Sol–gel-derived nanocrystalline gold–silicate composite biosensor

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A new class of sol–gel-derived electrochemical biosensors is introduced and demonstrated for glucose sensing. The electrode material comprises an interconnected dispersion of gold nanocrystals imbedded in a porous methyl silicate matrix. The gold nanoparticles provide electrocatalysis and electric conductivity; the methyl groups endow hydrophobicity and limit the active area of the exposed gold, thus decreasing the background current; the silicate provides a transparent and porous backbone; and the encapsulated glucose oxidase provides biocatalysis.

Sol–gel processing offers a simple and versatile route to combining the favorable properties of inorganic materials with the bioactivity of enzymes and proteins.1–5 Braun et al.6 demonstrated successful sol–gel encapsulation of enzymes in silicates, which triggered a spurt of research activity in sol–gel biosensing and bioelectronics.1,5–7 Initially, sol–gel encapsulation of enzymes was used, mainly for photometric biosensing. Very few reports described amperometric biosensing due to the harsh conditions that are needed for thin film formation by sol–gel processing.8 High concentrations of organic solvents and the acidic conditions that are frequently used for thin film formation led to denaturation of the enzymes and loss of most or all of the enzymatic activity during film formation or its subsequent drying.

Several methods of overcoming this problem were employed, including the use of layers of doped particles held by a permeable membrane,8 application of wet gels,9 encapsulation of the enzymes between two sol–gel layers10 and use of intercalated materials such as vanadium pentoxide-based sensors.11 Recently, our group introduced a new sol–gel–derived composite, ceramic–carbon electrodes (CCEs) for electrochemical and electrocatalytic applications.12,13 It was found that sol–gel processing is suitable for the preparation of large monoliths and thin film biomaterials, probably because the graphite particles adsorb and thus stabilize the enzymes during sol–gel processing.12,14,15 A series of articles by different groups described different applications of CCEs for biosensing and other electrocatalytic applications.12–23 This communication describes a method of replacing the graphite particles in bio-CCEs by a network of gold nanoparticles. This construction opens the door to the implementation of recent developments in the fast growing field of nanocrystalline materials in biosensing and bioceramic applications.

We have recently demonstrated a method for the encapsulation of gold nanoparticles (ca. 4–6 nm in diameter) in porous aminosilicate films.23 This communication describes the utilization of this synthesis method for obtaining glucose oxidase (GOD) and nanocrystalline gold co-dispersions in transparent, porous and rigid silicate films. In this way one can benefit from the biocatalytic properties of the enzyme and the conductivity and electrocatalytic properties of the interconnected gold dispersion while still maintaining (some) transparency of the composite bioceramic material.

Experimental

Reagents

N-[3-(Trimethoxysilyl)propyl]ethylene diamine (EDAS) and hydrogen tetrachloroaurate were purchased from Aldrich (Milwaukee, WI, USA). GOD (EC 1.1.3.4 from Aspergillus niger, 50 000 u g−1) was obtained from Sigma (St. Louis, MO, USA). Methyltrimethoxysilane (MTMOS) was from ABCR (Karlsruhe, Germany). Triply distilled water was used unless otherwise stated.

Apparatus

An EG&G PARC (Princeton, NJ, USA) model 273 potentiostat controlled by a PC was used for cyclic voltammetric studies. A single-compartment cell with a three-electrode system was used. The counter electrode was a platinum wire and a saturated Ag/AgCl electrode was used as reference electrode.

Preparation of nanocrystalline gold–silicate sol

Gold nanocrystalline dispersion in a silicate sol was prepared by a recently described procedure.23 EDAS and hydrogen tetrachloroaurate were mixed together in a molar ratio of 5:1, followed by the addition of a fixed quantity (3–5% by volume) of 0.1 M HCl and water (1–3% by volume). This homogeneous mixture was sonicated for 10 min and then 0.3 M NaBH₄ (3–5% by volume) was added dropwise. The mixture immediately turns purple, indicating formation of nanocrystalline gold dispersion. The sol was stable for at least three months when kept under ambient conditions as confirmed by transmission electron microscopy (TEM) and spectroscopic studies.

Preparation of nanocrystalline gold–silicate film

Thin films were prepared from the sol by dip coating of indium tin oxide (ITO)–glass substrate (ITO from Delta Technologies, MN, USA; CG-90IN-1510; resistivity, R < 100 Ω m–1). The films were dried at room temperature for 3 h followed by drying at 50–60 °C for 6 h. For the electrochemical studies the thin films were prepared by co-polymerization with 20 to 40% (m/m) MTMOS (with respect to Au sol) to endow hydrophobicity and to ensure good stability of the films in aqueous environments. Film thickness was ca. 2 μm.

