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Dynamic Process of the Formation of Collagen-DNA Complex

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Fibrillogenesis of collagen is significantly affected by the addition of DNA. Collagen fibrils formed in the presence of DNA exhibit an increase in size, and a distinct cross-banding pattern is observed. This result suggests that DNA binds to collagen directly to form the collagen-DNA complex. In order to clarify the mechanism of the complex formation, quartz crystal microbalance (QCM) and dynamic light scattering (DLS) measurements were carried out. The samples were pepsin-digested type I collagen, double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA). The resonance frequency of collagen-deposited QCM decreased in response to the addition of aqueous DNA solution. The adsorption of DNA per deposited collagen of 1 mg was calculated, and it was revealed that ssDNA is adsorbed to collagen ten times as much as dsDNA. In DLS measurements, scattered light intensity <I> for the collagen solution without DNA increased gradually with the increase of the elapsed time. On the contrary, <I> for the collagen solution with DNA was saturated immediately after the mixing. This result indicates that collagen fibrillogenesis is enhanced significantly by the addition of DNA. Temporal evolution of the decay time distribution revealed that both dsDNA and ssDNA bind to collagen to form the complex, and ssDNA had a higher ability of complex formation compared with dsDNA.

Key words: collagen, DNA, quartz crystal microbalance, dynamic light scattering

1. INTRODUCTION

Collagen is easily extracted and purified from the connective tissues, and it has been utilized as biomedical materials, *e.g.* skin, tendon and vessel replacement. The collagen molecule is consisted of three polypeptide chains with triple-helix, and the molecules assemble to form specific fibrils under physiological conditions. The structure of collagen fibrils affects its biological activity, such as proliferation of fibroblasts [1] and activation of platelets [2]. On the other hand, collagen molecules interact with various biological molecules; such as phosphoproteins [3,4], proteoglycans [5,6] and chitosan [7], resulting in a change of the structure of collagen fibril. Previously, we reported the marked effects of DNA on collagen fibrillogenesis [8]. Pepsin-digested collagen without terminal non-triple-helical sequences is known to form the fibril without a distinctive cross-banding pattern. However, the collagen fibrils formed in the presence of DNA showed a very clear pattern as shown Fig. 1. This result strongly suggests that DNA interacts with collagen molecules and regulates collagen fibrillogenesis.

In the present study, the interaction between collagen and DNAs were examined by using a quartz crystal microbalance (QCM). Furthermore, dynamic light scattering (DLS) measurements were carried out to investigate the dynamic process of the collagen-DNA complex formation.

2. EXPERIMENTAL

Two types of DNA (double-stranded DNA; M = *ca.* 5×10^6, Yuki Fine Chemical Co. Ltd., Japan and single-stranded DNA; M = *ca.* 2.5×10^5, Sarude Co. Ltd., Japan) extracted from salmon milt were dissolved in distilled water, respectively. Pepsin-digested type I collagen in HCl solution was purchased from Koken Co. Ltd., Japan.

The QCM measurements were made by the commercially available QCM-system (AFFINIXQ, Initium Co. Ltd., Japan). Collagen-deposited quartz crystal (27 MHz, 4.9 mm^2 Au electrode) was immersed in 8 ml of phosphate buffered saline (PBS), and then 100 μl of DNA solution (0.4 mg/ml) was injected with stirring.

DLS measurements were carried out using a homemade spectrometer and an ALV-5000 multiple-tau digital correlator to obtain the correlation function of scattered $g^{(2)}(t)$, where t is the delay time. The decay time, τ, distribution function $\tau G(\tau)$ was obtained from $g^{(2)}(t)$ using a CONTIN program. The light source was an Ar ion laser with a wavelength λ = 488 nm, and the details of the apparatus was described elsewhere [9]. Collagen solution (4 μg/ml), DNA solution (0.3 μg/ml)

and PBS were passed through a membrane filter of 0.8 μm pore size. Immediately after mixing these solutions at the volume ratio of 1:1:2, the measurements of the correlation function were started by using the homodyne mode at a scattering angle of θ=30°. All the measurements of QCM and DLS were performed at 27°C.

