Transactions of the Materials Research Society of Japan 31[3] 739-741 (2006)

Mono- and multilayered aluminum ion-induced liquid crystalline gel of DNA

Yoshiyuki Minamisawa, Kazuya Furusawa, Takao Yamamoto* and Toshiaki Dobashi
Department of Biological and Chemical Engineering, Faculty of Engineering, Gunma University, 1-5-1, Tenjin-cho, Kiryu, Gunma 376-8515, Japan
Fax:0277-40-1377, e-mail:dobashi@bce.gunma-u.ac.jp
*Department of physics engineering, Faculty of Engineering, Gunma University, 1-5-1, Tenjin-cho, Kiryu, Gunma 376-8515, Japan

ABSTRACT

Mono- and multilayered DNA liquid crystalline gel (LCG) films were prepared by means of dialysis of concentrated DNA solution into concentrated aluminum chloride solution. To characterize the DNA LCG, the condition for forming LCG, its stability and acridine orange adsorption were measured using DNA LCG beads prepared using insolubilization reaction. The condition for forming LCG was determined as a function of concentrations of DNA and aluminum cations. Four different phases appeared depending on the concentrations: (a) precipitated micro-gel (b) isotropic gel bead, (c) gel bead with clouded core and transparent corona with liquid crystallinity and (d) transparent gel bead with liquid crystallinity (LCG). The stability of the DNA LCG was examined in various conditions: DNA LCG was stable in polar and non-polar solvents and pure water at the boiling point, while it was disintegrated in strong acid and strong base. DNA LCG was shown to adsorb one of carcinogenic agents, acridine orange.

Key word: liquid crystalline gel, multilayer, aluminum ion, DNA

INTRODUCTION

DNA has attracted as a functional biomacromolecular material; DNA is an adsorbent of environmental pollutant such as endocrine disrupter and carcinogenic agent through the intercalation [1-3], and complexes of DNA and surfactant, ion etc. are used as photo- and electro-conductive polymers [3-7]. For these purposes DNA needs to be insoluble in water. Typical methods of insolubilization of DNA reported until now are cross-linking of thymidine residues by exposure to ultraviolet ray [2], forming a complex with polyelectrolytes [8] and surfactants [6]. Recently, we showed that simple dialysis of one of polysaccharides, curdlan, into ionic solutions induce liquid crystalline gel (LCG) formation [9-11]. In the present study, we demonstrate an alternative method of insolubilization of DNA; aluminum cations induce mono- and multilayered DNA LCG by dialysis, similar to curdlan LCG.To characterize the DNA LCG, the condition for forming LCG, stability and efficiency of adsorbing one of carcinogenic agents, acridine orange (AO) are also studied.

EXPERIMENT

Sodium type DNA (Na^+DNA)(Lot 02037) provided by Nippon chemical foods Co. Ltd. was dissolved in 40mM sodium tetra-borate aqueous solution (pH 9.2) at various concentrations. Limiting viscosity number of DNA in the solution was determined as 32.1ml/g. In order to prepare the LCG film, 10wt% DNA solution was sandwiched between two slide glasses with 18mmϕ and immersed into aluminum chloride aqueous solution at 600mM. Immediately after the immersion, cross-linking reaction occurred at the surface of the DNA solution and the resultant thin membrane played the role of dialysis membrane, and mono-layered DNA LCG was prepared. The photographs of DNA LCG observed under natural light and crossed nicols at the optimum condition are shown in Fig. 1(a). When we change the temperature of the aluminum chloride solution stepwise between 20°C and 50°C for each 20 min, we obtained multilayered DNA LCG, as shown in Fig. 1(b). These results suggest that diffusion of aluminum ion induces the unique structure of DNA.

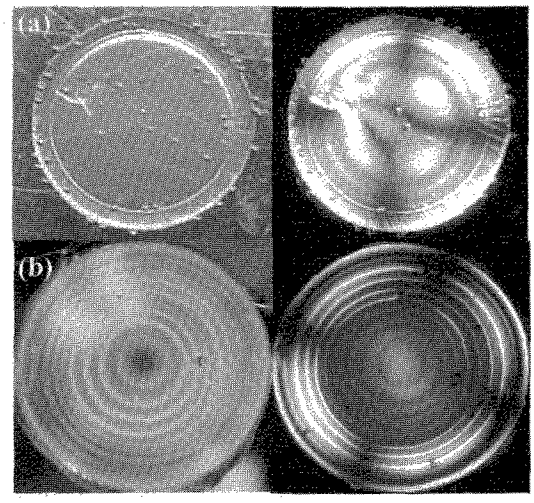

Fig.1 (a) Mono-layered and (b) multilayered DNA LCG films observed under open and crossed nicols prepared in the condition of 10 wt% DNA and 600 mM aluminum chloride.

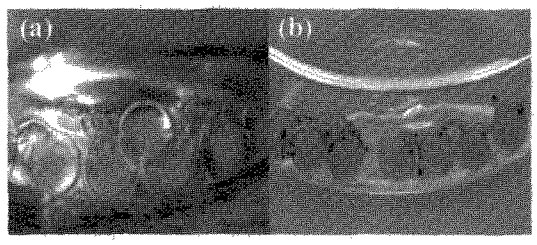

Fig.2 DNA LCG beads observed (a) before and (b) after immersion in acridine orange solution.

