THE AFFINITY ELECTRODE

Application to the assay of human serum albumin

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1. Introduction

In recent years there has been a remarkable growth in demand for rapid and precise analytical data from clinical and other laboratories. This demand has been partly satisfied by the development of enzyme electrodes [1–4]. An enzyme electrode is a marriage of an enzyme specific for the substance to be measured and an appropriate electrochemical sensor to monitor the products of the enzymic reaction. However, whilst enzyme electrodes are applicable to the measurement of a wide range of metabolites there have been difficulties in the specific electrochemical assay of proteins and peptides. The application of immunochemical methods to the measurement of such substances has culminated in the development of enzyme immunoelectrodes [5–7]. Such sensors combine the unique sensitivity and specificity of enzyme-linked immunoadsorbent assay (ELISA) with the advantages of the conventional enzyme electrode technique [5–7] or enzyme thermistor system [8].

Simple and direct electrochemical sensors have been less explored [9] although electrical potential changes have been noted on combination of antigen with immunologically sensitised metal electrodes [10]. The present paper proposes a novel type of direct electrode, the 'affinity electrode' comprising a biospecific ligand covalently attached to a metal electrode. A potential is generated on combination with the complementary protein. This concept is exemplified by the binding of human serum albumin to Cibacron Blue F3G-A immobilised directly to oxidised metal electrodes.

2. Materials and methods

2.1. *Materials*

Cibacron Blue F3G-A was obtained from Ciba-Geigy (UK) Ltd, Manchester. Human serum albumin (A grade) was from Calbiochem, San Diego, CA. Bovine serum albumin (fraction V; 96%) and lysozyme (3 × crystallised) were from BDH Chemicals (Poole) Ltd. Myoglobin (Type I, equine skeletal muscle) and ovalbumin (crystallised) were from Sigma (London) Ltd and rabbit γ-globulin was from Miles Labs., Stoke Poges, Bucks. Titanium wire (0.500 mm diam.; >99.6% pure) was purchased from Goodfellow Metals, Cambridge. All other chemicals were obtained from BDH Chemicals (Poole) Ltd.

2.2. *Preparation of metal electrodes*

Titanium wire (7 cm) was immersed in 1 M HCl for 20 h at 20°C, washed thoroughly with distilled water and heated electrically (5.6 A/5 min) in air to produce a bluish-white colour due to oxide film formation between the terminals placed 5 cm apart. The electrodes were placed in water (50 ml) containing Cibacron Blue F3G-A (500 mg) and the solution stirred for 5 min prior to adding solid NaCl to give a final conc. of 2% (w/v). After 30 min, solid Na_2CO_3 was added to give a final conc. of 1% (w/v) and the solution bathing the electrodes stirred for 5 days at 20°C. The electrodes were washed thoroughly with water to remove unbound dye and stored in 20 mM potassium phosphate buffer (pH 7.0). The reference electrode was prepared as described for the Cibacron Blue electrode except that the dyestuff was omitted.

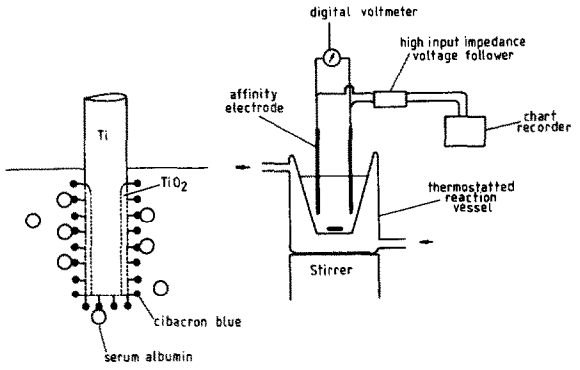

Fig.1. The principle of the affinity electrode and the electrode arrangement.

