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DNA–collagen complex as a carrier for Ag^+ delivery

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Abstract

The possibility of DNA–collagen complex as a drug carrier was investigated. The interaction between DNA and silver ions was proved by CD spectra. The release property of the complex of DNA–Ag^+ was measured through turbidity of PBS solution to indicate that silver ions could coordinate with base pairs of DNA, and be released slowly from the complex of DNA–Ag^+. Collagen film, collagen–Ag^+ film, DNA–collagen film and DNA–collagen–Ag^+ film were prepared, and studied through SEM. Particles were found present in DNA–collagen–Ag^+ film by SEM. These show that silver ions may be enclosed inside these particles, which led to the slow release of Ag^+ to the environments. Two bacteria, *Eschericia coli* and *Staphylococcus aureus*, were used to study the antibiotic properties of the complex films. The growth of *E. coli* and *S. aureus* could be inhibited by these films. It indicates that DNA–collagen may be a good drug carrier for the drug-controlled release.

Keywords: Collagen; DNA; Silver ions; Drug carrier

1. Introduction

Clinical drugs for anti-bacteria and germicides are usually in the form of liquid, pill, capsule and unguentum. Silver or silver ions have long been known to have powerful antibacterial activity. Silver is widely applied in some medical fields for its high antimicrobial activity and low concentration [1]. However, few examples could be seen for the use with bio-controlled release carrier. DNA, consisting of four kinds of bases, is not only important as genetic material, but is also useful as functional material [2]. The special double helical structures endue DNA a special ability to complex with other materials. It is expected that DNA could be effectively used as a controlled release material.

Collagen is of particular interest as a natural polymer for drug delivery since it is a major natural constituent of connective tissue and a major structural protein of any organ [3]. Collagen has long been used as a biocompatible material [2], and its usefulness and safety have been verified with many examples [4–6]. Biomaterials made of collagen offer several advantages: they are biocompatible and non-toxic and have well-documented structural, physical, chemical, biological and immunological properties [7–9]. Additionally, drug release kinetics can be influenced by modification of the matrix characteristics (porosity, density) or by different chemical treatment regimes affecting its degradation rate [3]. Many attempts have been made to utilize collagen as a carrier of sustained release preparations, but because of the difficulty in controlling release, the technology to put this substance into practice has yet to be developed [6].

The utilization of DNA from salmon has been investigated for the testes as functional films and gels [10–15]. It has been reported that that DNA could adsorb collagen, that offers the promise for the combined use of DNA and collagen as drug delivery material [14]. In the present study, the controlled release and anti-bacterial properties of the complexes of Ag^+, DNA and type I collagen that 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 [14] were investigated.

2. Materials and methods

2.1. Materials

Double stranded DNA from salmon milt (M.W. ca. 5×10^6) was kindly supplied by Yuki Fine Chemical, Tokyo, Japan. The

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molecular weight of *ds*.DNA was examined by 1% agarose gel electrophoresis. The content of *ds*.DNA was examined by using a fluorescence reagent (PicoGreen dsDNA Quantitation Kit, Molecular Probes Co., Ltd., USA). Type one Collagen (M.W. over 3×10^5) was purchased from Funakoshi.

2.2. Methods

2.2.1. CD spectra measurement

The concentration of DNA was adjusted to 0.1 mol/l, and circular dichroism (CD) spectra were obtained with different ratios of Ag^+ on a Jasco J-720 CD spectropolarimeter (Japan spectroscopic, Japan). Measurements were carried out using a quartz cell of 1 cm path length at 4 °C.

2.2.2. Measurement of turbidity

Phosphate buffered saline (PBS) was prepared by adding NaCl (1.745 g) and anhydrous Na_2HPO_4 (0.568 g) into water (70 – 80 ml) and then the pH was adjusted to 7.4 with 1N HCl. The final amount of the buffer was adjusted to 100 ml with water. The turbidity measurements were spectrophotometerically carried out at 400 nm using a UV–vis spectrophotometer (Jasco V-560, Jasco Co., Ltd., Japan). Ten minutes after DNA solutions (without or with Ag^+) were mixed with PBS, a solution containing Cl^- was added. The time course of the optical density was continually measured at 37 °C.

2.2.3. Observation of fibrils by scanning electron microscopy (SEM)

The films of collagen, collagen–Ag^+, DNA–collagen and DNA–collagen–Ag^+ were prepared. These films were observed using a S-2300 electron scanning microscope (Hitachi Co., Ltd., Japan).