To characterize the novel materials, DNA LCG beads were prepared by dropping DNA solutions of 0.9-13 wt% into aluminum chloride solutions (1-800mM) by insolubilization reaction at the surface of the droplets. The elasticity and liquid crystallinity of the beads were observed by hand touch and with crossed nicols. Four different types of gel were formed depending on the concentrations of DNA and aluminum chloride; (a) precipitated micro-gel, (b) isotropic gel bead, (c) gel bead with clouded core and transparent corona with liquid crystallinity and (d) transparent gel bead with liquid crystallinity.

To examine the stability of DNA LCG, the beads were immersed in boiling water during 10 minutes, strong acid (12N hydrochloric acid aqueous solution), strong base (100mM sodium hydrate aqueous solution), weak base (10mM sodium hydrate aqueous solution), and several organic solvents (ethanol, hexane, and 2-propanol), respectively, for one day. The swelling ratio ϕ was estimated from

$$\phi = \left(\frac{a_1}{a_0} \right)^{3/2}$$

where a_0 and a_1 are the average cross sectional areas of the beads before and after the treatment.

Table.1 Swelling ratio of DNA LCG beads in various solvents

solvent	ϕ
hexane	0.92
ethanol	0.86
2-propanol	0.84
water(at 95°C)	0.39
10mM NaOH	1.51
100mM NaOH	-
HCl	-

To examine the adsorption behavior, 0.5g of LCG beads prepared from 13wt% DNA solution by a dialysis into 500mM aluminium chloride solution were immersed into 1.5ml of AO solution at AO concentrations of 3.3, 8.8, 20, 42, and 63 μg/ml, respectively, in a polypropylene tube for five days. The concentration of AO after the immersion was determined by measuring optical absorbance of the solution at 495nm using a HITACHI U-2000 spectrophotometer. Thus the absorption coefficient defined by

$$\psi = \frac{(c_0 - c_1)}{c_0}$$

was obtained. Here, c_0, and c_1 are the concentrations of AO before and after the immersion. Figure 2 shows DNA LCG beads observed (a) before and (b) after immersion in acridine orange solution for one day.

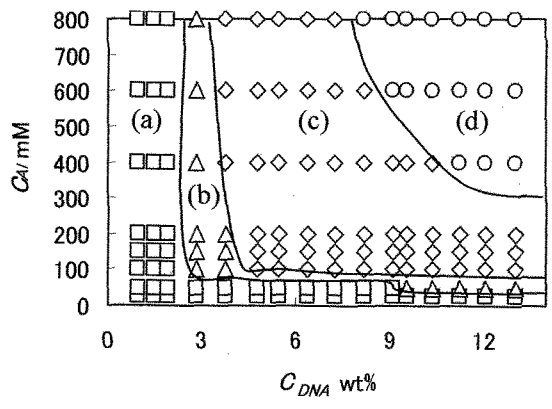

Fig. 3 Phase relationship of DNA dialyzed into aqueous aluminum chloride: (a) precipitated micro-gel, (b) isotropic gel bead, (c) gel bead with clouded core and transparent corona with liquid crystallinity and (d) transparent gel bead with liquid crystallinity

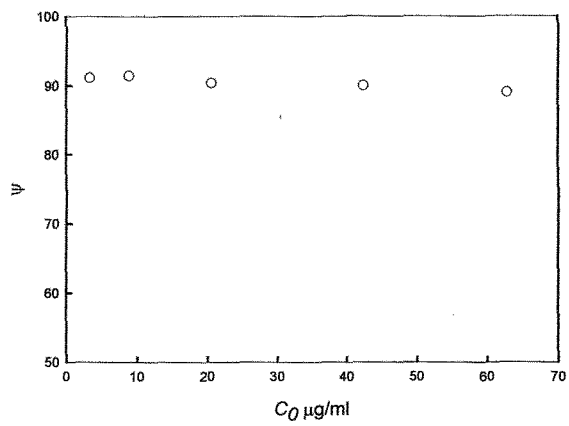

Fig.4 Acridine orange adsorption efficiency of DNA LCG bead

RESULTS

Phase relationship

Figure 3 shows the phase relationship expressed as a function of concentrations of DNA and aluminum chloride. In the regions (a), (b), (c), and (d) in Fig. 3, the phase consisted of precipitated micro-gels, isotropic gel beads, gel beads with clouded core and transparent corona with liquid crystallinity and transparent gel beads with liquid crystallinity, respectively. Except for the region (a), bulk gels appeared. The liquid crystallinity for (c) and (d) was observed at concentrations of DNA more than 13 wt% and aluminum chloride more than 500 mM. When the concentration of aluminum chloride is low in the region (a) and (c), the central clouded part was sol state.

Stability and shrinking of LCG beads

The swelling ratio ϕ at various conditions is shown in Table.1. The liquid crystalline structure was maintained even after immersing the beads into the solvents used except for 100mM NaOH aq. and 12N HCl aq.