3. RESULT AND DISCUSSION

Figure 2 shows typical time courses of frequency changes ΔF of the collagen deposited QCM in response to the addition of aqueous ssDNA (a) or dsDNA (b) solution. Immediately after the addition of 100 μl of DNA solution, the frequencies abruptly decreased and were saturated at -ΔF=380 Hz within 50 min for dsDNA and at -ΔF=200 Hz within 30 min for ssDNA, respectively. The relation between ΔF and mass change Δm of the adsorbed materials onto the electrode is expressed by the following Sauerbey equation [10]:

$$\Delta F = \left(-2F_0^2 / A\sqrt{\rho\mu}\right) \cdot \Delta m \qquad (1)$$

where F_0 is the fundamental frequency of the QCM (27 MHz), A is the area of electrode (4.9 mm^2), ρ and μ are the density (2.65 g/cm^3) and shear modulus (2.95×10^{11} dyn/cm^2) of quartz, respectively. Thus, a frequency decrease of 1Hz corresponds to a mass increase of 30 pg on the QCM. The amount of collagen deposited on the QCM was obtained from the difference in frequency between the deposited and the bare QCM. The adsorption of DNA per the deposited collagen of 1 mg was 0.16 mg for dsDNA or 0.08 mg for ssDNA. Thus, the adsorbed number of ssDNA molecules was calculated to be ten times as much as that of dsDNA molecules taking account of the respective molecular weights. It was confirmed that both DNAs have affinity with collagen molecules and the ssDNA binds to collagen more easily as compared with dsDNA. In this context, it is noteworthy that the annealing of dsDNA

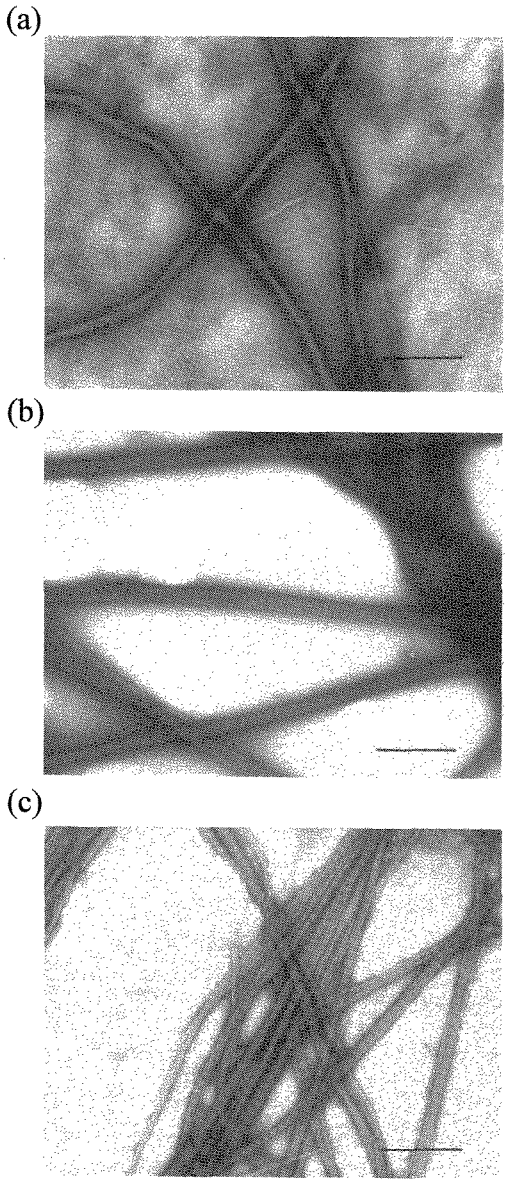

Fig. 1. Transmission electron micrographs of pepsin-digested type I collagen fibrils. (a), without DNA; (b) and (c), with dsDNA and ssDNA, respectively. The mixtures of collagen and DNA were incubated at 37 °C for 60 min.

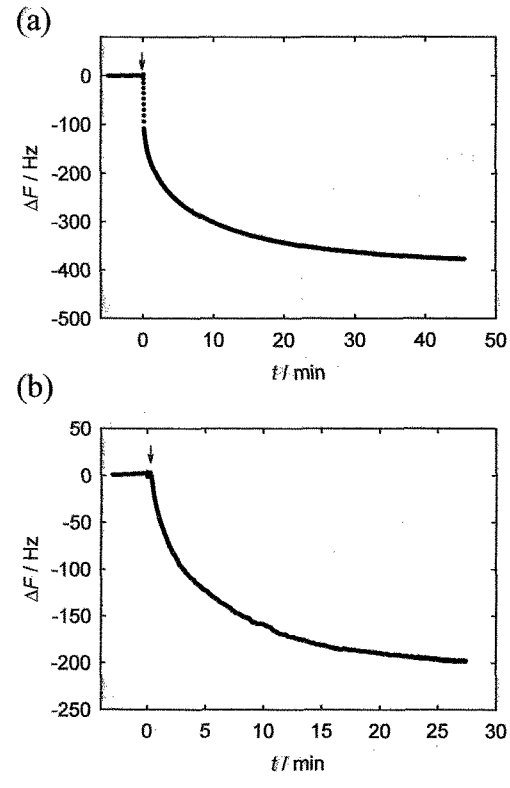

Fig. 2. Typical time course of frequency change of collagen deposited QCM in response to the addition of dsDNA (a) and ssDNA (b). 100 μl of DNA solution was added at the time indicated the arrow.

