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Synthesis of water-soluble and cross-linkable ferrocenyl redox polymers for uses as mediators in biosensors

Huimin Deng, Wei Shen, Zhiqiang Gao*

Department of Chemistry, National University of Singapore, Singapore 117543, Singapore

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ABSTRACT

A series of ferrocenyl redox polymers to be used as electron transfer mediators in biosensors were synthesized. Ferrocene loading in the redox polymers varied from 3 to 14%, depending on the polymerization conditions. Flourier transform infrared (FT-IR) and UV–visible (UV–vis) spectrophotometry were performed to confirm the presence of the monomeric units in the redox polymers and the saturated character of the synthesized redox polymers. Gel permeation chromatographic technique was applied to reveal the molecular weights of the redox polymers. The redox polymers were cross-linkable with proteins. For example, glucose oxidase and the redox polymers were cross-linked using poly(ethylene glycol) diglycidyl ether and bovine serum albumin (BSA), forming stable and highly effective biosensing membranes on an electrode surface. The electron-mediating efficiency of the redox polymers was assessed using cyclic voltammetry, based on the sensitivity of catalytic oxidation of glucose and reproducibility of the results obtained.

1. Introduction

In recent years, polymeric materials have gained wide theoretical interest and practical applications in biosensors [1]. They can be used for many different purposes and may offer unique possibilities as interfaces between smart sensors and intelligent molecular receptors. For example, the enzymatic oxidation of glucose to gluconolactone by glucose oxidase (GOx) has been applied to many amperometric glucose biosensors. In the natural enzymatic reaction, glucose is oxidized by O_2 to gluconolactone and O_2 is reduced to H_2O_2. Since neither glucose nor gluconolactone is electroactive within the working potential window from −0.5 to 1.0 V, either the increase in H_2O_2 concentration or the decrease in O_2 concentration is measured to quantify the glucose concentration. The detection of H_2O_2 by its oxidation at a platinum electrode requires a working potential of 0.5–0.6 V (vs. Ag/AgCl), and thus is subjected to interferences of electroactive species in blood, such as ascorbic acid and uric acid. To circumvent the above-mentioned problems associated with glucose monitoring involving O_2 or H_2O_2, redox-active mediators are proposed as artificial electron acceptors in place of O_2 molecules, as illustrated in Fig. 1.

Two groups of mediators were extensively investigated, namely, transition metal complexes and ferrocenyl materials. For example, polyferrocenyl compounds have been used as redox indicators in molecular recognition [2], as mediators in biosensors [3], and as active coating to electrode surface [4]. However, only few ferrocenyl and polyferrocenyl materials are water-soluble [5]. Water-soluble ferrocenyl materials are of particular interest as redox mediators in biosensors. By co-polymerizing alkene substituted ferrocenes, such as vinylferrocene (VFc), with an appropriate water-soluble polymer, it is possible to prepare ferrocenyl materials that are readily soluble in water. But it has been shown that the free radical initiated polymerization of VFc is unusual [6,7]. And co-polymerization of VFc is known to be difficult because the ferrocenium plays as a radical scavenger in the polymerization system, leading to deactivation of the polymer chain [8]. On the other hand, polyacrylamide (PAA) is widely used as support matrix in enzyme immobilization and biosensing because of its good chemical and mechanical stability and its inertness to microbial degradation [9]. However, attempts of co-polymerization of VFc and acrylamide (AA) and its derivatives were not very successful [10]. Instead, to by-pass the inefficient co-polymerization of VFc, chemical grafting procedures were proposed in preparing ferrocenyl materials [11–13]. In two recent reports [14,15], VFc co-polymers were synthesized, but minute loading of ferrocene and lack of cross-linkable groups in these polymers restricts their use in biosensors.

In this work, we investigated the possibility of co-polymerization of VFc with AA and other acryl compounds. It was found that water-soluble and cross-linkable redox polymers were readily prepared in a mixture of ethanol and water with

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* Corresponding author. Tel.: +65 65163887; fax: +65 67791691.
E-mail addresses: chmgaoz@nus.edu.sg, chmgaoz@hotmail.com (Z. Gao).

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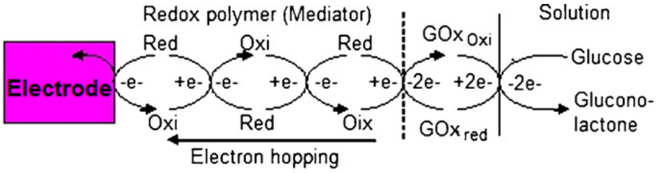

Fig. 1. Illustration of redox polymer mediated biosensing process.

a persulfate salt as a radical initiator. A schematic illustration of the redox polymers is given in Fig. 2. The chemical structure and composition of the redox polymers were analyzed using various techniques such as UV–vis, FT-IR and Gel permeation chromatography (GPC). Electrochemistry tests revealed that the redox polymers are highly electrochemically reversible and efficient in mediating enzymatic reactions.

2. Experimental

2.1. Reagents

Glucose oxidase (GOx, EC 1.1.3.4, from Aspergillus niger, 191 units/mg) was purchased from Fluka (CH-9470 Buchs, Switzerland). Ferrocene (Fc), VFc, AA, acrylic acid (AC), acrylamidosulfonic acid (AS) and persulfate salts were purchased from Sigma–Aldrich (St. Luis, MO, USA). All other chemicals used were of certified analytical grade and all solutions were prepared with Millipore water.

