Self-assembled mono- and bilayers on gold electrodes to assess antioxidants—a comparative study

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Abstract

Oxidative stress is considered as an imbalance of reactive species over antioxidants, leading to diseases and cell death. Various methods have been developed to determine the antioxidant potential of natural or synthetic compounds based on the ability to scavenge free radicals. However, most of them lack biological relevance. Here, a gold-based self-assembled monolayer (SAM) was compared with a gold-supported lipid bilayer as models for the mammalian cell membrane to evaluate the free radical scavenging activity of different antioxidants. The oxidative damage induced by reactive species was verified by cyclic and differential pulse voltammetry and measured by the increase of electrochemical peak current of a redox probe. Trolox, caffeic acid (CA), epigallocatechin gallate (EGCG), ascorbic acid (AA), and ferulic acid (FA) were used as model antioxidants. The change in the decrease of the electrochemical signal reflecting oxidative membrane damage confirms the expected protective role. Both model systems showed similar efficacies of each antioxidant, the achieved order of radical scavenging potential is as follows: Trolox > CA > EGCG > AA > FA. The results showed that the electrochemical assay with SAM-modified electrodes is a stable and powerful tool to estimate qualitatively the antioxidative activity of a compound with respect to cell membrane protection against biologically relevant reactive species.

Keywords Electrochemical assay · Reactive species · Radical scavenging activities · SAM · Lipid bilayer · Antioxidant activity

Introduction

Redox biology plays an important role in healthy or diseased cell function [1]. The cellular redox systems were found to be more complex than first anticipated, but its modulation by antioxidants is assumed to hold potential for the prevention or treatment of degenerative diseases [2, 3]. For example, the naturally occurring polyphenol kaempferol has been suggested to hold some potential for the clinical application in both tumor prevention and therapy [4]. Another example is Gynenosides, the main components from *Gynostemma*

pentaphyllum Makino, a plant widely used in traditional Chinese medicine, which has been attributed to anti-cancer effects [5]. However, even though a great variety of approaches to assess the antioxidant activity of plant compounds or extracts exists, its informative value for biological systems is often limited. Highly artificial in vitro antioxidant assays are tools often applied in the food industry or for compound screening purposes. They mainly focus on the ability of a substance to neutralize or reduce the number of active radicals in a system. From a mechanistic point of view, the common assays can basically be divided into two main groups: assays that rely on electron transfer reaction (ET) and assays that are based on hydrogen atom transfer (HAT) reactions [6]. Popular examples for ET assays are as follows: TEAC (Trolox equivalent antioxidant capacity) [7], FRAP (ferric ion reducing/antioxidant power) [8], DPPH (1,1-diphenyl-2-picrylhydrazyl) reduction capacity assay [9], *p*-NDA (*N,N*-dimethyl-4-nitrosoaniline) oxidation assay [10], PCL assay (photo chemiluminescence) [11], the DMPD (*N,N*-dimethyl-*p*-phenylenediamine) assay [12], or the total phenol assay by Folin-Ciocalteu reagent. Among the HAT assay are the ORAC (oxygen radical absorbance capacity) [13], the crocin

The authors dedicate this work to Professor Fritz Scholz on the occasion of his 65th birthday.

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bleaching method [14], inhibition of lipid oxidation, and the TRAP (total radical trapping antioxidant parameter) [15]. Most of them measure the reaction of a target substance with a radical. In some cases, like in the DPPH assay, they are rather stable artificial radicals that have no biological relevance. Other shortcomings that make it difficult to obtain biological relevant data are non-physiological pH values or the absence of complex reaction media that could affect reactions *in vivo*.

Electrochemical methods offer the possibility to investigate the antioxidant capacity of natural or synthetic substances in four ways. First, the antioxidant potential of a substance can be evaluated by the determination of the redox properties at relevant pH values [16]. It is a valuable tool for a quick screening; however, the obtained potentials depend on the selection of the electrolyte. Therefore, the predictive value for biological systems is often limited. The second option is to determine the interaction of reactive species in general, or as a third option reactive species that play a role in human redox systems, like reactive oxygen species (ROS), with a potential antioxidant and follow the reaction with electrochemical methods like cyclic voltammetry [16–19]. However, since it is only a two-component system, the results cannot be compared with the activity of such antioxidants *in vivo*.

The fourth option is to use systems that model biological or biochemical effects, i.e., sensor systems for ROS detection, utilizing the antioxidants capacity to reduce the ROS concentration and decrease the effect of ROS on the model system. One class of sensors relies on enzymes that are immobilized on the electrode surface, e.g., cytochrome c [20, 21] and superoxide dismutase (SOD) [22, 23]. However, using these sensors, specific experimental conditions that enable proper enzyme activity (e.g., pH, temperature, right location of the active center) are required. Other sensors that concentrate on the interaction of ROS with DNA use immobilized double-stranded DNA. Scholz et al. [24] developed an electrochemical assay to quantify oxygen radicals. Here, gold electrodes are modified with a self-assembled monolayer (SAM) that is degraded upon exposure to oxygen radicals proportional to their concentration and exposure time. The extent of damage of the SAM can be assessed by voltammetric techniques in an electrolyte containing a redox probe such as $\text{Ru}(\text{NH}_3)_6^{3+/2+}$ or $[\text{Fe}(\text{CN})_6]^{3-/4-}$. It has been demonstrated that the electrochemical assay can be used to determine antioxidative activities of plant extracts [25], as the scavenging activity is reflected in the inhibition of the SAM degradation. The main advantage of the method is the easy preparation of the SAM and that the interaction of biologically relevant radicals with SAMs as well as with antioxidants can be measured. As expected, the assays rank the antioxidant potential of individual substances differently from established antioxidant assays. Nevertheless, the question remains how efficient the

