Fabrication of Ultrathin, Protein-containing Films by Layer-by-Layer Assembly and Electrochemical Characterization of Hemoglobin Entrapped in the Film

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Multilayers of hemoglobin were uniformly deposited on a pyrolytic graphite electrode surface through a layer-by-layer self-assembly approach. The well-known anionic polyelectrolyte, DNA, was used to attract cationic hemoglobin due to the strong electrostatic interaction between DNA and the protein. Interestingly, the electron transfer reactivity of hemoglobin was significantly improved in the film, which in turn facilitated the electrocatalytic reduction of nitric oxide.

The interaction between DNA and proteins is one of the principal biological interactions in vivo. Not surprisingly the in vitro studies of this DNA-protein interaction remain an interesting topic in biochemistry. Specifically, the heme protein-DNA complexes have attracted much recent interest, which demonstrates novel enzyme properties not available in uncomplexed proteins.[1,2] Rusling and coworkers have provided an elegant example that the electron transfer (eT) reaction of hemoglobin (Hb) might be ''turned on'' upon entrapped in DNA chains.[3] The ''promoting effect'' of DNA has later proven to be applicable to a few other heme proteins.[4,5] While the normally employed ''casting'' approach has been successful in preparing these protein-DNA films, which have also found applications in biosensing,[6] the question remains that if the architecture of these films can be precisely controlled.

The layer-by-layer (LbL) assembly was first proposed by Decher.[7] This novel film assembly approach has been widely employed in various areas due to the simplicity and beauty of this method and the precisely controlled quality of the assembled films. Herein we report the assembly of the DNA and Hb multilayer films through the LbL approach, i.e., the cationic Hb and the anionic DNA were alternatively deposited on a pyrolytic graphite (PG) electrode through electrostatic interaction. Electrochemical techniques were employed to confirm the formation of the multilayers and study the eT properties of Hb as well as its catalysis towards a physiologically important messenger, nitric oxide (NO).

Hb (Serva) was dissolved at a concentration of 1 mg/mL in a pH 5.0 phosphate buffer containing 0.5 M NaCl. The concentration of DNA (Salmon sperm) solution is 1 mg/ml (containing 0.5 M NaCl). Water was purified with a Milli-Q purification system to a specific resistance $>16\,M\Omega cm^{-1}$ and was used to prepare all solutions. The working electrode was a pyrolytic graphite (PG) disk electrode ($A = 6.38\,mm^2$). A saturated calomel electrode (SCE) was used as the reference electrode and all potentials reported here were referred to SCE. A platinum wire electrode served as the counter electrode. The substrate PG was first polished using rough and fine sand papers. It was then polished to a mirror smoothness with an alumina (particle size of about $0.05\,mm^3$)/water slurry on silk. After that, it was ultrasonicated in both water and ethanol for about 2 min. LbL films of positively charged Hb and negatively charged DNA were assembled on PG surface via alternate 20-min adsorption from the appropriate solutions (We monitored the relationship between the peak current and the protein adsorption time, which showed that a 20-min immersion time guaranteed saturated protein adsorption). Electrochemical experiments were performed on Potentiostat/Galvanostat Model 263A (EG&G, PARC, USA). The modified electrode was cycled in phosphate buffer solution with pH 6.0. Prior to the experiment, the buffer was bubbled thoroughly with high purity nitrogen for 5 min. Then a stream of nitrogen was blown gently across the surface of the buffer solution in order to maintain the solution anaerobic during the experiment. All experiments were done at ambient temperature ($18 \pm 2\,^{\circ}C$).

As shown in Figure 1a, a pair of well-defined peaks can be observed at the PG electrode with one DNA layer and one Hb layer films. The anodic and cathodic peaks are located at $-290.0\,mV$ and $-235.0\,mV$ at a scan rate of 100 mV/s, respectively. The formal potential E^0, estimated from its half wave peak potential $E_{1/2}$, is $-262.5\,mV$, similar to previous studies on Hb entrapped in the DNA film.[6] The peak separation is 55.0 mV, indicative of a quasi-reversible one-electron heterogeneous eT process. Note that Hb cannot exhibit electroactivity in the absence of DNA (Figure 1b), which is consistent with

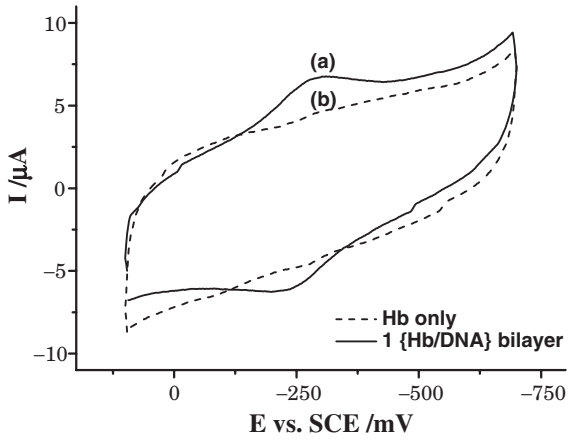

Figure 1. Cyclic voltammograms of (a) one bilayar of {DNA /Hb} and (b) Hb alone-modified PG electrode in a phosphate buffer solution with pH 6.0. Scan rate: 100 mV/s. Temperature: $18 \pm 2\,^{\circ}C$.

previous studies.[6,8] Essentially no electroactive Hb can be adsorbed at the bare PG electrode with the same immersion time in Hb solutions. Therefore, the adsorption of the first DNA layer is essential to attract Hb from aqueous solutions, in the meantime it provides a favorable microenvironment for Hb to exchange electrons with the underlying PG.