2.2. Apparatus

Electrochemical tests were performed with an AutoLab potentiostat/galvanostat running under the general purpose electrochemical system manager version 4.9. A three-electrode system cell, housed in a Faraday cage, was employed with an Ag/AgCl reference electrode, a platinum wire counter electrode and a gold working electrode (surface area of 7.94 mm^2). UV–vis spectra were recorded on an Agilent 8453 UV–vis spectrophotometer. Molecular weights were determined with a Toyo Soda high performance gel permeation chromatograph in water and standard poly(ethylene oxide) and poly(ethylene glycol) for calibration.

2.3. Preparation of the redox polymers

The water-soluble and cross-linkable redox polymers were synthesized according to the following procedure: 1.0 g AA and its derivatives were dissolved in 10 ml of mixture solvent of ethanol/water (3/1). To initiate polymerization, a 0.20-ml aliquot of 0.10 g/ml oxygen-free persulfate solution was added to the reaction mixture after 10 min of deoxygenating. Then 0.05–0.16 g VFc dissolved in degassed ethanol was added. The mixture was refluxed for 24 h under nitrogen. After cooling, the reaction mixture was added drop-wisely to rapidly stirred acetone to precipitate the redox polymer. The precipitated redox polymer was washed with acetone and purified by multiple water-dissolving acetone-precipitating cycles. The purified product was then dried under vacuum at 50 °C. Typical yields were between 55 and 90%.

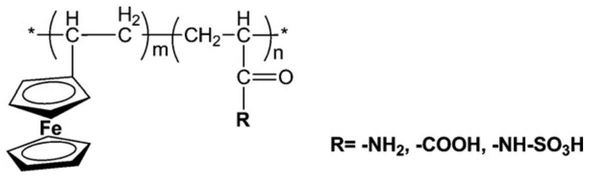

Fig. 2. Structure of water-soluble and cross-linkable ferrocenyl redox polymer.

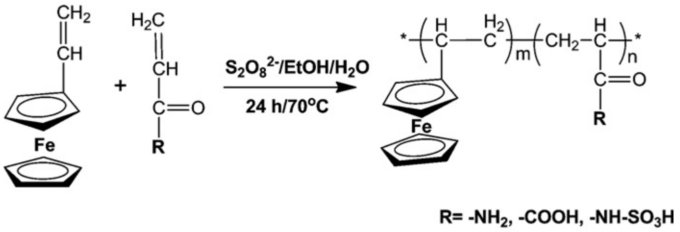

Fig. 3. Polymerization mechanism of the redox polymers.

3. Results and discussion

Co-polymerization of VFc with AA and its derivatives was carried out using peroxide (persulfate) initiated radical polymerization, as depicted in Fig. 3. However, in order to successfully co-polymerize the monomers, great attention must be paid to the terminating effect of VFc in the system. As mentioned early, VFc usually acts as a radical scavenger in the co-polymerization system. It was found that the amount of radical initiator needed in our system is substantially less that these needed in previously reported VFc polymerization systems [8]. Larger amounts of radical initiator significantly reduced the polymerization efficiency and the molecular weight of the product. Moreover, it was observed that the addition sequence also affects the polymerization efficiency. Less that 20% of polymerization was observed when adding the persulfate radical initiator to the mixture of VFc and AA. This is probably because the formation of ferrocenium in the reaction mixture which resulted in the retardation of polymerization rate and much early termination of the polymer chain growth process. To further confirm the termination effect of VFc in the mixture of VFc and AA, various amounts of ferrocene were added to this mixture. It was observed that significantly less polymer formation was obtained when a larger amount of ferrocene was added.

As shown in Table 1, under optimal conditions, relatively high yields were obtained. However, the polymer yield decreased with increasing VFc feeding ratio, indicating that the terminating effect of VFc in radical polymerization still exists although great care has been take in the polymerization process. It was found that minute yields were obtained once the reaction mixtures became blue which is due to the formation of a considerable amount of ferrocenium in the polymerization solution. Ferrocene loading in the redox polymer was determined from elemental analysis. It was found that the ferrocene loading varies from 3 to 14%, which is always less than ferrocene content in the monomer feedings. The molecular weights and the molecular weight distributions of the redox polymers were determined by GPC using standard polystyrene as a reference. It was showed that the products of the AA, AC, or AS and VFc co-polymerizations all have monomodal elution peaks, confirming that PAA–VFc, PAC–VFc, and PAS–VFc being co-polymers rather than blends of two homopolymers. The weight-average (M_w) molecular weights of the co-polymers were found to be in the range of 180,000–420,000 and the polydispersity indices (PDI) were from 2.4 to 3.3 (Table 1). Generally, the redox polymers prepared with higher ferrocene feeding ratios had lower molecular weights and broader molecular weight distributions.

3.1. Characterization the redox polymers

The co-polymers obtained were light-yellow colored powdery materials. To further confirm the formation of the redox polymers, one of the redox polymers, PAA–VFc was examined in great details.