Reversible Conformational Changes in a Poly(amino acid) Film Electrode with Attached Electroactive Groups

Ajit M. Abeysekera and James Grimshaw*

Department of Chemistry, Queen's University, Belfast BT9 5AG, N. Ireland

Conformational changes in a poly(N^{ε}-4-nitrobenzoyl-L-lysine) film coated on platinum become evident during continuous sweep cyclic voltammetry with the development, after 2–3 cycles, of clusters of redox groups with differing apparent redox potentials; resting the uncharged film restores the original simple redox behaviour during a single c.v. run.

Electrodes modified with a poly(amino acid) film containing electroactive groups are a particularly attractive possibility for the mediation of electron transfer between the electrode and a redox enzyme due to the compatibility of the peptide-like modified electrode surface and the enzyme. Non-peptide polymers bearing electroactive groups have recently been the subject of much research.[1—5] The ability of polypeptides to adopt one of several stable conformations depending on the molecular weight, the side chain, and the solvent, offers the intriguing possibility of changing the properties of the electrode by controlling the conformation of the poly(amino acid) film. The transesterification of poly(methyl glutamate) by ferrocenylmethanol has been used to obtain an electroactive poly(amino acid) film whose permeability by ions depends markedly on its redox state.[6]

We report here the first synthesis of a high molecular weight (1.1×10^5) poly(N^{ε}-4-nitrobenzoyl-L-lysine) and the properties of the modified electrode prepared from it. This is the first reported example of a polymer modified electrode displaying reversible conformational changes during the charging and discharging cycle. We also report a new method using salicylaldoxime for the sequestration of Cu^{II} ions from complexes with amino acids which gives high purity material essential for preparing high molecular weight polymers from amino acids with redox active groups on the side chain. Hydrogen sulphide causes unwanted side reactions of a redox

Table 1. Cyclic voltammetry of a poly(N^{ε}-4-nitrobenzoyl-L-lysine) electrode after initial 'breaking-in,' variation of parameters with successively slower scan rates (v).

Scan No.[a]	v/mVs^{-1}	i_{pc}/uA	i_{pa}/μA	$i_{pc}/(v^{\frac{1}{2}})$/μA s$^{\frac{1}{2}}$ V$^{-\frac{1}{2}}$	ΔE/mV	½(E_{pc} + E_{pa})/ mV *vs.* s.c.e.
1	100	37.4	38.4	118	156	1053
2	81	36.1	35.8	127	158	1055
3	49	26.9	26.8	122	148	1058
4	25	23.8	19.5	150	118	1043
5	16	18.1	13.7	143	116	1042
6	9	11.2	7.8	118	111	1044
7	100	34.2	17.0	108	246	1044

[a] Solvent acetonitrile, 0.1 M tetrapropylammonium fluoroborate; electrode area (A) = 8.7 × 10^{-2} cm^{-2}.

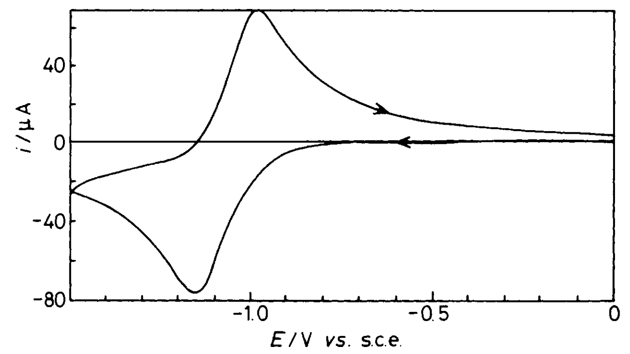

Figure 1. Cyclic voltammogram of a freshly 'broken-in' poly(N^{ε}-4-nitrobenzoyl-L-lysine) modified Pt electrode; scan rate 0.1 V s^{-1}, solvent acetonitrile containing 0.1 M Pr_4NBF_4.

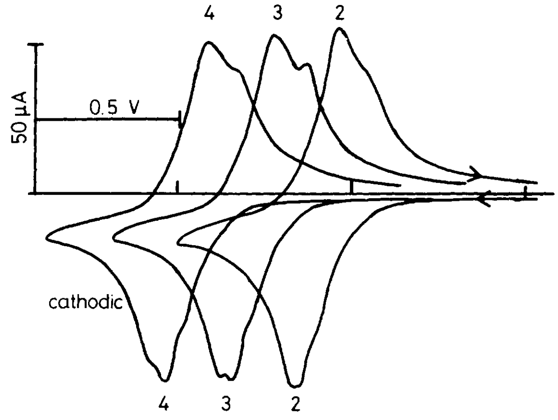

Figure 2. Scans 2, 3, and 4 of repetitive cyclic voltammetry of the 'broken-in' electrode of Figure 1. Scan rate 0.1 V s^{-1}. Each scan begins at 0 V and has a maximum excursion to −1.5 V *vs.* s.c.e. Consecutive scans are displaced by −0.2 V for clarity.

type.[7] The N-carboxyanhydride (NCA) prepared by reaction between N^{ε}-4-nitrobenzoyl-L-lysine and phosgene was polymerized with sodium hydride (NCA : NaH = 8 : 1) in dioxane–dimethylacetamide (2 : 1).

The poly(amino acid) film electrode was prepared on a platinum sphere sealed into a glass support by dip coating from a 0.3% solution in dimethylacetamide. Peptides are known to be adsorbed onto solid electrodes.[8] The coated electrode was studied by cyclic voltammetry [0 → −1.5 → 0 V *vs.* standard calomel electrode (s.c.e.)] in dry acetonitrile containing 0.1 M tetrapropylammonium tetrafluoroborate as the supporting electrolyte. After an initial 'breaking-in' period during which the coat became swollen with solvent and electrolyte,[3,4] the c.v. developed a smooth normal shape (Figure 1).

