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Enzymatic collapse of artificial polymer composite material containing double-stranded DNA

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ABSTRACT

Large amounts of DNA-enriched biomaterials, such as salmon milts and shellfish gonads, are discarded as industrial waste around the world. Therefore, the utilizations of DNA with the specific function are important for the biomaterial science and the curce technology. We could convert the discarded DNA to an enzymatic collapsible material by the addition of DNA to the artificial polymer material, such as nylon. Although these DNA–artificial polymer composite materials were stable in water, these materials indicated the collapsibility at the DNA-hydrolyzed enzyme, such as *Micrococcal nuclease*, condition. Additionally, these collapsibilities under enzyme condition were controlled by the number of imino groups in the components of the artificial polymer. Furthermore, these composite materials could create the fiber form with a highly ordered molecular orientation by the reaction at the liquid/liquid interface. The DNA–artificial polymer composite materials may have the potential utility as a novel bio-, medical-, and environmental materials with the enzymatic collapsibility and degradability.

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1. Introduction

DNA is one of the most important materials for the genetic process of living organisms. Therefore, DNA research has been investigated in the life sciences, such as the biochemical, medical, or pharmaceutical fields. However, since double-stranded DNA has highly specific functions, such as the accumulation of intercalating compounds, the complementary interaction between nucleic acid bases, or chiral recognition, it has the potential ability to be used as a functional polymer material [1–5]. Additionally, DNA is readily purified from salmon milts or shellfish gonads, but large amounts of the DNA-enriched materials have been discarded as waste in the industry. Therefore, DNA is interesting as a material from an environmental science point of view. The utilization of DNA as a functional material has been reported for the protein binding DNA columns [6,7], DNA–biopolymer composite materials [8–10], and electroconductive DNA films [11]. Recently, we also prepared a water-insoluble and nuclease-resistant DNA film by UV irradiation [12], as a result, these DNA films were useful as environmental materials, such as the selective accumulation of DNA-intercalating compounds [13] and heavy metal ions [14].

Artificial polymer materials, such as nylon or polystyrene, have been used around the world. However, these polymer materials are stable, non-degradable, and non-collapsible in the natural world and often cause environmental pollution. In contrast, being a biopolymer indicates degradation in nature. Additionally, the biopolymers are non-hazardous and environmentally benign. Therefore, a composite material formed by mixing an artificial polymer and biopolymer is effective as a biodegradable material [15,16]. However, a degradable composite material including DNA, one of the most famous biopolymers, has not yet been reported to the best of our knowledge. Additionally, DNA-hydrolyzed enzyme, such as *Micrococcal nuclease*, has widely existed in the human body or environmental field. Therefore, the degradable and collapsible composite materials by the DNA-hydrolyzed enzyme are important and new concepts. So, we examined the enzymatic collapse of an artificial polymer composite material containing DNA.

In the present study, we prepared the DNA–artificial polymer composite material by the mixing of DNA and the components of nylon 66. These composite materials formed fibers by interface polymerization and were stable in an aqueous solution. Additionally, these materials indicated the collapsibility in the presence of the DNA-hydrolyzed enzyme, such as *Micrococcal nuclease*. Furthermore, these collapsibility under enzyme conditions were controlled by the number of imino groups in the components of the artificial polymer. These DNA–artificial polymer composite materials may

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have the potential utility to be used as biomedical, bioengineering, and environmental materials.

2. Material and methods

2.1. Materials

Double-stranded DNA (sodium salt from salmon milt, molecular weight; $>5 \times 10^6$) was obtained from Yuki Fine Chemical Co. Ltd., Tokyo, Japan. Adipoyl chloride (AD), hexamethylenediamine (Hex), bis(3-aminopropyl)amine (NBis), and *N*,*N*′-bis(3-aminopropyl)ethylenediamine (NNBis) were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan or Strem Chemicals Inc., Newburyport, MA. Their molecular structures are shown in Fig. 1. *Micrococcal nuclease*, a DNA-hydrolyzed enzyme, was purchased from Worthington Biochemical Corp., Lakewood, NJ.

2.2. Preparation of DNA–artificial polymer composite materials

DNA–artificial polymer composite materials were prepared by the molecular ratio of DNA (nucleotide)—diamino-molecule: AD = 3:43:30. A DNA aqueous solution (100 μl, 10 mg/ml) and aqueous diamino-molecules, such as Hex, NBis, or NNBis, solutions (100 μl, 4.3×10^{-1} M) were mixed in a test tube. These mixed solutions were added to a 0.3 M AD hexane solution (100 μl) and vigorously mixed by the touch mixer. As a result, the DNA–artificial polymer composite materials were formed in the solution. These DNA–artificial polymer composite materials were rinsed with pure water (5× 5 ml) and hexane (5× 5 ml) then stored in water. The amount of DNA in the composite material was determined by the following procedure: the composite material was hydrolyzed with 6 M HCl solution at 100 °C for 1 h and quantified by absorbance at 260 nm using a U-2010 UV-Vis spectrophotometer (Hitachi Co. Ltd., Tokyo, Japan).

