Electron Transfer between Electrodes and Heme Proteins in Protein−DNA Films

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Electrodes with immobilized DNA can be used as DNA sensors, and for many other applications.[1] Herein we report stable films of calf thymus (CT) double stranded (ds) DNA and proteins on pyrolytic graphite (PG) electrodes in which direct electron transfer involving heme protein Fe(III)/Fe(II) couples is achieved. DNA films on PG also extracted heme proteins from solution. Myoglobin diffused into pure DNA films much faster than hemoglobin.

DNA has been immobilized on surfaces by covalent bonds,[1a] by adsorption to Pd[1b] or to Al(III) alkylphosphonate films on Au,[1c,d] and by binding to polymers.[2] Detection was achieved by voltammetry and electrogenerated chemiluminescence, both utilizing metal complexes bound to DNA.[1a,c,d] Fast electron transfer between two intercalated metal complexes can be mediated by DNA.[3]

Electron transfer rates between electrodes and heme proteins in liquid crystal films of surfactants are enhanced compared to proteins in solution.[4,5] Electron transfer is facilitated partly by adsorbed surfactant, which blocks adsorption of inhibiting macromolecules.[5c] The relative independence of kinetics on surfactant type suggests that rate enhancement might occur with a range of surface active materials.

Ordered films have been made from DNA and polycations,[2b,c] and from proteins and polyions.[7] Ikeda et al.[8] promoted electron transfer of cytochrome *c* in solution with nucleic acids, which they proposed were adsorbed onto the electrode. Thus, we felt that DNA and redox proteins, both polyions, might provide films that could facilitate electron transfer between electrodes and proteins.

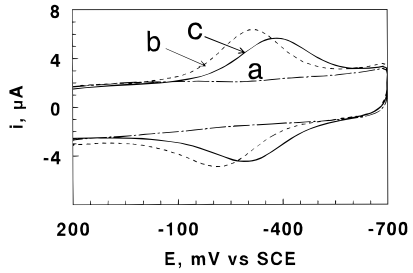

Figure 1. CVs at 25 °C and 100 mV s^{-1} for the following: (a) DNA film on a PG electrode in buffer solution, (b) Mb-DNA film on a PG electrode in acetate buffer pH 5.5 + 100 mM NaBr, and (c) Mb-DNA film on a PG electrode in tris buffer 7.5 + 100 mM NaCl. (Buffers contain no protein.)

Electrophoresis[9a] of both CT ds-DNA and DNA−protein solutions indicated MW = 15 × 10^6, showing that ds-DNA remained intact when bound to proteins. Aqueous mixtures[9b] of 7 μL of 0.5 mM myoglobin (Mb) or 0.1 mM hemoglobin (Hb) and 3 μL of 1 μM ds-DNA were cast onto PG disk[9c] electrodes. Alternatively, only DNA was cast onto electrodes. Films were dried in air (22 °C). The average thickness from specular reflectance[9d] was 40 ± 12 μm. Some 15 μm films were also used.

Protein−DNA films in deoxygenated buffers gave nearly reversible cyclic voltammograms (CV) at scan rates (ν) of 75−500 mV s^{-1} (Figure 1). Peaks appeared at potentials slightly negative of those of the heme Fe(III)/Fe(II) couples of Mb and Hb in solution (Table 1). Peaks shifted negative with increasing pH, as in surfactant films.[4,5] Dissolved Mb or Hb gave no peaks on bare PG between 300 and −800 mV. Electrodes coated with DNA alone gave no peaks in protein-free buffers in this potential range.

Between 75 and 500 mV s^{-1}, peak currents for Mb and Hb in DNA films were proportional to $\nu^{1/2}$ suggesting diffusion control.[10,11] At ν < 6 mV s^{-1}, symmetric peaks were found with peak current proportional to ν, as expected for thin-layer electrochemistry.[11] CV and specular reflectance showed that films in buffers lost about 5% of their original signals in 1 week, and 40% in 3−4 weeks.

Since the diffusion layer in CV is much thinner than film thickness (40 μm), diffusion-kinetic theory[10] was used to estimate electrochemical parameters.[5] Formal potentials ($E^{\circ\prime}$) of proteins were slightly negative in DNA films compared to solutions (Table 1), possibly reflecting interactions within the film environment.[12b] Electron transfer rate constants ($k^{\circ\prime}$) were much larger than in similarly purified protein solutions on bare electrodes, even compared to the most active surfaces (e.g. In_2O_3) or when using mediators. Charge transfer diffusion coefficients (D_{ct}) of Mb in DNA films were 5-fold smaller than in solution. D_{ct} of Hb, which has four Mb-like subunits, was much smaller than for Mb. This is consistent with Hb moving much more slowly within the DNA film.

