 (1996).

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