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Dendritic Polyglycerol–Poly(ethylene glycol)-Based Polymer Networks for Biosensing Application

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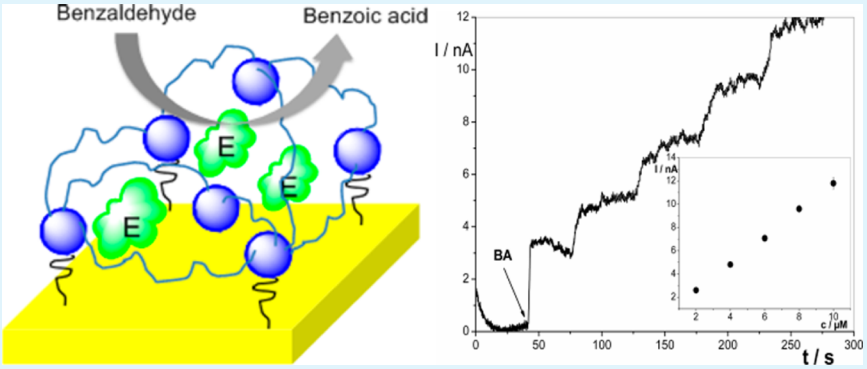
Supporting Information



ABSTRACT: This work describes the formation of a new dendritic polyglycerol–poly(ethylene glycol)-based 3D polymer network as a matrix for immobilization of the redox enzyme periplasmatic aldehyde oxidoreductase to create an electrochemical biosensor. The novel network is built directly on the gold surface, where it simultaneously stabilizes the enzyme for up to 4 days. The prepared biosensors can be used for amperometric detection of benzaldehyde in the range of 0.8–400 μM.

KEYWORDS: *biosensors, hydrogel, amperometry, dendritic*

INTRODUCTION

The main challenge for the development of small enzyme-based bioelectronic devices (such as biosensors or self-powering devices) lies in the long-term stability of an efficient biocatalyst on the electrode surface. The enzymatic activity is mainly retained by the immobilization of enzymes in hydrogel matrixes, which provide them physiological conditions and minimize denaturation.[1] The type of polymer also plays a vital role in controlling the catalytic activity as well as the biosensor performance. Polymers containing hydroxy groups are an especially good choice for such an application because they provide a suitable microenvironment for enzymes.[2] A sufficient amount to maintain an enzyme excess is typically immobilized in films of those polymers in separate membranes. On small electrode surfaces, however, a direct binding of the functional biomolecule in high density is desired. Self-assembled monolayer (SAM) procedures were developed, but normally it is difficult to achieve sufficient stability for large and complex enzymes with only monolayer immobilization.[3,4] The amount of enzyme immobilized in a monolayer is also limited. Therefore, 3D architectures are of interest.

In this regard, dendritic polymers are attractive because of their structural homogeneity, integrity, controlled composition, and highly dense multidentate homogeneous ends, which can be utilized for consecutive bioconjugation reactions.[5,6] To date, only poly(amidoamine) dendrimers have been used to construct enzyme-based electrochemical biosensors by a direct layer-by-layer deposition of dendrimers and enzymes like glucose oxidase, tyrosinase, and horseradish peroxidase, using reductive amination and imine bond formation.[7−9] There is a need for the development of a multilayer network architecture with an easy and fast cross-linking chemistry that does not affect the original activity of the enzymes. The enzyme must be adequately accessible, i.e., for a fast substrate diffusion, in order to control the biosensors' sensitivity.[10]

Considering all of the above-mentioned issues, this paper presents a new approach for the development of a polymer network on gold surfaces using dendritic polyglycerol (dPG) as bioinert multifunctional building blocks with poly(ethylene glycol) (PEG) as a macromolecular cross-linker. The dPG–PEG-based polymer network has been used to construct a biosensor by immobilizing a model enzyme, periplasmatic aldehyde oxidoreductase (PaoABC)[11,12] from *Escherichia coli*, for benzaldehyde (BA) detection. dPG is a class of highly biocompatible polyether–polyols with a globular dendritic structure and was prepared by anionic, ring-opening multi-

