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Effect of DNA structure on the formation of collagen–DNA complex

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Abstract

Using various types of DNAs prepared from plasmid DNA, complete double-stranded DNA (ds.DNA) with linear and cyclic forms and double-stranded DNA coexisting with single-stranded DNA (ss.DNA), the structure and fibrillogenesis of the collagen–DNA complex were investigated by means of turbidity, transmission electron microscopy, and confocal laser-scanning microscopy. The rate of fibrillogenesis of the collagen–DNA complex significantly depends on the DNA structure. The structure of the fibrils formed in the complexes showed a marked difference between the ds.DNA and ss.DNA complexes with collagen. Spatial distribution of the DNA and collagen in the complexes suggests that the characteristic collagen–DNA interaction depends on the DNA forms.

Keywords: Collagen; DNA; Fibrillogenesis

1. Introduction

There are various types of collagens that have different structures, properties, and tissue distributions, and their applications have been studied by various approaches [1–5]. Among them, the type I collagen is the commonest one with a molecular weight of 300 kDa and is an essential component of tissues in animals, such as the skin, tendons, and bones. Type I collagen consists of triple helix and non-helical telopeptides on both ends, which is known to have an antigenecity, as shown in Fig. 1. Type I collagen forms straight fibrils with a clear cross-banding pattern under physiological conditions. The cross-banding pattern arises from the regularly arranged collagen assemblies and exhibits characteristic changes depending on the solution conditions, such as ionic strength, pH, coexisting components in the solution, and so on [3,4]. The fibril structure of collagen is important for the biological functionalities and applications of collagen. Complexes of collagen with biomaterials have already been reported and proposed as a novel biological functional material by many researchers [6–9]. Some of these collagen complexes were found to induce biological activity, such as proliferation of fibroblasts and activation of platelets [8,9]. Recently, it has been reported that atelocollagen could be utilized in the delivery systems for proteins and genes [10,11]. In atelocollagen, the telopeptides at the ends of the collagen molecules are removed. Atelocollagen is a harmless biomaterial, because the telopeptides of collagen are the antigenic sites, and is a valuable candidate for the development of novel biomaterials.

DNA is not only important as a genetic material, but is also useful as a functional material, because of its unique structure and properties. We have studied the preparation of functional materials using DNA, such as an insoluble DNA film for the use in the environmental field [12–15], a DNA–chitosan bilayer membrane for use in the medical field [16], and a DNA-based complex gel for a lactic bacteria delivery system [17]. The complexes of DNA with positively charged materials such as a colloid [18], lipid [19,20], chitosan and

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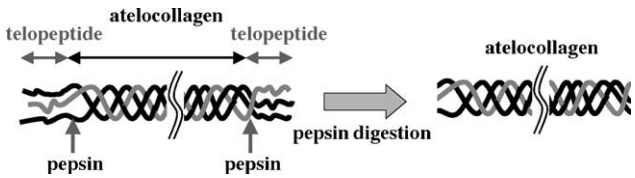

Fig. 1. Schematic illustration of atelocollagen.

gelatin [21,22] have been studied and useful results have been obtained for their application in the biological and medical fields.

Complexes of the DNA-positively charged colloid can be used in the biological and genetic fields, because of the resemblance of these complexes to the histone–DNA complex. Histone is a positively charged protein, interacts electrostatically with DNA in vivo, and forms complexes. The relation of histone–DNA is especially similar to that of the collagen–DNA. The collagen–DNA complex is postulated to be a unique material with unique applications. In fact, the atelocollagen–plasmid DNA complex was recently reported as a valuable material in a gene delivery system [10,11].

Previously, we reported that collagen binds to DNA originating from salmon milt (DNA_{salmon}) and forms a complex [23–25]. The collagen–DNA_{salmon} complex formed straight fibrils with a clear cross-banding pattern ascertained by TEM observations even when using atelocollagen, although the atelocollagen without telopeptides has been considered to form thin fibrils without a clear cross-banding pattern. These facts mean that DNA molecules mediate the regular arrangement of collagen molecules through a complex formation.

