Formation of a DNA/polygalactosamine Complex and Its Interaction with Cells

Toshinori Sato, Nobuaki Shirakawa, Hirotaka Nishi, and Yoshio Okahata*
Department of Biomolecular Engineering, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226

(Received April 16, 1996)

DNA complexes with naturally occurring polysaccharides, polygalactosamine or chitosan, were formed in water. Thermal profiles, CD spectrum , zeta-potentials, and cell uptake were investigated. The DNA/polygalactosamine complex showed higher cell uptake than DNA/chitosan complex did.

Recently, a lot of new techniques have been developed to introduce foreign DNA into cells. One of basic requirements for the therapeutic use of nucleotides is efficient cell uptake. Encapsulation of nucleic acid into a liposome resulted in the increase of the macrophage activation.[1] Bindings of nucleic acid to polycation such as a cationic liposome,[2] lipopolyamine,[3] poly(L-lysine),[4] and DEAE-dextran[5] have been developed for the efficient cell uptake. These cationic DNA complexes are considered to internalize into cells by ionic interactions with negatively charged cell membranes. In the previous paper, we have prepared DNA complexes with lipoglutamate[6] or lipoglutamide having ethylene glycol tails.[7] These DNA complexes showed efficient cell uptake by non-specific interaction through hydrophobic alkyl chain or ethyleneglycol chain. Exploration of a DNA complex that can specifically internalize into cells is indispensable to the progress of DNA delivery system. Several polysaccharides have been used as cell recognition devise.[8,9] Though DNA/DEAE-dextran complex is used as DNA delivery system, it has some problems such as strong cell toxicity and no cell specificity. It has been known that animal cells have lectin-like receptors, which can bind to galactose/*N*-acetylgalactosamine etc, on their cell membranes.[10] Polygalactosamine from *Paecilomyces* sp. I-1[11] was found to be non-toxic and show macrophage activation.[12] We could expect the specific interaction of polygalactosamine with tumor cells or white blood cells which have a receptor against galactose. Since polygalactosamine has a primary amine on 2-C position of galactose, hydrochloride salt of the polygalactosamine can bind to polyanion DNA. From these reasons, formation of DNA complex with polygalactosamine would be an effective method to obtain cell-specific DNA complex.

In this paper, we investigate formation and cell uptake of several DNA/polysaccharide complexes. Chemical structures of polysaccharides used in this paper are shown in Figure 1. Polygalactosamine (average molecular weight, 250 kD) was kindly gifted from Higeta Shoyu Co., Ltd., Tokyo, Japan. Chitosan (average molecular weight, 245 kD) was purchased from Funakoshi Co. Ltd., Tokyo, Japan. Hydrochloride salts of polygalactosamine and chitosan were prepared by the addition of 12 N HCl to the polysaccharides suspended in water until the solution became transparent. A commercial transfection reagent, DEAE-dextran (average molecular weight 500 kD, Amres Co., Ohio, USA) was used to make a comparison with polygalactosamine and chitosan. Substitution degree of cationic DEAE group was 55%.

Aqueous solution of a sonicated DNA from salmon sperm (average molecular weight, 236 kD) was mixed with

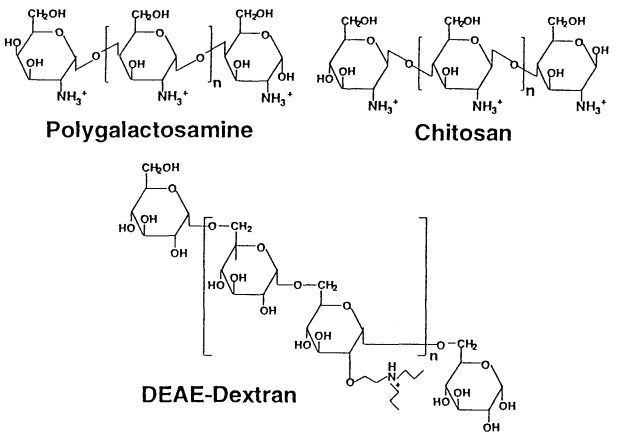

Figure 1. Chemical structures of polygalactosamine, chitosan, and DEAE-dextran employed in this study.

polysaccharide solutions. The molar ratios of the phosphate anion of DNA to the cation of the polysaccharides were prepared to be 1:1 and 1:2. The mixing of aqueous solutions of DNA/polysaccharide 1:1 complex resulted in precipitate. The mixture of DNA and polysaccharides at 1:2 was turbid, but showed no precipitates during experiments.

The thermal stability of the DNA/polysaccharide 1:2 complexes were estimated from hyperchromicity at elevated temperature (Figure 2). Thermal profiles of the DNA/polysaccharide complexes showed that the DNA complexes were more stable than simple DNA. The DNA/polysaccharide complexes showed CD spectrum characteristic to C-form.

