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Preparation and assembly of collagen–DNA complex on an electrode surface and its application to protein analysis

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ABSTRACT

In this work, we have prepared collagen–DNA complex on the surface of an electrode through electrostatic interaction and hydrogen bonding between collagen triple helix and DNA double helix. The prepared tubular material is then deposited on the electrode surface to form a film. Due to the excellent biocompatibility of collagen and DNA, the prepared biomaterial can provide a new platform for protein analysis. On the one hand, since collagen in the complex is the substrate of matrix metalloproteinases-2 (MMP-2), a new kind of electrochemical biosensor for the detection of MMP-2 can be developed. On the other, the collagen–DNA complex can be used to entrap proteins onto the electrode surface with the activity of the target proteins being well maintained, thus further analysis can be conducted on the target proteins.

1. Introduction

Collagen is the major structural protein in mammals. It is one of the main components of basement membranes (BMs) which provide substrata for cells and important signals for differentiation, maintenance, and remodelling of tissues [1]. It is also an attractive biomaterial for its excellent biocompatibility, biodegradability as well as ease of extraction, purification and processing [2]. With respect to that, complexes of collagen with biomacromolecules like gelatin [3,4], chitosan [5–7], proteins [8,9] and nucleic acids [9–12] have been developed for a variety of medical applications ranging from hemostatic materials and biocompatible coatings to drug delivery and tissue engineering [13]. Among them, the complex of collagen with DNA attracts more and more interest, which is postulated to be a unique material with an excellent assembled molecular structure. The similarity of collagen triple helix and DNA double helix in topology may contribute to the self-assembly of collagen–DNA complex. Such self-association of the complex is firstly induced by electrostatic interactions between neutral collagen cylinders with strong dipole moment and negatively charged DNA cylinders and formed by H-bonds between specified donor groups of collagen and phosphate acceptor groups of DNA [14]. In this final complex, each segment of DNA can interact with five collagen molecules, thus for DNA, it is embedded in the collagen molecules. So, protection against nucleases can be achieved [9,11], and the complex has been developed as a kind of splendid gene carrier.

In this work, the collagen–DNA complex is developed as an ideal biomaterial for protein analysis, and the collagen–DNA complex is prepared on an electrode surface so as to be further developed as new kind of biosensors. In this study, collagen is fixed onto an electrode surface through the interaction with DNA which has been previously immobilized on a gold electrode surface. The complex forms spontaneously on the electrode surface with DNA as template, which results in the generation of an excellent biomaterial that is made up entirely of collagen and DNA. Furthermore, application of this biomaterial in protein analysis is investigated. Firstly, with collagen in the complex as the substrate of matrix metalloproteinases (MMPs), a simple but sensitive biosensor for the assay of MMPs is developed. MMPs are a family of zinc-dependent endopeptidases [15], which are essential for the breakdown of extracellular matrix (ECM) in embryonic development, morphogenesis, reproduction, and tissue resorption and remodelling [16,17]. So, many efforts have been made for the assay of MMPs with collagen as substrate, in which collagen are usually modified covalencely through thiol-functionalization [18–20] or deposited onto a solid surface [21]. So, in this work, we have made use of the prepared collagen–DNA complex to propose a simple and sensitive method for the assay of MMPs without any further chemical modification of collagen. Secondly, based on the high biocompatibility and

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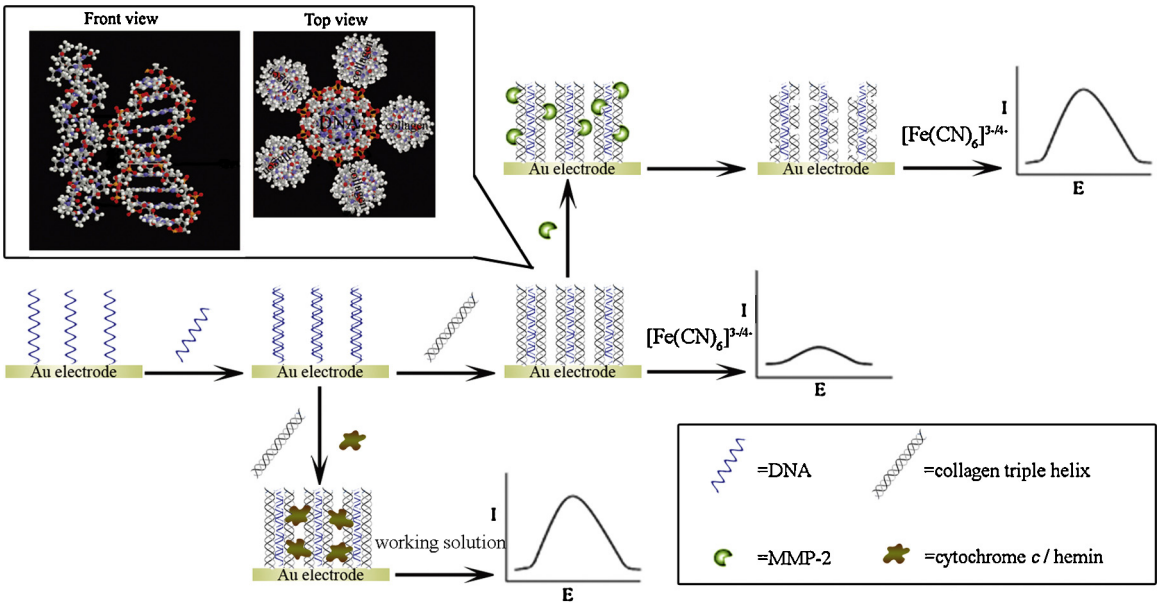