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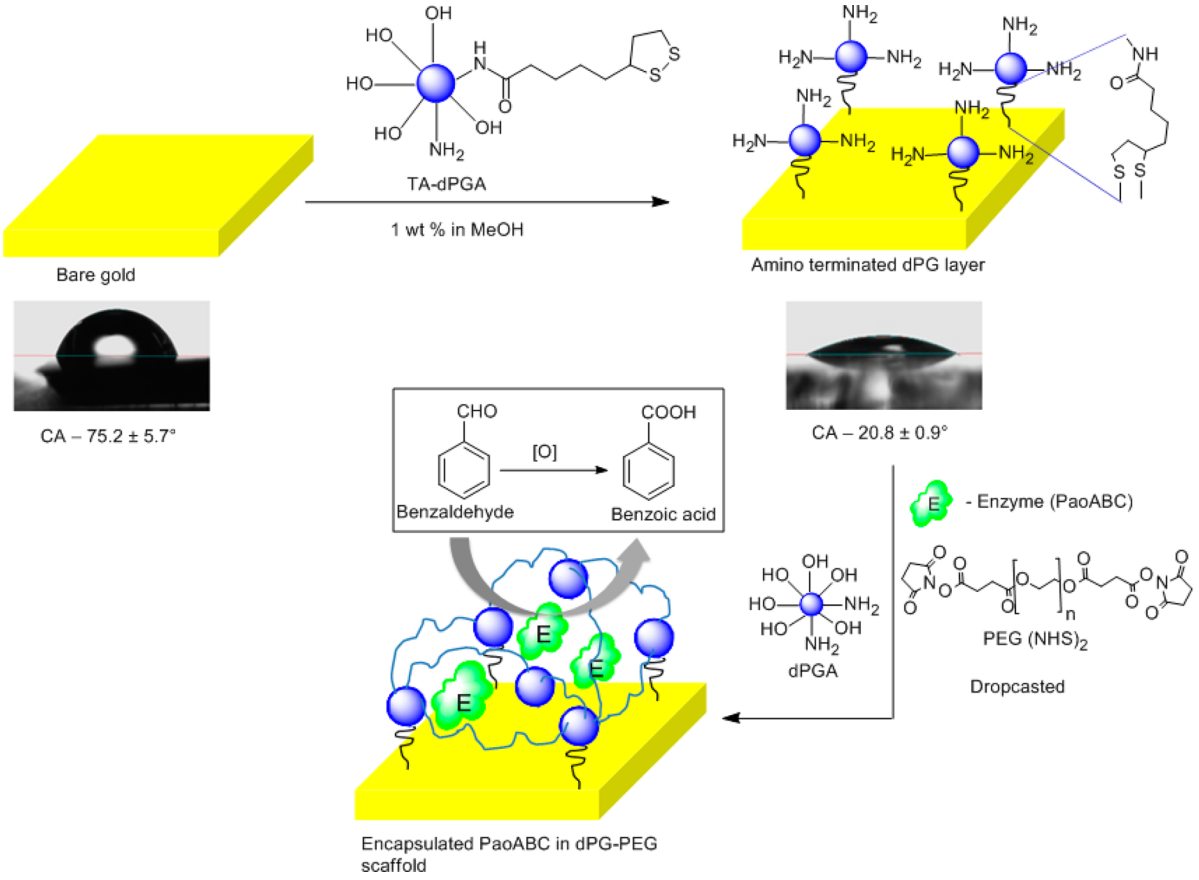

Figure 1. Scheme displaying the modification steps involved in the construction of a dPG–PEG scaffold (n = 34, 136, and 227; PaoABC electrochemical biosensor) on gold surfaces. Inserted pictures: static water contact angles of the bare and TA-dPGA-modified gold surfaces.