The results from successive c.v. scans at progressively slower sweep rates are collected in Table 1. The value of $i_p/v^{\frac{1}{2}}$ is approximately constant over the range, indicating that charge transfer through the film is a diffusion phenomenon (associated diffusion coefficient D). Application of the Randles–Sevcik equation† for reversible charge transfer gives $D^{\frac{1}{2}}\ C_o$ = 0.54 × 10^{-8} mol cm^{-2} s$^{-\frac{1}{2}}$, where C_o is the initial concentration of electroactive sites. The peak separation is large, probably due to a high Ohmic resistance within the coat. The value of $\frac{1}{2}(E_{pc} + E_{pa})$ is that expected for the 4-nitrobenzoyl group. C.v. at a very slow sweep rate indicates close to complete saturation of redox sites (plateau currents approach the base line) and integration gives a surface coverage of 2.9 × 10^{-8} mol cm^{-2}. A subsequent scan (no. 7) at 100 mV s^{-1} indicates that the coat has now acquired a much higher Ohmic resistance. The electrode film is prepared from a solution where the stable form is the α-helix.[9] Taking the known dimensions of poly(N^{ε}-benzyloxycarbonyl-L-lysine) as a model, a monolayer of close packed α-helices would give a surface coverage of 6.6 × 10^{-10} mol cm^{-2} of active sites. Thus the electrode film must be a multilayer coat.

Direct evidence for reversible conformational changes during c.v. was obtained from repetitive scanning of the electrode without a time gap between scans. This resulted in the development of multiple charging and discharging peaks (Figure 2). We suggest that conformational changes of the peptide bring about different environments for clusters of nitro groups with slightly different apparent redox potentials. After resting, the electrode no longer shows multiple peaks on the next scan. The process can be repeated indicating that the conformational changes taking place within the film are reversible. The existence of electrochemically non-equivalent sites in polymer films has been noted before[5] but we are not aware of another system which shows this reversible behaviour. Reversible conformational changes in polypeptide membranes have been induced photochemically.[10]

It is known for poly(lysine) that the α-helix is stable in alkaline solution where the N^{ε}-group is uncharged but that the polymer reverts to a random coil in acid solution where the N^{ε}-groups are charged.[11] Thus we expect that when poly(N^{ε}-4-nitrobenzoyl-L-lysine) is in the radical-anion form, strain will be introduced into the α-helix resulting in a conformational change over some sections. In the repetitive scan experiment,

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† $i_p = 2.72 \times 10^5\, n^{3/2} A D^{\frac{1}{2}} C_o v^{\frac{1}{2}}$: see refs. 3 and 4.

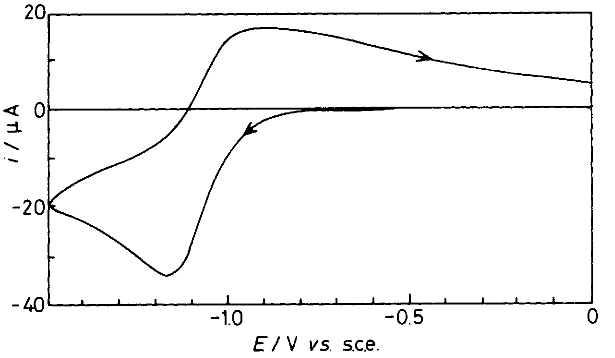

Figure 3. Cyclic voltammogram, scan rate 0.1 V s^{-1}, of a poly(N^{ε}-4-nitrobenzoyl-L-lysine) modified Pt electrode previously subjected to scans at 0.016 V s^{-1}.

the peptide is not given time to come back to its equilibrium conformation in the uncharged state. Anson has prepared a poly(vinylpyridine) coated electrode bearing Fe^{II} centres which displays the development of a new peak in the c.v. on repetitive cycling. This represents a conformational change in the electrode film resulting from the protonation of unco-ordinated pyridine sites which cannot be reversed other than by neutralizing the film with a base.[4]

Chronoamperometry with a potential step from 0 to −1.5 V *vs.* s.c.e. was carried out on a freshly 'broken-in' film. The current transient was fitted to the Cottrell equation‡ for bounded diffusion, layer thickness l. From the data, $D^{\frac{1}{2}} C_o = 1.75 \times 10^{-8}$ mol cm^{-2} $s^{-\frac{1}{2}}$ and $l^2/D = 3.5$ s. It is only possible to fit the first 1.5 s of the current transient.

Ohmic resistance has a less important effect on chronoamperometry than c.v. so this value of $D^{\frac{1}{2}} C_o$ is more reliable than the one obtained from c.v. measurements. Propagation of charge through these films resembles that through other redox polymer films and the diffusion coefficient is associated with polymer motion and the diffusion of counter ions. We have so far been unable to obtain a value for C_o.

Repeated charging and discharging of the electrode eventually causes a permanent change characterized by a decrease in peak currents on c.v., an increase in peak separation, and the development of marked tailing in the discharge peak (Figure 3). These effects may reflect irreversible conformational changes taking place in the charged polymer. The abstraction of a proton from the peptide bond by the nitrobenzoyl radical-anion, followed by further reaction and perhaps cross linking, cannot be excluded.

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‡ $i = (nFAD^{\frac{1}{2}} C_o/\pi^{\frac{1}{2}} t^{\frac{1}{2}})\,[1 + 2\sum_{k=1}^{\infty}(-1)^k \exp(-k^2 l^2/Dt)]$, easily derived from the double exponential form given in refs. 3 and 4.