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The Anodic Voltammetry of Deoxyribonucleic Acid at a Glassy Carbon Electrode

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Synopsis. Deoxyribonucleic acid was oxidized in two steps at a glassy carbon electrode. The peak currents had the characteristics of adsorption currents, but if the surface of the electrode was not fully covered by the adsorbed polynucleotide molecule the peak currents had rather the characteristics of diffusion-controlled currents.

Electrochemical methods have been used for the investigation of the chemical and physical properties of solutions of deoxyribonucleic acids (DNA). It has been established that two of the four bases usually found in every DNA, adenine and cytosine, are reducible at the dropping mercury electrode, both in a free form[1-3] and bound to the nucleotide chain.[4-6] However, the two reducible bases are reduced only in a pH range where they are converted to the protonated form,[7] and their half-wave potentials are very close.

A few studies of the electrochemical oxidation of the purine bases were done at the pyrolytic graphite electrode[8-10] and the glassy carbon electrode (GCE).[11] Guanine and adenine, which are constituent bases of DNA, were voltammetrically oxidized at different potentials over the pH range of 0—14. In the present work, the electrochemical oxidation of DNA and its adsorption behavior on the electrode surface were investigated by means of a linear-sweep voltammetry using a stationary GCE.

Experimental

Chemicals. The herring-sperm DNA used throughout this work was obtained from the Wako Pure Chemical Co. All the other reagents were of a reagent grade.

Apparatus. The anodic voltammograms were recorded using a Yanagimoto P-8 type Polarograph at a scan rate of 3.3 mV/s. A three-electrode voltammetric cell maintained at (20±0.1) °C was used for all the experiments. An aqueous saturated calomel electrode (SCE) was used as the reference electrode, and a platinum wire served as the counter electrode. The working electrode was constructed from a glassy carbon rod 3 mm in diameter (Grade GC-20, Tokai Electrode Co.). The rod was cut into a length of 10 mm and was sealed in one end of a glass tube 3 mm in diameter with epoxy cement. The end of the glass tube-carbon rod assembly was polished with 400-grade emery paper until it was quite smooth.

Voltammetric Procedure. In order to obtain reproducible results, a standard pretreatment procedure was applied before recording each voltammogram. The GCE was polished for 30 s with 1500-grade emery paper, and then for 60 s with a paste of sedimented calcium carbonate. The surface of the electrode was washed with a fine spray of distilled water. Any water remaining on the electrode surface was then removed by touching the surface very gently with a piece of soft absorbent paper. The test solutions were not deaerated. The GCE was inserted into the test solutions, and then the solutions were stirred gently for a few seconds. The voltage scan was commenced after quiescency for 20 s at the starting potential (always 0.0 V *vs.* SCE). At least three replicate voltammograms were recorded for each test solution.

Results and Discussion

Figure 1 shows the typical anodic voltammograms of DNA obtained at the stationary GCE. The voltammetric oxidation of DNA took place in two steps; the first and second peaks can be attributed to the oxidation of the guanine and adenine contained in the DNA molecules. The half-peak potentials ($E_{P/2}$) of the voltammetric oxidation peaks shifted linearly towards negative potentials with an increase in the pH; $E_{P/2}$(*vs.* SCE)/V= 1.06−0.060 pH for the first peak and $E_{P/2}$(*vs.* SCE)/V= 1.41−0.065 pH for the second peak over the pH range of 2—12. The peak current (i_P) did not change much with the pH.

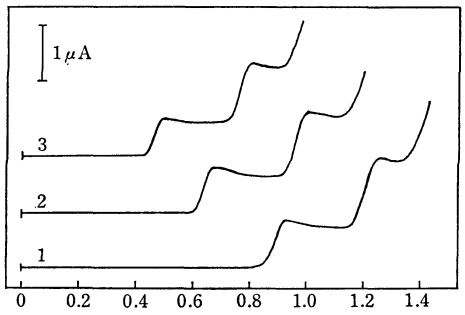

Fig. 1. Anodic voltammograms of DNA at different pH values. pH(Britton-Robinson buffer): curve 1; pH 3.02, curve 2; pH 6.98, curve 3; pH 10.03.
DNA concentration; 0.4 mg/ml, GCE geometric area; 7.1 mm², scan rate; 3.3 mV/s.