Intercalation efficiency of LCG beads

The adsorption efficiency ψ for DNA LCG bead was shown as a function of initial AO concentration in Figure 4. ψ was roughly constant of approximately 90% in the experimental AO concentration range.

DISCUSSION

Insolubilization reaction is one of microencapsulation techniques used for such as alginic acid microcapsules. Aluminium cations are known to induce counter-ion condensation of DNA due to the strong Coulombic force, and incompatible to DNA solution. When concentrated DNA solution is dropped into concentrated aluminum chloride solution, cross-linking reaction of aluminum cations into DNA molecules occur on the surface of the DNA droplets, and DNA in the circumferential portion of the droplets shrink to globules. Here we note that Figure 3 is not the "real phase diagram" at the equilibrium state but the abscissa means simply the initial concentrations. At this stage we have no detailed structural data, but we can still speculate the mechanism of forming each phase in analogy to curdlan LCG [9], as follows. At low concentrations of both aluminum cation and DNA, precipitated micro-gels appeared. This is consistent with the common rule of gelation that enough amounts of cross-linking functional groups are required to form a bulk gel. Micro-gel formation by the addition of multivalent cations has been often observed in the other works [12,13]. Once an interface is formed by the insolubilization reaction on the surface of the droplets, it plays the role of a dialysis membrane. Then the aluminum cations diffuse through the membrane into the direction of center of the droplet, while DNA molecules diffuse to the direction of interface of DNA droplet, since each DNA molecule shrinks, gets together and forms a liquid crystalline phase. In the case of high aluminum chloride concentration, the aluminum cations quickly reach the center of the DNA droplet because of large concentration gradient. Then liquid crystalline phase is incomplete even when the whole droplets are gelled. In the case of extremely low aluminum chloride concentration, the aluminum cations slowly diffuse to the center of the DNA droplet. Since the liquid crystalline gel develops with a slow dynamics, the density of DNA in the LCG layer can be raised. Therefore, the LCG forms only at the outer layer of droplet because of exhaustion of DNA molecules.

When the DNA LCG beads were immersed into a non-polar organic solvent of hexane, neither shrinking nor swelling was observed. This indicates that DNA and H_2O molecules were not released into hexane phase from DNA LCG beads because of low affinity. DNA LCG is stable in polar organic solvents and water, but disintegrated in strong base and acid.

AO is one of carcinogenic agents, and has aromatic plane structure. As expected, DNA LCG beads adsorbed AO with high efficiency.

CONCLUSION

To characterize DNA LCG, DNA beads were prepared by insolubilization reaction with aluminum chloride. The LCG beads were formed at high DNA concentration. The liquid crystalline structure of the beads was still maintained after immersing them into several organic solvents, boiling water, and weak basic solvent. The DNA LCG beads effectively adsorbed AO in water. This adsorption was expected to result from intercalation. Thus, DNA LCG beads could be applied to adsorb other environmental pollutants that have aromatic plane structures.

ACKNOWLEDGEMENTS

This work was partly supported by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists. (#00006825)

REFERENCES

[1] Kitamura, H.; Iwamoto, C.; Sakairi, N.; Tokura, S.; Nishi, N. Int. J. Biol. Macromol., 20, 241-244 (1997).
[2] M. Yamada, K. Kato, K. Shindo, M. Nomizu, M. Haruki, N. Sakairi, K. Ohkawa, H. Yamamoto, N. Nishi, Biomaterials., 22, 3121-3126 (2001).
[3] M. B. Lyles, I. L. Cameron, Biophysical Chemistry., 96, 53-76 (2002).
[4] M. F. Lecompte, D. Muller, and M. Jozefowicz, Biopolymers., 11, 2563-2575 (1972).
[5] J.D. Magan, W. Blau, D.T. Croke, D.J. McConnell, J.M. Kelly, Chem. Phys. Lett., 141, 489-492 (1987).
[6] Y. Okahata, T. Kobayashi, K. Tanaka, and M. Shimomura, J. 5Am. Chem. Soc., 120, 6165-6166 (1998).
[7] R. Bruinsma, G. Gruner, M. R. D'Orsogna, and J. Rudnick, Phys. Rev. Lett, 85, 4394-4396 (2000).
[8] W. Liu, S. Sun, Z. Cao, X. Zhang, K. Yao, W. Wilu, and K. D. K. Luk, Biomaterials, 26, 2705 (2005).
[9] T. Dobashi, M. Nobe, H. Yoshihara, T. Yamamoto, and A.Konno, Langmuir, 20, 6530-6534 (2004)
[10] T. Dobashi, H. Yoshihara, M. Nobe, M. Koike, T. Yamamoto, and A. Konno, Langmuir, 21, 2-4 (2005).
[11] M. Nobe, T. Dobashi, and T. Yamamoto, Langmuir, 21, 8155-8160 (2005).
[12] L. C. Gosule, J. A. Scellman, Nature, 259, 333-335 (1976).
[13] V. A. Bloomfield, Biopolymers, 31, 1471-1481 (1991).

(Received January 31, 2006;Accepted June 5, 2006)