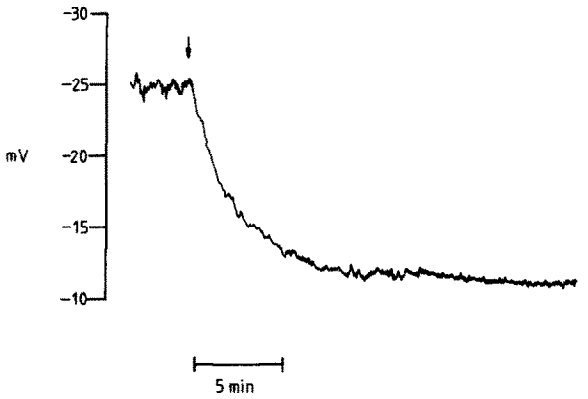

Fig.2. The time course of the Cibacron Blue affinity electrode responding to human serum albumin at 6 μg/ml in 20 mM potassium phosphate buffer (pH 7.0) and 30°C.

2.3. *The electrode assembly and operation*

Figure 1 illustrates schematically the principle of the 'affinity electrode' and of the electrode arrangement. The protein–dye interaction was monitored in 20 mM potassium phosphate buffer (pH 7.0) at 30°C by means of the change in electrode potential between the Cibacron Blue electrode and the reference electrode with a Sinclair PDM 35 Digital Multimeter. The potential was recorded by means of a high input impedance ($\sim 10^{12}$ Ω) voltage follower containing a MOSFET integrated circuit and run off 2 × 9 V batteries to isolate it from mains pickup and earthing problems, connected to a Vitatron VI2001 chart recorder containing a multirange input module (0.2 mV–10 V). The sample was loaded into a thermostatted vessel containing 20 ml 20 mM potassium phosphate buffer (pH 7.0) at 30°C with constant agitation. The Cibacron Blue electrode was photosensitive and hence all experiments were performed at constant illumination.

3. Results

Figure 2 illustrates a typical response of the Cibacron Blue 'affinity electrode' when human serum albumin (12 μl of a 10 mg/ml solution) is added to 20 mM potassium phosphate buffer (pH 7.0, 20 ml) at 30°C. The potential difference between the working and reference electrodes decreases from −25 mV to −12 mV within 10 min and remains unaltered thereafter. The electrode is regenerated by immersing in 8 M urea/20 mM potassium phosphate buffer (pH 7.0) for 10 min followed by washing to remove excess urea. The electrode can be used continuously over a period of many months without apparent loss in response. The electrode is relatively specific for serum albumin. Table 1 demonstrates that the Cibacron Blue electrode responds to final concentrations of 10 μg/ml of both bovine and human serum albumin with a potential change of +17 mV in 10 min but that this potential change is significantly less when equivalent final concentrations of the other proteins were tested. The electrode response to serum albumin is sensitive to both ionic strength and temperature. The response to human serum albumin is abolished completely in

Table 1
The specificity of the response of the Cibacron Blue affinity electrode to several proteins

Protein	Electrode potential[a] (ΔmV/10 min)
Human serum albumin	+17
Bovine serum albumin	+17
Myoglobin	+ 2
Lysozyme	0
Ovalbumin	0
Rabbit γ-globulin	+ 3

[a] Electrode potential measured as described in section 2.3 in 20 mM potassium phosphate buffer (pH 7.0, 20 ml) at 30 ± 0.2°C. Protein samples were added to give a final concentration of 10 μg/ml

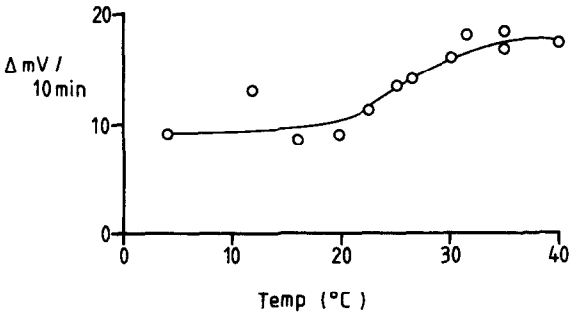

Fig.3. The effect of temperature on the electrode response to human serum albumin (10 μg/ml).