branching polymerization of glycidol.[13,14] We have already shown the protein resistance properties of oligoglycerols and dPG by studying their interaction of different human plasma proteins like fibrinogen and albumin.[15−17] The rationale for designing a dPG–PEG-based polymer matrix for enzyme encapsulation is that the enzymes (proteins) do not alter their secondary structure within the polymer matrix and lead to maximum protein stabilization, as has been recently proven by encapsulating proteins like lysozyme and asparaginase in a dPG nanogel.[18]

RESULTS AND DISCUSSION

Significant effort has been devoted to designing dPG-based hydrogels using different chemical reactions like thiol Michael addition, copper(I)-catalyzed click chemistry, and redox- or UV-initiated radical coupling, etc.[19−21] However, all of the above-mentioned reactions have limitations for encapsulating redox enzymes during the network formation. For example, photopolymerization by UV denatures enzymes,[2] and copper-(I)-catalyzed click reaction generates a reducing environment, which may affect the catalytic site of the enzyme. This demands a cross-linking approach with an insignificant amount of influence on the enzymatic activity. Figure 1 schematically illustrates our new cross-linking approach and immobilization strategy on gold surfaces for the formation of dPG–PEG-based polymer networks by reacting dPG amine with homobifunctional PEG *N*-hydroxysuccinimide (PEG NHS) ester. dPG amine (dPGA) and homobifunctional PEG NHS ester with different numbers of ethylene glycol units (n = 34, 136, and 227) were synthesized from dPG and respective PEGs, as reported previously.[22−24] The detailed synthetic procedures are described in the Supporting Information (SI).

At first, the gold surfaces were activated for amide bond formation by the formation of an amino-terminated SAM of thioctic acid functionalized dPG amine (TA-dPGA). Then the dPGA and homobifunctional PEG NHS ester solution were dropcast onto the amino-terminated dPG surface and kept in air for 1 h to create a homogeneously distributed polymer network. Performing a similar procedure and mixing the enzyme with the polymer solutions led to formation of an enzyme-entrapped polymer network.

The amino-terminated SAM surfaces were characterized by static contact-angle measurement, ellipsometry, and atomic force microscopy (for AFM images, see SI Figure S16). The static water contact angle of an amino-terminated SAM surface was 20.8 ± 0.9°, whereas bare gold had a static water contact angle of 75.2 ± 5.7°. This clearly indicates formation of a hydrophilic dPGA layer. These observations were further proven by the thickness measurement of the TA-dPGA layer using ellipsometry. The thickness of the amino-terminated dPGA layer was 1.8 ± 0.1 nm, whereas the hydrodynamic diameter of TA-dPGA was 4.7 nm (see SI Table S3 and Figure S7). The thickness value from ellipsometry was consistent with the actual size of TA-dPGA, considering the fact that the dendrimer collapsed because of the large number of interaction sites with the substrate, which led to formation of a densely packed layer.[25,26]

Having established efficient cross-linking chemistry to form dPG–PEG-based polymeric networks on gold surfaces, we next