2.2.4. Antibacterial test

Two bacteria, *Eschericia coli* and *Staphylococcus aureus*, were incubated with the films of DNA–collagen–Ag^+ on the medium containing beef extract (0.0105 g), bacto peptone (0.015 g), NaCl (0.0105 g) and agar (0.225 g) in deionized water (15 ml) at 28 °C for 24 h. The inhibitory effects were observed and evaluated by the inhibition zone.

3. Results and discussion

3.1. Interaction between DNA and Ag^+

Metal–DNA interactions have been the subject of extensive study because metal ions play very important role in the process of DNA synthesis and cleavage [16,17]. It has been proposed that Ag^+ binds more strongly with nucleobases than phosphates [18] and also proved that the binding could reduce overall negative charge of DNA and increase molecular mass of DNA [19].

To our knowledge, no reports were published about the interaction between Ag^+ and DNA studied through CD spectroscopy. The interaction between DNA and silver ions is disclosed herein by CD spectroscopy (Fig. 1). By the addition of silver ions, obvious changes in the CD spectrum of DNA are observed.

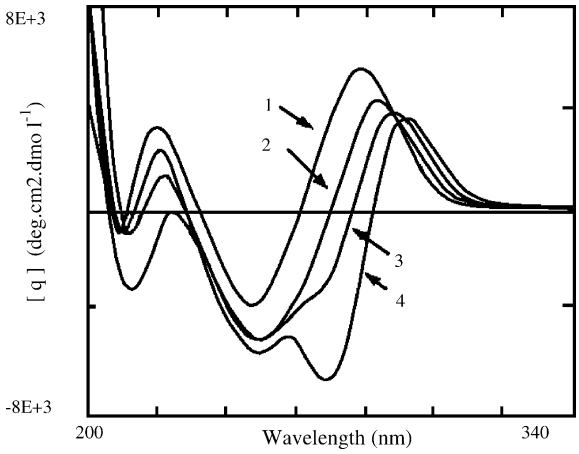

Fig. 1. CD spectra of double-stranded DNA in the absence (1) and presence of silver ions. Residual molar ratios of DNA to silver ions are 6:0.25 (2), 6:0.5 (3), and 6:1 (4).

In the absence of silver ions there are two peaks in CD spectrum of double-stranded DNA at 219.0 and 277.5 nm, respectively. In the presence of silver ions the two peaks move to longer wavelengths, at 220.2 and 282.8, 221.3 and 287.5, 222.5 and 291.3 nm with the molar ratios of DNA to silver ions at 6:0.25, 6:0.5 and 6:1, respectively. The shift distance of first peak is correlated well with the amount of DNA added, about 1.2 nm between neighboring peaks. The shift of second peak, however, is not correlated well with the amount of DNA. The shift distances are 5.3, 4.7 and 3.8 nm, respectively. [θ] is lowered by the addition of silver ions, the more added, the more decreases. The result implies that silver ions can enter into the DNA molecules, and coordinate with base pairs of DNA. The reason may arise from the conformation change caused by the binding of silver ions to nucleobases in DNA [20].

3.2. Release of Ag^+

The controlled release of silver ions enclosed by DNA was studied by turbidity, because the amount of silver ions can be quantitatively measured with chlorine ions in PBS by Turbidity Method. According to Fig. 2 when DNA is absent from the solution, the turbidity of the PBS solution increases immediately after the addition of silver ions, and then drops down slowly by the formation of AgCl precipitate (curve 1). Whereas the

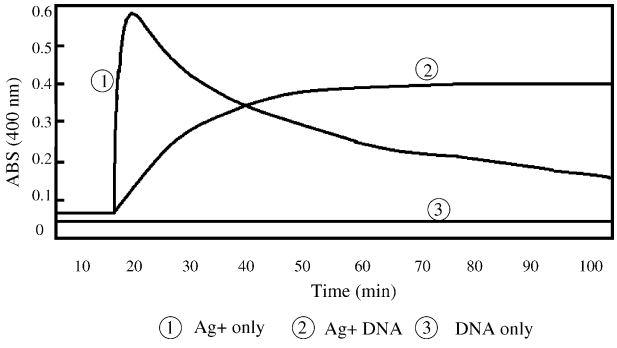

Fig. 2. Time course of turbidity due to the formation of AgCl precipitate.