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Single layer and bilayer bienzyme electrodes based on the combination of a 3-dimensional redox epoxy network that electrically connects redox centers of bound horseradish peroxidase (HRP) to electrodes with a hydrogen peroxide generating enzyme, the redox centers of which are not connected to the redox epoxy network, are described. In the single layer electrodes, H_2O_2 generated by the first enzyme oxidizes the second enzyme HRP, which oxidizes the redox polymer network, that is electrochemically reduced at 0mV (SCE). When the redox centers of the H_2O_2 generating enzyme are also electrically connected to the redox epoxy network, the substrate reduced redox centers are oxidized by the redox polymer network, thus lowering the cathodic current. Such attenuation is avoided in bilayer electrodes, where the H_2O_2 producing enzyme and the redox-epoxy-HRP network are not electrically connected.

The single-layer bienzyme electrodes extend the range of amperometric biosensors based on directly redox-epoxy "wired" enzymes to enzymes that are difficult to electrically

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connect to redox polymer networks and whose preferred or only co-substrate is oxygen. For a difficult to wire enzyme, choline oxidase, the cathodic current density in the single-layer peroxidase and choline oxidase containing electrode is 80μA cm^{-2} at 10mM choline concentration, while the anodic current density of the directly wired enzyme is only 5 μA cm^{-2}. Alcohol oxidase is an enzyme that could not be electrically connected to the HRP wiring 3-dimensional redox epoxy network. The anodic current density of its redox epoxy wired electrodes is close to nil, while the cathodic current density observed in alcohol oxidase and wired peroxidase containing single layer electrodes at 10mM ethanol is 5μA cm^{-2}. When well-wired enzymes such as glucose oxidase or lactate oxidase are utilized in single layer electrodes, limiting cathodic current densities of 60 μA cm^{-2} are observed for both. These currents are much lower than those observed in the directly wired enzyme anodes.

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BIENZYME SENSORS BASED ON "ELECTRICALLY WIRED" PEROXIDASE

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Bienzyme Sensors Based on
"Electrically Wired" Peroxidase

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ABSTRACT

Single layer and bilayer bienzyme electrodes based on the combination of a 3-dimensional redox epoxy network that electrically connects redox centers of bound horseradish peroxidase (HRP) to electrodes with a hydrogen peroxide generating enzyme, the redox centers of which are not connected to the redox epoxy network, are described. In the single layer electrodes, H_2O_2 generated by the first enzyme oxidizes the second enzyme HRP, which oxidizes the redox polymer network, that is electrochemically reduced at 0mV (SCE). When the redox centers of the H_2O_2 generating enzyme are also electrically connected to the redox epoxy network, the substrate reduced redox centers are oxidized by the redox polymer network, thus lowering the cathodic current. Such attenuation is avoided in bilayer electrodes, where the H_2O_2 producing enzyme and the redox-epoxy-HRP network are not electrically connected.

The single-layer bienzyme electrodes extend the range of amperometric biosensors based on directly redox-epoxy "wired" enzymes to enzymes that are difficult to electrically connect to redox polymer networks and whose preferred or only co-substrate is oxygen. For a difficult to wire enzyme, choline oxidase, the cathodic current density in the single-layer peroxidase and choline oxidase containing electrode is 80μA cm^{-2} at 10mM choline concentration, while the anodic current density of the directly wired enzyme is only 5 μA cm^{-2}. Alcohol oxidase is an enzyme that could not be electrically connected to the HRP wiring 3-dimensional redox epoxy network. The anodic current density of its redox epoxy wired electrodes is close to nil, while the cathodic current density, observed in alcohol oxidase and wired peroxidase containing single layer electrodes at 10mM ethanol is 5μA cm^{-2}. When well-wired enzymes such as glucose oxidase or lactate oxidase are utilized in single layer electrodes, limiting cathodic current densities of 60 μA cm^{-2} are observed for both. These currents are much lower than those observed in the directly wired enzyme anodes.