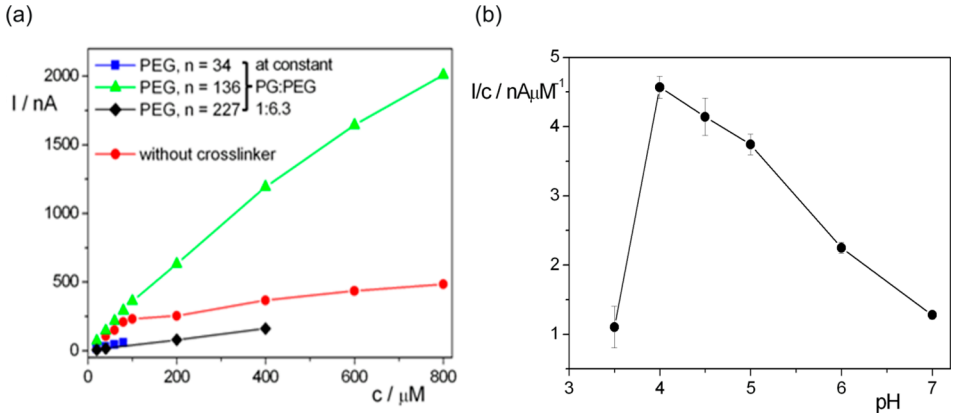

Figure 2. (a) Effect of the length of the cross-linker used for PaoABC biosensor preparation on the amperometric signal of BA with a concentration range of 20−800 μM. (b) Effect of the pH on the sensitivity of the amperometric BA measurement at the PaoABC biosensor. Measurements were performed in a citrate phosphate buffer with pH 4.5 in the presence of 2 mM mediator HCF at a potential of +250 mV vs Ag/AgCl.

explored their capability of accommodating complex redox enzymes in a biosensor configuration. As a model enzyme, we chose the molybdenum hydroxylase PaoABC. PaoABC catalyzes the oxidation of aromatic aldehydes to the respective aromatic carboxylic acids using synthetic electron acceptors (M_{ox}) according to[11]

$$\text{R-CHO} + M_{ox} \rightarrow \text{R-COOH} + M_{red}$$

This enzyme has recently been demonstrated to be active in a poly(vinyl alcohol) (PVA) membrane sensor. However, the biosensor had considerably longer response times because of its higher diffusion resistance.[27]

To build up the enzyme-modified dPG−PEG scaffold on gold, experiments were started initially by applying 96 pmol of PaoABC at different dPG:PEG (with n = 136) ratios ranging from 1:5 to 1:7. The ratios varied because of the presence of 17 amino groups on dPGA. BA addition generated a clear oxidation current in the presence of potassium hexacyanoferrate(III) (HCF). This result confirms the presence as well as the catalytic activity of enzyme entrapped in the scaffold. The best results were obtained with a dPG:PEG ratio of 1:6.3. There was no BA signal with the dPG−PEG scaffold without either PaoABC or a mediator. A relatively high amperometric signal of BA was obtained (3 nA/μM) by dropcasting PaoABC onto a clean as well as dPGA-modified electrode, but the amperometric signal already decreased to 35% and 60%, respectively, after 10 consecutive measurements. These different behaviors can be explained by the fact that the dPGA-modified electrode was weakly positively charged because of the amino groups, which helped bind the enzymes to the surface more tightly than to the bare electrode surface, where there were no such interactions. The enzyme loading was optimized by varying the amounts of enzyme between 2.4 and 192 pmol of PaoABC to reach the highest sensitivity, which was obtained with a biosensor containing 24 pmol of enzyme (see SI Figure S17). Using the optimum amount of enzyme and keeping the dPGA amount constant, the cross-linker [PEG $(NHS)_2$, n = 136] amount varied from 10.9 to 70.7 nmol. The amount of cross-linker had a significant effect on the sensitivity of BA detection, particularly for BA > 100 μM (see SI Figure S18). Increasing the amount of cross-linker resulted in a decrease of the signal from the limited diffusion of analyte to the electrode surface. This is also supported by an observed increase in the response time. The best results were obtained with a PEG amount of 32.8 nmol, i.e., 1:6.3 dPG:PEG.