GOD-doped Au–methyl silicate composite electrode

GOD was immobilized in the Au–silicate sol by the following procedure. One millilitre of Au–silicate sol (stored at 4 °C) and 0.2 ml of GOD (20 mg ml−1) were mixed and stored at 4 °C overnight. This sol (0.8 ml) was mixed with 0.2 ml of MTMOS and 0.05 ml of 0.1 M HCl and shaken thoroughly. The mixture was stored at 4 °C. Thin film bioceramic films were deposited on an ITO substrate by dip coating. The films were dried at room temperature for 3 h and then transferred to a desiccator at 4 °C for an additional 72 h. After drying, electrodes were stored at 4 °C in 0.1 M KH₂PO₄ solution at pH 5.8.
Results

Fig. 1, curve ‘A’, presents a typical absorbance spectrum of an Au–aminosilane sol. The surface plasmon absorption peak at ca. 520 nm indicates the nanocrystalline nature of the gold dispersion. TEM investigations of sol deposited on a copper grid have shown that the average crystalline size is 4 to 6 nm and that the sol is stable for at least several months. Dip-coated films from this sol maintained the transparency and the optical spectra as demonstrated by curve ‘B’, Fig. 1. The similarity of the absorption spectra of the sol and the film clearly indicates that the gold nanocrystalline dispersion is maintained in the dried xerogel as well. The insert of Fig. 1 depicts the optical spectrum of a 1 cm thick gel prepared using the sol for the preparation of the GOD–Au–silicate film. An overlap of the absorption peak of the FAD (in the oxidized form) and the 520 nm peak of the nanocrystalline gold is clearly observed.

Electrochemical characterization of the active gold surface

Only the wetted and interconnected gold particles in the Au–organically modified silicate electrode are electrochemically active. Thus, only electrochemical methods can be used to probe the active (i.e., wet and conductive) surface area of these electrodes. We have used well accepted electrochemical methodologies of Cu under potential deposition (UPD) and monolayer oxide formation (and coulometric stripping) in order to determine the surface area of the exposed gold. Fig. 2 demonstrates a typical blank and UPD (50 mV s\(^{-1}\)) voltammograms of the Au–silicate electrode in 0.5 M H\(_2\)SO\(_4\) and 1.0 mM CuSO\(_4\). The stripping peak around 130 mV vs. Ag/AgCl is characteristic of UPD dissolution\(^{24}\) and the charge associated with this peak was unaffected by a wide range of voltammetric attributes (e.g., scan rate, deposition potentials and hold time at the vertex potential). The calculated exposed gold surface area by Cu UPD was 80 \(\times\) 10\(^{-6}\) m\(^2\) and the gold oxide-based estimate gave 89 \(\times\) 10\(^{-6}\) m\(^2\). Cu UPD usually gives a lower estimate of the specific surface area, which is attributed to surface irregularities and edge effects. The estimated active surface area corresponds to roughly 10% of the geometric cross section area and shows that only 1.5–2% of the total gold surface in the film is active. This rather low exposure is attributed to the hydrophobicity of the film, which prevents complete wetting of the gold dispersion, and to the partial blocking of the gold surfaces by the aminosilane groups. Full characterization and a more detailed description of the effect of the various preparation parameters will be presented elsewhere.

Glucose biosensing

The gold-dispersed sol–gel film exhibited good catalytic activity for oxidation and reduction of hydrogen peroxide. Fig. 3 depicts cyclic voltammograms recorded with different concentrations of glucose in 0.1 M KH\(_2\)PO\(_4\), pH 5.8 solution for both oxidation and reduction of the H\(_2\)O\(_2\) by-product. Deaeration by nitrogen bubbling restored the blank voltammograms and confirmed that the response is due to oxidation or reduction of the hydrogen peroxide, which is formed by oxidative regeneration of the flavin centers of the GOD by oxygen.

A typical glucose calibration curve based on steady state measurements is depicted in Fig. 4, along with some dynamic response curves shown in the inserts. The dynamic range of the electrode covers the physiologically relevant range (1–20 mM). The calibration curve follows Michaelis-Menten kinetics. The rather noisy (unfiltered) response shown in Fig. 4 and the relatively fast response time (ca. 3 s) show that only a very thin layer of the hydrophobic Au–silicate film is active and contributes to the electrochemical signal. Similar behavior was
reported for the hydrophobic CCEs, where only the outermost section of the electrodes was wetted and active. The electrodes were stable for at least one month when stored in a refrigerator; during that time the electrodes lost only 10% of their response.

Conclusion

This communication demonstrates that it is possible to combine the favorable catalytic properties of biochemicals with the good electrochemical properties of high dispersion of gold nanoparticles and still enjoy the good optical characteristics of silicates. Though this communication centers only on a single and rather robust enzyme, GOD, we expect that this biosensing tool will become useful for other analytes as well. This communication also paves the way for the construction of three-dimensional networks of self-assembled monolayers on nanocrystalline metal dispersions encapsulated in porous silicates and their use for biosensing.

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References


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