EFFECT OF CARBON SURFACE ON THE ENANTIOSELECTIVITY OF AN AMPEROMETRIC BIOSENSOR

J. McBride, Y. Suida, G.W. Hanlon, A.J. Hutt and C.J.Olliff, Pharmaceutical Sciences Research Group, Brighton Polytechnic. Brighton BN2 4GJ, UK.

There is presently considerable interest in the pharmacological properties of the enantiomers of chiral drugs. This interest has been partially stimulated by the development of analytical methodologies for the determination of the stereochemical composition of chiral drugs (Hutt & Caldwell 1989). We have previously reported on the construction and stereoselectivity of an amperometric biosensor for the determination of the enantiomers of warfarin by the coupling of *Nocardia corallina* to carbon electrodes (Hyland et al 1990). It was decided to extend our investigations by using alternative biological systems as chiral selectors, and the present report describes the construction of an amperometric biosensor by coupling horseradish peroxidase (HRP) to carbon electrodes.

The electrodes were prepared as described previously (Hyland et al 1990). HRP was coupled to platinised carbon felt and carbon cloth, by the carbodiimide method, in the presence of the mediator ferrocene. Platinisation methods employed were those described by Feltham & Spiro (1971) and platinum deposits were identified by electron probe microanalysis. The electrodes prepared were washed throughly with distilled water and stored at 4°C in phosphate buffered saline (PBS) pH 7.3. The activity of the coupled HRP was verified by enzyme assay.

Determinations were carried out at 25°C using a PAR 174 polarographic unit and the three electrode mode with a fixed potential of 400mV. Linear responses (response time 2 mins) were obtained for current produced as a function of concentration for dopamine, D-, L- and D,L-dopa over the range 10^{-6} to 5 x 10^{-5} M ($r > 0.998$), higher concentrations resulted in curvature. Response factors using the platinised carbon felt electrode for L-, D,L- and D-dopa were 2.44 x 10^3, 1.44 x 10^3 and 0.61 x 10^3 mAM^{-1} respectively. After storage for one week, linear responses with reduced slopes were obtained, and after two weeks these became the same as for the carbon felt without the coupled enzyme. For the carbon cloth electrode differentiation between the enantiomers of dopa was only clearly observable at high concentrations, ie 5 x 10^{-5}M, due to the very large background current present for this system; this background is due to oxidation of dopa at the electrode surface, a linear response being obtained over the concentration range studied with no enantioselectivity.

These results show that enantiodifferentiation is possible using enzyme coupled biosensors, but that the selectivity is dependent on both the carbon surface and its treatment.

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