priori to mixing with collagen solution is necessary to attain the firm fiber formation. Without annealing, fibrillogenesis of collagen occurs only weakly. Further, the results of QCM used for plasmid DNA were likely no adsorption to collagen. These findings strongly suggest that the presence of single strand part of DNA could be essential for the complex formation with collagen. By annealing, single stranded DNA should be formed more or less at some portion, and such a portion plays an important role in the bindings. This picture should be verified in more detail further.

The scattered light intensity <I> for collagen solution without DNA gradually increased with increasing the elapsed time t_e. However, <I> for the collagen solution with DNA attained a saturated value even at t_e <10 minutes. This result indicates that collagen fibrillogenesis is markedly enhanced by the addition of DNA. Figure 3 shows the time evolution of the decay time distribution function $\tau G(\tau)$ with τ being the decay time. The $\tau G(\tau)$ for collagen solution without DNA showed essentially a unimodal distribution. The magnitude of τ corresponding to the peak of distribution was about 24 ms at t_e=7 min, and it coincided with that of the collagen-HCl solution at pH = 3. The peak of $\tau G(\tau)$ shifted to a longer decay time region with increasing t_e, due to collagen fibrillogenesis. On the other hand, the $\tau G(\tau)$ for collagen solution with DNA showed a bimodal distribution. The second peak of $\tau G(\tau)$, which appeared in a slower decay time region, was due to the formation of a collagen-DNA complex and the intensity for collagen solution with ssDNA was fairly larger than that for collagen solution with dsDNA. These results indicate that DNA binds to collagen directly inducing the formation of large aggregates of collagen-DNA complexes. Clarkson et al. reported that collagen fibrils formed in the presence of phosphoprotein were significantly wider and the cross-banding was more distinct [4]. Since DNA consists of a series of phosphate groups bound to each other in a regular way, its effects on the collagen fibrillogenesis seem to be significantly larger.

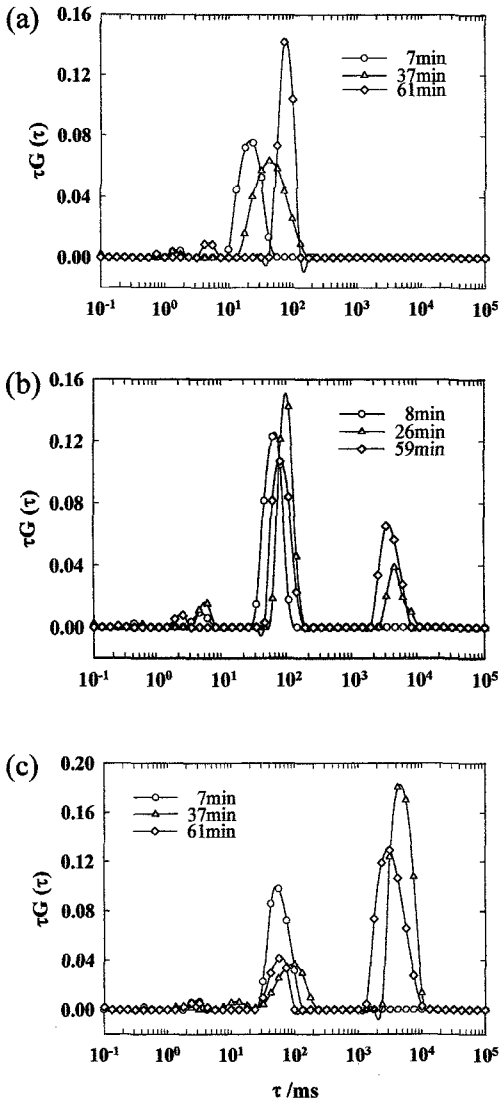

Fig. 3. Decay time distribution function of the collagen solution without DNA (a) and with dsDNA (b) or ssDNA (c).

In conclusion, we investigated the mechanism of collagen-DNA complex formation by means of QCM and DLS measurements. The ability of complex formation was examined for dsDNA and ssDNA. The obtained results showed clearly that both DNAs bind to collagen molecules directly to form the complex, and ssDNA has a higher ability of the complex formation compared with dsDNA.

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