We then employed square wave voltammetry (SWV) to detect the growth of multilayer films and notice that the Hb and DNA can be further assembled at this {Hb/DNA} bilayer. As illustrated in Figure 2, the peak current, which indicates the amount of electroactive Hb in the film, increases nearly proportionally in the first five bilayers. Nevertheless, the peak current has only a slow increase after the fifth bilayer and finally saturates at the seventh bilayer. It seems that although Hb obtains its eT reactivity in the presence of DNA, it gradually loses its electroactivity in film far away from the electrode surface. This suggests that electron hopping might be responsible for the electrical communication between PG and intercalated Hb layers. The increased layers of Hb add the amount of electroactive Hb which produces larger electrical current, meanwhile when the film gets thicker, the distance between the added Hb layers and the electrode also increases, which significantly lowers the efficiency of the unidirectional eT.

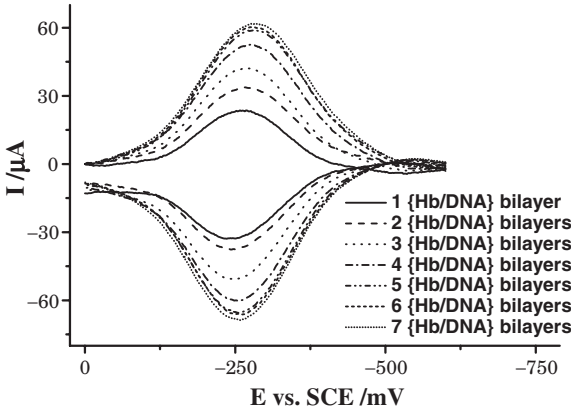

Figure 2. A series of SWVs with different number of {DNA/Hb} bilayers. Both the forward scan and the reverse scan are shown in the figure. Other Conditions are the same as those in Figure 1.

To test the electrocatalytic effect of these Hb-containing multilayer films, 5 μM NO was added into the phosphate buffer. As shown in Figure 3, it can be observed that a new reduction peak appears at about −760 mV, in the negative direction of the redox peaks of Hb. Given the similarity of this peak to those reported in our previous studies,[6,9] we believe that this peak comes from the reduction of NO that is facilitated in the presence of electroactive Hb. The possible mechanism may be expressed in the following equations:[10]

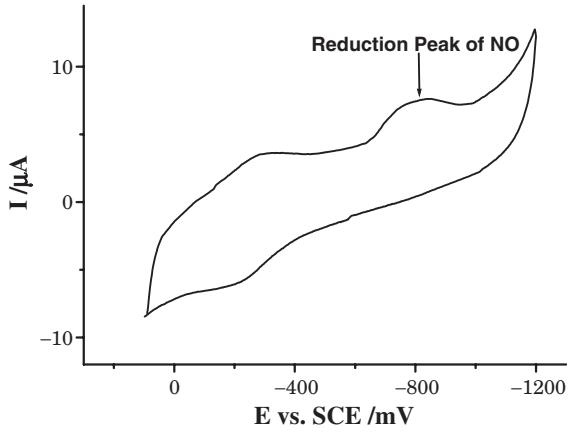

Figure 3. Cyclic voltammograms obtained at three {DNA/Hb} bilayers modified PG electrode in a phosphate buffer solution (pH 6.0) containing 0.002 mM NO. Scan rate: 100 mV/s.

$$\text{Hb Fe(II)} + \text{NO} \rightarrow \text{Hb Fe(II) NO}$$

$$2\text{Hb Fe(II) NO} + 2\text{H}^+ + 2\text{e} \rightarrow 2\text{Hb Fe(II)} + \text{N}_2\text{O} + \text{H}_2\text{O}$$

Stability of these films was also tested. The multilayer films were dried and stored in air, and CV was run periodically after returning the dry films to buffer solution. CV reduction peaks decreased slowly during the first 3 days and afterwards, the peak potentials and currents were essentially stable for at least 1 month.

In summary, we have fabricated well-structured, multilayer {Hb/DNA} films via layer-by-layer self-assembly technique. The electron transfer rate of Hb is greatly facilitated, and this Hb containing film demonstrates nice catalytic capability towards NO, which shows promise in biosensing fields.

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