DNA as a Component of ER Materials

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Abstract. Deoxyribonucleic acid (DNA), which is known as a typical biopolymer, has been utilized for a few types of ER materials. Suspensions were prepared with the particles of DNA, DNA/lipid complexes, and LDH (layered double hydroxide)/DNA composites. The purified DNA showed larger ER effect than the others, but this particle tended to absorb water, which caused less stability. Preliminary experiments of preparing composite with LDH indicated that this inorganic material would be useful for hydrophobic modification of DNA particles, although further optimization of composite preparation is needed. In addition, the LDH/DNA suspensions showed interesting behaviours under some conditions, which indicated possibility for controlling ER property in a wide range.

1. Introduction
Deoxyribonucleic acid (DNA) is not only one of the most important biomolecules but also an interesting functional material because of its unique structure and property. The base pairs inside the double-strand specifically interact with appropriate chemical species, and also adsorb hydrophobic substances. The outside double helix is negatively charged, and binds cationic substances. For example, cationic lipids can form a complex with DNA, and its possible application as a functional material has been reported [1]. It is also known that the charge of DNA is used for separation of them according to size, i.e. electrophoresis, which is based on the movement of the charged polymer chain by an electric field. This electric-field response suggests utilization of DNA as a component of electrorheological (ER) fluids. We have focused on DNA as one of the series of study on polymeric ER materials [2, 3, 4]. The application of DNA as a bio-based functional material can also be effective use of the ‘waste natural product’. For example, salmon testes, which are produced in the process of food industry, are typical DNA-rich waste.

The salmon testes, and other commonly available DNA’s are combined with proteins, and purification and/or modification are necessary for utilization as a material. Here suspensions of DNA, DNA/lipid complexes, and LDH (layered double hydroxide)/DNA composites were prepared in order to evaluate the effects of chemical modification of DNA. Some cationic lipids are known to bind with DNA, which should be useful for DNA modification. LDH was also selected here because of its unique property to form composites with organic compounds. The resulting composites have the properties of both inorganic and organic components. For example, LDH/drug composites are expected to be useful for biomedical applications, which is another subject of our recent work [5] as the basis of this study.
2. Materials and methods

2.1. Preparation of DNA and complex particles
Salmon sperm DNA was commercially available as powders containing some amount of proteins. The as-received DNA, purified DNA, and DNA/lipid complex were compared here. The purification of DNA was carried out according to the standard methods of phenol/chloroform extraction and ethanol precipitation. DNA/lipid complex was prepared by mixing aqueous solutions of DNA and of n-hexadecylammonium bromide followed by filtration. The complex was freeze-dried and milled to give fine particles. The LDH/DNA composites were prepared according to the co-precipitation method. Aqueous MgCl2 and AlCl3 solutions (Mg/Al = 2, 3, or 4) were mixed in the presence of DNA. The obtained composite particles were filtered and dried in vacuum.

2.2. Characterization of particles
X-ray powder diffraction patterns of LDH samples were recorded on a Rigaku X-ray diffractometer. An optical microscope (Nikon) and a scanning electron microscope (Hitachi FE-SEM S-4700) were used for the observation of appearance of the particles.

2.3. Preparation of ER fluids
The as-received DNA, purified DNA, DNA/lipid complex, and LDH/DNA composite particles were respectively dispersed in silicone oil (DMS; 1000cSt) to examine the influence of proteins, the cationic lipid, and LDH bound to DNA.

2.4. ER measurement
The ER measurements were performed by use of a parallel-disc rotation rheometer (Rheometric ARES) equipped with a high-voltage power supply. The disc diameter and the gap between the electrodes were 40 mm and 1.0 mm, respectively. Steady shear viscosity and dynamic viscoelasticity were measured in the presence and absence of a d.c. electric field.

3. Results and discussion

3.1. DNA and DNA/lipid particles
Figure 1 (a-c) shows the appearance of the DNA particles prepared in this study; i.e. (a) as-received DNA, (b) purified DNA and (c) DNA/lipid complex. It is found that all these particles have similar irregular shape and rather wide size distribution. Figure 2 exemplifies the results of the ER measurements with the suspensions of purified DNA and DNA/lipid complex (particle concentration: 20wt%). Here the shear rate dependence of the shear stress is plotted in the absence and presence of an electric field ranging between 0 and 2kV/mm. The both fluids behaved like a Newtonian and a Bingham fluid in the absence and presence of an electric field, respectively. This ER effect was caused by the formation of chain-like clusters of DNA particles bridging the electrodes under the electric field as shown in Fig. 1(d).