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Table 1. Electrochemical Parameters from CV for Mb and Hb at 25 °C

pH	sample/ electrode	$10^7 D$,[b] $cm^2\ s^{-1}$	$E^{\circ\prime}$, V/NHE	$10^3 k^{\circ\prime}$, $cm\ s^{-1}$	% electroactive protein[d]	ref
7.0	aq Mb/bare In_2O_3	5	0.05	0.007		12a
7.0	aq Hb/Pt		0.14[c]	0.003[c]		13
5.5[a]	Mb-DNA/PG	1.2	−0.01	1.1	67	tw
7.5[a]	Mb-DNA/PG	0.9	−0.072	2.6	39	tw
7.5[a]	Hb-DNA/PG	0.013	−0.10	1.4	55	tw

[a] Included 100 mM NaBr at pH 5.5 and 100 mM NaCl at pH 7.5, but no proteins; tw = this work. All values were averages of two films; uncompensated ohmic drop <1 mV. [b] D for films is charge transport diffusion coefficient. [c] $E^{\circ\prime}$ from mediated potentiometry, values up to 0.1 V more negative were found by spectroeletrochemistry.[13c,d] Value of $k^{\circ\prime}$ on Azure A-coated Pt.[13d] [d] Estimated as amount of electroactive protein in the film found by integration of CVs at $\nu < 6\ mV\ s^{-1}$ divided by the amount of protein deposited on the electrode.

Square wave voltammetry (SWV) was used after protein-free DNA films (15 μm) were placed into protein solutions. A SWV peak for $MbFe^{III}$ appeared within 10 s (Figure 2), and increased with time. From the relation of root mean square displacement Δ^2 to diffusion coefficient (D)[10]

$$\Delta^2 = 2Dt$$

we estimated the breakthrough time t to pass through a 15-μm film. For Mb, $D_{ct} = 1.2 \times 10^{-7}\ cm^2\ s^{-1}$ (Table 1), and $t = 9$ s. This agreement with experiment suggests that Mb can diffuse into the DNA films.[14] For DNA films in Hb solutions, initial growth of an Fe(III) peak was also found. Subsequently, the peak did not increase significantly for several minutes. Results suggested more complex diffusion/charge transport for Hb in DNA films.

Heme Soret absorbance bands provided information about protein denaturation. Bands at 410 nm for Mb-DNA on quartz were consistent with the absence of large secondary structural differences from the native protein. Urea denaturation caused a 10 nm blue shift.[5] In contrast, Hb-DNA at pH 5.5 gave a precipitate suggesting gross denaturation. The Hb Soret band at pH 7.5 shifted 5 nm to the blue compared to a native Hb film, suggesting a conformational difference.

(14) This result does not necessarily mean that diffusion is the only mode of charge transport during voltammetry of the cast protein−DNA films.

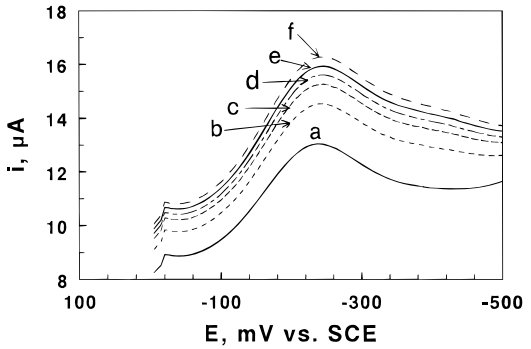

Figure 2. Square wave voltammograms of a 15 μm thick DNA film on a PG electrode at 25 Hz, 2 mV step, 15 mV pulse after immersion into a pH 5.5 buffer containing 0.5 mM Mb + 100 mM NaBr for (a) 10, (b) 60, (c) 80, (d) 102, (e) 130, and (f) 180 s.

Circular dichroism (CD) of Soret bands[15] of free and DNA-bound proteins in solution showed small differences at $T < 40$ °C, suggesting small conformational changes for bound proteins. At $T > 40$ °C, differences in CD spectra suggested that protein bound to DNA is significantly unfolded compared to native protein.

Our results show that electrons can be exchanged between electrodes and heme proteins bound to DNA. The proteins bind to DNA films, and electron transfer rates are enhanced compared to bare electrodes. Small conformational changes in proteins and DNA upon binding are likely, as suggested by visible spectra and CD. Protein−DNA films on electrodes may be useful as biosensors, and for studies of protein−DNA interactions.

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