The interaction between the DNA/polysaccharide (1:2) complexes and tumor cells was investigated. Hela cells (2×10^5 cells), which is one of tumor cells, were co-incubated with the complex of FITC-labeled DNA and polysaccharide ([DNA] = 40 $\mu g\ ml^{-1}$) for 6 h at 37 °C in 1 ml of serum-free culture medium (ASF104, Ajinomoto Co. Inc., Japan). No cell damage was not observed in the DNA/polygalactosamine and DNA/chitosan complexes at a given concentration, while DNA/DEAE-dextran complex showed strong cytotoxicity. The cells were washed five times with phosphate buffered saline (pH 7.4), thereafter collected by treating with the aqueous solution of 0.05% trypsin-0.02% ethylendiamine tetraacetic acid. The cells loading FITC-DNA were detected by flow cytofluorometer (EPICS-XL, Coulter Co., USA). Uptake efficiencies were calculated from the ratio of cell number that showed the significant increase of fluorescence intensity in all cells. The results are summarized in Table 1. Cell uptake efficiency of the DNA/polygalactosamine complex was higher than that of the DNA/chitosan complex. When incubation time was 2 h, uptake efficiency of the DNA/polygalactosamine complex was 31%. Interactions of the DNA complexs were depend on incubation times. In the case of

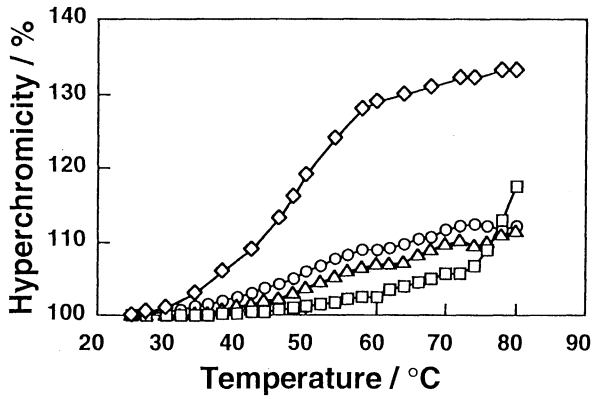

Figure 2. Melting curves (T_m) of simple DNA (◇), DNA/DEAE-dextran (□), DNA/chitosan (△), and DNA/polygalactosamine (○) complexes in aqueous solution ([DNA] = 50 μg ml^{-1}).

DNA/DEAE-dextran complex, fluorescence histogram was very broad at the present condition (data not shown) because of the cytotoxicity of the DEAE-dextran.

When these DNA/polysaccharide complexes ([DNA] = 40 μg ml^{-1}) incubated with human blood cells (500 μl) for 2 h at 37 °C, the DNA/polygalactosamine complex showed the significant uptake by blood monocytes, while uptake of the DNA/chitosan complex was almost equivalent to that of simple DNA as shown in Table 1. Since the DNA complexes had no interaction with lymphocyte, the DNA/polygalactosamine complex is considered to be specific to monocytes.

Zeta-potentials of the DNA/chitosan and the DNA/polygalactosamine complexes were +18 and +20 mV, respectively. There were no significant differences in the surface charge between the two DNA complexes. Since Hela cells and blood monocytes are known to have membrane lectin that can specifically bind to galactose,[10] efficient cell uptake of DNA/polygalactosamine complex may be caused by receptor-mediated interaction.

Through the present paper, it was suggested that DNA/polygalactosamine complex will be good vehicle for the delivery of DNA into animal cells.

This work was partly supported by Terumo Life Science Foundation.

Table 1. Uptake efficiencies of DNA and DNA/polysaccharide complexes by Hela cells and blood monocytes

Cells	Samples	Uptake efficiencies[a]
Hela cells	DNA	3%
	DNA/chitosan	44%
	DNA/polygalactosamine	85%
Blood monocytes	DNA	10%
	DNA/chitosan	9%
	DNA/polygalactosamine	58%

[a]Experimental condition and calculation of uptake efficiency was described in the text.

References and Notes

1 M. Akashi, H. Iwasaki, N. Miyauchi, T. Sato, J. Sunamoto, and K. Takemoto, *J. Bioactive and Compatible Polym.*, **4**, 124 (1989).
2 P. L. Felgner, T. R. Gadek, M. Holm, R. Roman, H. W. Chan, M. Wenz, J. P. Northrop, G. M. Ringold, and M. Danielsen, *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 7413 (1987).
3 J.-P. Behr, B. Demeneix, J.-P. Loeffler, and J. Perez-Mutul, *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 6982 (1989).
4 J. P. Lemaitre, B. Bayard, and B. Lebleu, *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 648 (1990).
5 K. Ohtani, M. Nakamura, S. Saito, K. Nagata, K. Sugamura, and Y. Hinuma, *Nucleic Acids Res.*, **17**, 1589 (1989).
6 T. Sato, T. Kawakami, N. Shirakawa, and Y. Okahata, *Bull. Chem. Soc. Jpn.*, **68**, 2709 (1995).
7 T. Sato, H. Akino, and Y. Okahata, *Chem. Lett.*, **1995**, 755.
8 T. Sato and J. Sunamoto, *Prog. Lipid Res.*, **31**, 345 (1991).
9 T. Sato and J. Sunamoto, in "*Liposome Technology, 2nd edition*," ed by G. Gregoriadis, CRC press, London (1993) Vol III, p180.
10 M. Monsigny, C. Kieda, and A-C. Roche, *Biol. Cell*, **47**, 95 (1983).
11 H. Takagi and K. Kadowaki, *Agric. Biol. Chem.*, **49**, 3159 (1985).
12 K. Ishitani, S. Suzuki, and M. Suzuki, *J. Pharmacobio-Dyn.*, **11**, 58 (1988).