The spatial distributions of collagen and DNA_{salmon} in the complex gel were not homogeneous according to the CLSM measurement. The dynamic process of the complex formation in the collagen–DNA_{salmon} mixture was initially investigated by dynamic light scattering (DLS) and a quartz crystal microbalance (QCM) [25]. However, it was difficult to elucidate the mechanism of the collagen–DNA complex formation in detail using DNA_{salmon}, because the DNA_{salmon} has a very wide molecular weight distribution range and the content of the double-stranded DNA was not perfect due to the substantial coexistence of the single-stranded DNA. The structure and fibril formation process of the collagen–DNA complex must be affected by the molecular weight distribution of DNA and the type of DNA, such as the single- or double-stranded DNA. Therefore, it is desirable to use a DNA sample having a narrow molecular weight distribution and distinct strand form.

In the present study, a complete double-stranded DNA with a monodisperse molecular weight distribution prepared from plasmid DNA was investigated for the interaction between collagen and DNA. Turbidity has been measured in order to clarify the fibrillogenesis and to evaluate the condition of fibril assembly in the collagen–DNA complex. The fibril and fibril network were observed using a confocal laser-scanning microscope (CLSM) as well as a transmission electron microscope (TEM).

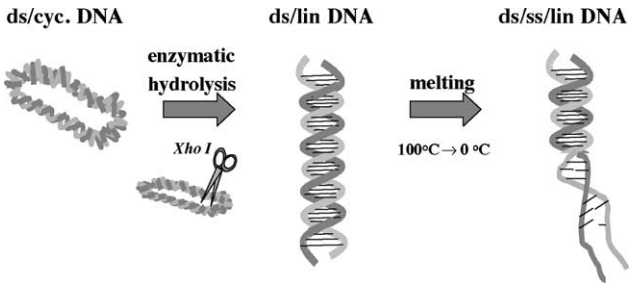

Fig. 2. Preparation of three types of DNA.

2. Experimental

2.1. Materials

The pepsin digested type I atelocollagen (3 mg/ml, pH 3) dissolved in hydrochloric acid was purchased from KOKEN Co., Ltd., Japan. The plasmid DNA (pBluescript (+) SKII, TOYOBO Co., Ltd., Japan) was extracted from *Escherichia coli* and purified using an ion-exchange column (NUCLEOBIND, Macherey-Negel Co., Ltd., Germany). Three types of DNA with different structures were prepared from the plasmid DNA as shown in Fig. 2. The plasmid DNA was used as a double-stranded cyclic DNA (ds/cyc.DNA). The double-stranded linear DNA (ds/lin.DNA) was prepared by treating ds/cyc.DNA with Xho I. A partially single-stranded linear DNA (ds/ss/lin.DNA) was prepared by heating ds/lin.DNA at 100 °C for 10 min followed by immediate cooling at 0 °C. The molecular size of these DNAs were examined by 1% agarose gel electrophoresis. The content of the double-stranded DNA of these DNAs were examined using a fluorescence reagent (PicoGreen dsDNA Quantitation Kit, Molecular Probes Co., Ltd., USA). These DNAs were dissolved in phosphate buffered saline (PBS; 137 mM NaCl, 8.10 mM $Na_2HPO_4{\cdot}12H_2O$, 2.68 mM KCl, 1.47 mM KH_2PO_4, pH 7.4), and the concentration of the DNA solution was determined from the absorbance at 260 nm. The DNA solutions were heated at 70 °C for 5 min in order to accomplish the molecular dispersion before mixing with collagen. The melting temperature of the DNA was confirmed using a spectrophotometer at 260 nm over the temperature range of 20–100 °C, and no melting of the double-stranded DNA in PBS was observed at 70 °C. The characteristics of these DNAs are summarized in Table 1.

2.2. Methods

2.2.1. Turbidity measurements

The turbidity measurements were spectrophotometerically carried out at 400 nm using a UV–vis spectrophotometer