Figure 2a shows the effect of the cross-linker length on the amperometric signal, where the length of the PEG chain (cross-linker) was varied (n = 34, 136, and 227) and the optimum cross-linking density was kept constant (dPG:PEG = 1:6.3). The biosensor without any cross-linker showed relatively high sensitivity for low concentrations of BA, but the signal already approached an almost stationary value above 100 μM BA. The resulting low apparent K_m value indicated kinetic control. Moreover, the stability of this sensor was not sufficient. Only very low signals were obtained using the short cross-linker (with n = 34), indicating a diffusion barrier because of the dense network. Using a cross-linker with n = 227 led to a long amperometric response time (>50 s) and small signals that were typically observed for systems with thick layers. Formation of the 3D scaffold with PEG (n = 136) resulted in high amperometric signals with a wide range of concentrations and with very short response times (<5 s). This cross-linker was used for the subsequent experiments. Multiple modification of one gold surface with two or three layers of dPG−PEG enzyme did not improve the performance.

In order to find an optimum concentration of the mediator HCF, amperometric measurements of 20 and 200 μM BA were performed with different HCF concentrations ranging from 0.1 to 6 mM. The maximum signal for both concentrations of BA was reached with 2 mM HCF. Higher concentrations of the mediator did not improve the response (see SI Figure S19). The applied potential of +250 mV had the highest sensitivity. Usually the pH of the medium controls the enzymatic activity, which then affects the sensitivity of the biosensor response. The dependence of the sensitivity of the amperometric signal of BA on the pH value was tested, as shown in Figure 2b. The maximum response was obtained at pH 4.0. The signal of BA decreased with an increase of the pH. At pH 3.5, on the other hand, the loss of the signal was caused by an irreversible denaturation of the enzyme. The results are in good agreement with the known behavior for PaoABC in solution[11] and in PVA.[27] A buffer with pH 4.5 was chosen for the next experiments in order to reach good sensitivity and long-term biosensor stability.

Amperometric detection of BA was performed using the optimized conditions for the measurement. The BA biosensor was very sensitive for a broad linear range of responses between

0.8 and 400 μM (as shown in Figure 3) and had a detection limit of 0.8 μM. The repeatability of the measurement can be

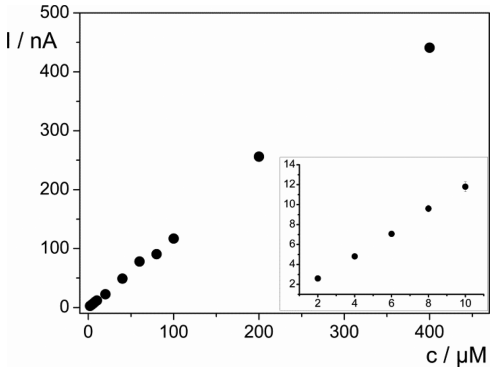

Figure 3. Calibration curve of the PaoABC biosensor for BA amperometric detection in the concentration range from 2 to 400 μM (slope = 1.13 and adj. R^2 = 0.9941). The inset plot shows the small concentration range from 2 to 10 μM. Measurements were performed in a citrate phosphate buffer of pH 4.5 in the presence of 2 mM mediator HCF at a potential of +250 mV vs Ag/AgCl.

characterized by the relative standard deviation of 5.6% that was obtained for detection of 20 μM BA (n = 6). The change in the sensitivity of the measurement was insignificant for the first 2 days. On the third day, 50% of the original signal could still be detected. This showed that the activity loss is mainly due to inactivation of the enzyme and not enzyme leaching.

CONCLUSION

In summary, we have demonstrated an easy and fast cross-linking approach to form dPG−PEG-based polymer networks to entrap and stabilize PaoABC on gold surfaces. The developed biosensor can be used to amperometrically detect BA in the concentration range of 0.8−400 μM. The biosensor performance was also successfully optimized by varying all of the parameters like enzyme loading, pH, cross-linking density, and cross-linker lengths. The presented approach will be extended to other enzymes and may also involve additional functional elements such as redox transfer units and further coenzymes. We foresee that this work will provide a new scaffold for the development of miniaturized biosensors and for applications in the field of biocatalysis.

ASSOCIATED CONTENT

Supporting Information

Experimental section, NMR data, electrochemical results, and AFM data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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