Scheme 1. Schematic illustration of the preparation of collagen–DNA complex at an electrode surface and the application of the biomaterial to protein analysis.

biostability of collagen, which make it to be a good candidate as a supporting structure for various proteins, the collagen–DNA complex is developed as molecular devices to entrap proteins on an electrode surface. When the target proteins are mixed with collagen, they are entrapped into the collagen–DNA films during the formation of collagen–DNA complex, and the biologic activity of the target proteins can be well maintained. Therefore, the complex may provide a gentle environment for the further study of the target proteins.

2. Experimental

2.1. Materials and chemicals

Collagen-like peptide (GPQGIFGQIGPQGIFGQIGPQGIFGQIGPQGIFGQ) designed as the optimal substrate for MMP-2 [22], was synthesized by China Peptides Co., Ltd. The thiolated DNA, 5′-HS-$(CH_2)_6$-CAC GAC GTT GTA AAA CGA CGG CCA GAG CAG-3′, and its complementary strand, 5′-CTG CTC TGG CCG TCG TTT TAC AAC-3′, were synthesized by Shanghai Invitrogen Biotechnology Co., Ltd. Recombinant Human MMP-2 was purchased from Sino Biological, Inc., Captopril and 4-an inophenylmercuric acetate (APMA) were obtained from Genmed Scientifics, Inc., USA. Cytochrome *c* and mercaptohexanol (MCH) were purchased from Sigma. All other chemicals were analytical grade.

The buffer solutions used in this work were as follows. DNA immobilization buffer: 10 mM Tris–HCl, 1 mM EDTA, 10 mM TCEP, and 0.1 M NaCl (pH 7.4); hybridization buffer: 10 mM phosphate buffered saline (PBS, pH 7.4) with 1 M NaCl; collagen-like peptide solution buffer: acetic acid solution (pH 4.5); MMP-2 reaction solution (TCNB): 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl and 0.05% Brig (pH 7.5).

All solutions were prepared with double-distilled water, which was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of 18 MΩ cm.

2.2. Preparation of collagen–DNA complex on an electrode surface

Before the preparation of the collagen–DNA complex, the gold electrode (3 mm diameter) should be thoroughly cleaned. Firstly, the electrode was soaked in piranha solution (98% H_2SO_4:30% H_2O_2 = 3:1) for 5 min (*Caution: piranha solution dangerously attacks organic matter!*). Then, it was polished carefully on P3000 silicon carbide paper and alumina slurry (1 μm, 0.3 μm, 0.05 μm), respectively. After that, it was thoroughly washed by sonicating in both ethanol and double-distilled water for about 5 min. After the above pretreatment, the electrode was soaked in nitric acid (50%) for 30 min and then electrochemically cleaned with cyclic voltammetry, scanning from 0 to 1.6 V for 20 cycles in 0.5 M H_2SO_4 to remove any remaining impurities. Finally, the electrode was thoroughly rinsed with pure water and dried with nitrogen, and was ready for further experiment.

To prepare the complex of collagen with DNA on the electrode surface, the template DNA should be firstly immobilized on the surface of the electrode. Herein the template DNA was thiolated and immobilized onto a gold electrode via gold-sulfur chemistry [23] by incubating the electrode with 1 μM thiolated DNA for 16 h. Then the electrode was immersed in an aqueous solution of 1 mM spacer thiol molecules, MCH, for 1 h to obtain well-aligned DNA monolayers [24] followed by the hybridization with 1 μM complementary DNA for another 1 h at 37 °C. After that, the electrode was further incubated in an acetic acid solution (pH 4.5) containing collagen-like peptide (20 μM) for 30 min. Thus, due to the electrostatic interaction between collagen and DNA, the self-assembly of the complex was triggered, and the complex was formed by hydrogen bonds.

2.3. Assays of MMP-2

Before assays of MMP-2, the pro-MMP-2 was firstly activated by incubating 1 mM APMA at 37 °C overnight [15]. Then it was diluted to various concentrations with TCNB buffer. The electrode, which has been modified with collagen–DNA complex, was then dipped into it for 2 h at 37 °C. The collagen in the complex could thus be degraded by MMP-2, which could lead to the change of the electrode surface, thus the change of electrochemical probes can be obtained to assay the activity of MMP-2.

Captopril was introduced to modulate the activity of MMP-2 [25,26]. After the pro-MMP-2 was activated, it was further treated with captopril (1 μL) for 30 min at 37 °C. Then the activity of MMP-2 (1 μg/mL) which had been treated with captopril was assayed