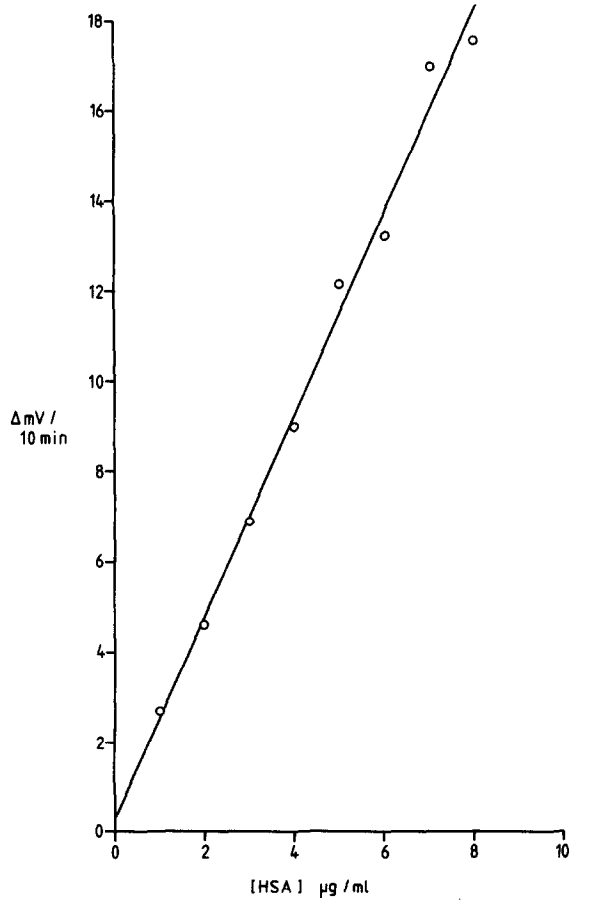

Fig.4. Standard curve showing the response of the Cibacron Blue F3G-A affinity electrode to human serum albumin at 30°C.

1.5 M KCl and increases as the temperature is increased. Thus, fig.3 demonstrates that the potential change (ΔmV/10 min) on binding human serum albumin (final conc. 10 μg/ml) to the Cibacron Blue 'affinity electrode' increases over 4–40°C. For these reasons, all measurements were performed at 30 ± 0.2°C in 20 mM potassium phosphate buffer (pH 7.0).

The 'affinity electrode' exhibits a linear response to added human serum albumin over 0–15 μg/ml (fig.4) but shows a progressive saturation effect at >15 μg/ml serum albumin. Linear regression analysis of the data in fig.4 could be fitted to a line corresponding to [ΔmV/10 min] = 2.2567 [HSA] + 0.2067 with a product moment correlation coefficient of 0.9959. A single sample of human serum albumin corresponding to a final concentration of 5 μg/ml and equivalent to a potential change (ΔmV/10 min) of 11.49 from the linear regression line gave a mean ± SEM of 11.80 ± 0.42 [10] for 10 separate response/regeneration cycles of the same electrode.

4. Discussion

The 'affinity electrode' is a novel concept for the direct electrochemical determination of substances of clinical significance. A ligand, which interacts biospecifically and reversibly with the protein to be assayed is covalently attached to the surface of an oxidised metal electrode and potential changes on binding the complementary protein recorded with a suitable high input impedance system. This principle is illustrated with the development of a specific electrode for serum albumin. The anionic triazine dyestuff Cibacron Blue F3G-A binds specifically to serum albumin and when immobilised to agarose can be used for the selective removal of albumin from other plasma proteins [11].

The Cibacron Blue 'affinity electrode' described here displays a relatively rapid response time, is reproducible and is relatively specific for serum albumin. The response of the electrode is linear up to 15 μg albumin/ml under the conditions cited. The enzyme immunoelectrode reported in [7] displays a non-linear response to albumin over the same concentration range of human serum albumin. Furthermore, with this electrode 100% response corresponds to absence of serum albumin whilst a 25% reduction in

response corresponds to albumin at 10 μg/ml. The electrode described here has zero response in the absence of serum albumin with the electrode potential increasing linearly with increasing albumin concentration. Furthermore, the 'affinity electrode' responds considerably faster than the immunologically sensitised metal electrodes reported in [10] and the enzyme immunosensor [6]. Efforts are currently directed at reducing both the response and regeneration times of the 'affinity electrode' in order to permit the electrode to monitor rapid consecutive samples or be fully automated in the determination of serum albumin.

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