As is shown in Fig. 2, the peak current reached a limiting value as the DNA concentration was increased, but in the concentration range below 0.8 mg/ml a linear relationship was observed between the peak current and the concentration. This behavior is probably connected with the coverage of the GCE surface by polynucleotide molecules. Also, the peak potentials for both peaks were dependent on the DNA concentration and shifted slightly to more positive values with the increase in the concentration. Over the concentration range of 0.1—1 mg/ml this shift was about 30—50 mV for both peaks.

In order to study the adsorption of DNA on the GCE surface, the effect of the voltage scan rate on the peak

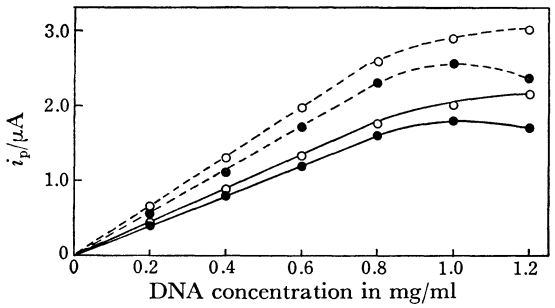

Fig. 2. Peak current-concentration curves for the voltammetric oxidation of DNA at the stationary GCE.
——; First peak, ----; second peak.
pH(Britton-Robinson buffer): ○; pH 3.02, ●; pH 7.00. GCE geometric area; 7.1 mm², scan rate; 3.3 mV/s.

current was examined. The electrochemical oxidation of DNA was irreversible; cyclic voltammetric experiments have shown that no cathodic peak corresponding to the rereduction of the oxidation product was observed at any scan rate. For a linear diffusion-controlled irreversible peak, the peak current function, $i_P/ACV^{1/2}$, should be a constant,[12] and a plot of this function *vs.* $V^{1/2}$ should be a straight line parallel to the $V^{1/2}$ axis, where A is the area of the electrode surface; C, the bulk concentration of the depolarizer, and V, the scan rate. At the various pH values, the peak current functions of both peaks were almost independent of the scan rate. However, at DNA concentrations higher than 1.0 mg/ml the peak current functions of the two peaks of DNA clearly increased with the increase in the scan rate. This behavior is typical of an electrode process where the reactant is adsorbed on the electrode.[13]

These effects of the concentration and the scan rate on the peak current imply that the voltammetric oxidation of DNA takes place in the adsorbed state on the surface of the electrode. The peak currents of DNA have the characteristics of adsorption currents when the surface of GCE is fully covered by adsorbed polynucleotide molecules. However, if the surface is not fully covered, the peak currents have rather the characteristics of diffusion-controlled currents.

In the DNA molecule, the number of guanine equals the number of cytosine, while the number of adenine equals that of thymine (Chargaff's rule). Thus, the ratio of the sum of the voltammetrically active groups of adenine and guanine to the sum of the nonactive groups of cytosine and thymine is approximately one. The herring-sperm DNA contained 14.5% (mass fraction) of guanine and 16.7% of adenine. The value of the diffusion coefficient of guanine was 1.22×10^{-5} cm²/s. The value of the diffusion coefficient of DNA, which was determined from the peak current of the guanine contained in DNA, was 7.3×10^{-7} cm²/s. Also, the value of the diffusion coefficient of DNA, computed with the aid of the Einstein-Stokes law, was 3.6×10^{-7} cm²/s,[4] from the presumed value of the molecular weight of 1×10^{6}.

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