2.3. Characterization of DNA–artificial polymer composite materials

The stability of the composite material in an aqueous solution was confirmed by the following method: the composite materials were incubated in ultra-pure water (20 ml) for various times. The absorbance at 260 nm of the solution was measured and the eluted DNA from the composite material was determined. The infrared (IR) absorption spectra of the composite material were measured by ATR methods using a FT-IR 8200 Fourier transform infrared spectrometer (Shimadzu Corp., Kyoto, Japan).

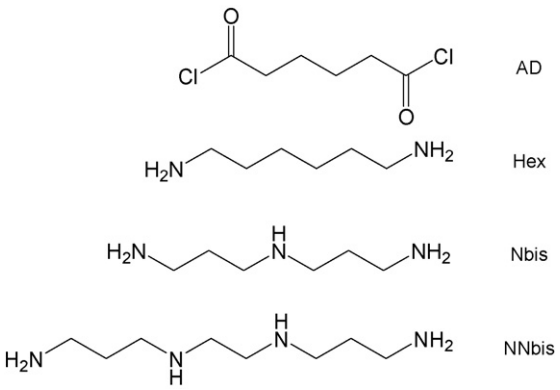

Fig. 1. Molecular structure of components of artificial polymer.

2.4. Preparation of DNA–artificial polymer composite fiber

The AD hexane solution was added to the mixed solution of DNA and diamino-molecules without mixing and the composite material at the liquid/liquid interface was drawn from the liquid/liquid interface by tweezers. The composite fiber was rinsed with pure water (5× 10 ml) and hexane (5× 10 ml). This fiber was dried in air and observed using an optical or polarization microscope (BX50, Olympus Optical Co. Ltd., Tokyo, Japan) with two polarizing filters.

2.5. Enzymatic collapse of DNA–artificial polymer composite materials by the DNA-hydrolyzed enzyme

The DNA–artificial polymer composite materials were stored in ultra-pure water for more than 1 day to remove the small amount of water-soluble DNA and then used for the sample of the enzymatic collapse. The degradation and collapse of the composite material by the nuclease was confirmed by the following method: the composite materials were added to 20 ml of 20 mM Tris–HCl buffer containing 5 mM NaCl and 2.5 mM $CaCl_2$ (pH 7.4) in the presence of nuclease (*Micrococcal nuclease*, 2 units/ml) at 37 °C [12]. The amount of hydrolyzed-DNA by the nuclease was measured by the absorption at 260 nm at various times.

3. Results and discussion

DNA–artificial polymer composite materials were prepared by the mixing of DNA, diamino-molecules, and AD solutions. Especially, we used diamino-molecules, such as Hex, NBis, and NNBis, with the different number of imino groups (please see molecular structures in Fig. 1). These white composite materials did not break by pinching with tweezers. Table 1 shows the yield of composite polymer and the amount of DNA in DNA–artificial polymer composite material. The yield of composite polymer was estimated by the following equation:

$$\text{yield} = \frac{\text{weight of dried composite material}}{\text{weight of initial mixing amount}} \times 100 \quad (1)$$

The yield of composite polymers was 23–36% and the yield of DNA–NNBis–AD composite material was lower than other composite materials. This lower yield is due to the molecular length of NNBis molecule. In fact, the yield of NNBis–AD composite material without DNA was lower than other materials (data not shown). The amount of DNA in DNA–artificial polymer composite material was 14–17% and almost similar value. However, the stability of DNA in composite materials showed a large difference (mention later).When the AD hexane solution was carefully applied on a mixed solution of DNA and diamino-molecules, a film of the DNA–artificial polymer composite material was formed at the liquid/liquid interface. This film was continuously produced at the interface by drawing the film, and the DNA–artificial polymer composite fiber was then prepared. Fig. 2(a) and (b) shows

Table 1
Yield of composite polymer and amount of DNA in DNA–artificial polymer composite material

	Yield (%)[a]	Amount of DNA (%)[b]
DNA–Hex–AD	34	14
DNA–NBis–AD	36	17
DNA–NNBis–AD	23	15

[a] Yield of composite material was determined by Eq. (1).
[b] DNA–artificial polymer composite material was hydrolyzed with 6 M HCl solution at 100 °C for 1 h and quantified by absorbance at 260 nm.

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