Fig.1  Optical micrographs of DNA particles: (a) as-received DNA, (b) purified DNA, (c) DNA/lipid complex, and (d) chain-like structure of purified DNA observed under 2kV/mm. Scale bar: 100µm.
Fig. 2 Flow curves of suspensions with DNA based particles. (a) purified DNA (20wt%) and (b) DNA/lipid complex (20wt%).

Fig. 3 Comparison of the ER effects of as-received DNA, purified DNA, and DNA/lipid complex suspensions. (a) Electric-field dependence of stress at a low shear rate (0.02 s\(^{-1}\)); (b) The ratio of stress obtained under a 2kV/mm electric field to that under no electric field measured at 1 s\(^{-1}\).

Figure 3 shows the ER effect of as-received DNA, purified DNA, and DNA/lipid complex suspensions. In Fig. 3(a), the stress values obtained at the lowest shear rate examined were plotted against the square of the electric-field strength. These values can be used to evaluate the 'absolute value' of ER effect. The stress values are proportional to the square of field strength, and the largest stress increase was obtained with the purified DNA suspension. The large ER effect of purified DNA is due to the removal of proteins shielding the charge of DNA molecules. The lipid molecules attached to DNA have similar effect to that of proteins. It is also noticed that the relative ER effect shows a maximum at the particle concentration of 20wt%. This result is due to an increase in the shear stress in the absence of an electric field at high particle concentrations. Although the purified DNA seemed to be best from these results, the purified DNA particle was found to form sticky aggregates due to the
adsorbed water from air when kept under open atmosphere. Thus the stability of this particle is not so good. It should also be noted that water affects the ER property. On the other hand, the lipid complex did not change its shape for long time, which indicates little influence of water from the air. This fact suggests that appropriate chemical modification can be used for utilization of DNA as stable particles. Further improvement is expected by using various DNA-binding substances.

3.2. LDH /DNA composite
The X-ray diffraction patterns of the particles obtained with the coprecipitation method indicated an increase in the distance between LDH layers. The increase of the layer spacing suggested that DNA molecules were intercalated into LDH layers. From the observation with optical microscopy, the average diameter of obtained LDH/DNA primary particles was about 5 μm, although the size varied with the reaction conditions. It was found that the LDH/DNA particles are in aggregated form, and the average diameter of the aggregates was typically about 25 μm. Suspensions of LDH/DNA with DMS were prepared and the dependence of ER effect on the LDH composition was examined. All samples varying Mg/Al ratio showed an ER effect: e.g., the shear stress of 20 wt% suspensions under a 2kV/mm field at 1 s⁻¹ for Mg/Al = 2, 3, 4 was 70, 50, and 40 Pa, respectively. The low Mg/Al ratio resulted in a larger ER effect. Although the present preliminary experiments searching for optimum conditions resulted in rather low stress enhancement under an electric field, this composite preparation method has the advantages that stable DNA-based particles can be obtained easily and that the compositions of LDH (metal species and ratio) and of composite (LDH/DNA ratio, etc.) can be widely changed. It was also found under some conditions that a ‘slipping layer’ of DMS by particle aggregation could be formed (Fig. 4), which decreases the apparent viscosity. The slipping occurs when the particles tend to aggregate rather than homogeneously dispersed. The shear rate and electric-field strength are also important. The control of slipping and non-slipping structures by the composition and field conditions makes it possible to vary the ‘positive and negative’ ER effects. From these results, LDH/DNA composites were found to be applicable to ER suspensions, and the property can be widely varied by changing the ratios of metals and DNA, and fluid composition. A variety of ER materials can be designed by use of this method.

4. Conclusion
DNA-based particles are applicable for ER suspensions. The enhanced ER effect of purified DNA indicated the potential usefulness of DNA as a component of ER materials. Though the particle stability is a problem, chemical modification with lipid can be a solution for it. The LDH composite also indicated another possibility of the use of DNA-based ER fluids having interesting properties.

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References
[1] Tanaka K and Okahata Y 1996, J. Am. Chem. Soc., 118 (44), 10679.
[2] Minagawa K and Koyama K 2005, Curr. Org. Chem., 9,1643.
[3] Minagawa K, Aoki Y, Mori, T, Tanaka M 2006, Int. J. Mod. Phys., 20 (25, 26, 27), 3987.
[4] Minagawa K, Aoki Y, Tanaka M 2007, Polym. Prepr. Jpn., 56, 2E18.
[5] Berber M R, Minagawa K, Katoh M, Mori T, Tanaka M 2008, Eur. J. Pharm. Sci. in press.