antioxidant activity of a substance is in terms of protecting a particular target molecule/structure such as a cell membrane. Here, supported lipid bilayers can provide a valuable *in vitro* platform for the investigation of a variety of biological questions associated with cell membrane function, structure, and composition.

In the current study, to mimic the biological system, a gold-supported lipid bilayer was used as a model for cell membrane, and cold physical plasma jet (kINPen®) was used for the formation of a mixture of biologically relevant reactive species. A gold-supported lipid bilayer of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was prepared by the potential cycle assisted vesicle fusion method [26, 27]. The bilayer was exposed to reactive species generated by cold physical plasma jet (kINPen®) in the presence and absence of different antioxidants, and electrochemical measurements were performed to follow the degradation of the bilayer. The obtained ranking of the antioxidant activity of four model substances as well as the concentration dependence of the protective action was compared with the results obtained with a SAM-modified electrode which was exposed to radicals produced by photolysis of H_2O_2 in presence and absence of the same compounds. By finding no significant difference in the outcome of the two assays, SAM-modified electrodes with electrochemical detection are powerful tools to estimate qualitatively the antioxidative activity of a compound with respect to cell membrane protection.

Experimental

Chemicals

Dry powder of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was from Avanti Polar Lipids. Chloroform, ethanol, 1-decanethiol, $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$, ascorbic acid (AA), and (–)-epigallocatechin gallate (EGCG) were purchased from Sigma-Aldrich. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97 %) was from Acros Organics. Potassium ferrocyanide ($\text{K}_4[\text{Fe}(\text{CN})_6]$), Na_2HPO_4 , NaH_2PO_4 , H_2SO_4 , H_2O_2 (30 %), acetic acid, and sodium acetate were obtained from Merck. Caffeic acid (CA, 98 %) was purchased from Roth. All chemicals employed were used without further purification. All solutions were prepared using ultrapure water (resistivity = 18.2 MΩ/cm).

Electrode preparation

Polycrystalline Au electrodes (Ø 2 mm; Metrohm) served as working electrodes. Before modification with self-assembled mono- or bilayers, the surface must be cleaned in order to get an ultra-flat surface. This was done based on the protocol by Thal et al. [28]. Briefly, electrodes were mechanically

polished with Al_2O_3 powder (Buehler; grain sizes 300 and 50 nm) and loosely attached alumina particles were removed in ultrasonic bath. Afterwards, electrodes were cycled from 0 to 1.5 V in 0.1 M H_2SO_4 at 0.1 V s^{-1} until a stable voltammogram was obtained (40 ± 10 cycles to obtain stable voltammogram). Au oxides were removed from the surface by additional 10 cycles in 0.1 M H_2SO_4 from 0.75 to 0.2 V at 0.1 V s^{-1} .

SAM formation

The cleaned gold electrodes were rinsed with water and ethanol and immersed into 2 mM ethanolic 1-decanethiol solution and kept at room temperature in the dark for 17 h. After the SAM formation process, the electrodes were again rinsed with water and ethanol. The integrity of the formed SAM was tested in differential pulse voltammetry (DPV) measurements in 1 mM $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$ solution in 0.1 M acetate buffer (pH = 4.7). The coating procedure was assumed successful in the case of the absence of the $\text{Ru}(\text{NH}_3)_6^{3+}$ reduction signal.

Bilayer formation

Bilayer formation was done by potential assisted vesicle fusion method [26]. Briefly, POPC liposomes were prepared by sonication. The desired amount of lipid was dissolved in chloroform. The solvent was evaporated under a constant stream of nitrogen yielding a homogenous lipid film. Remaining traces of solvent were removed by placing the samples under vacuum overnight. The lipid film was thereafter suspended in deaerated 50 mM phosphate buffer (pH 7.4) and sonicated with a tip-sonicator for 45 min in ice yielding small unilamellar vesicles (SUVs). Afterwards, the suspension was centrifuged for 10 min at 14,000 rpm to remove titanium debris of the sonication procedure.

The size distribution of the obtained SUVs was measured by dynamic light scattering (DLS) using a NICOMP 380 Particle Sizer (Particle Sizing Systems). The final lipid concentration in the samples was 0.5 mg mL^{-1} .

Lipid bilayer on the gold surface was formed by slow potential scans (5 mV s^{-1} cycling between 0.4 and -0.8 V vs Ag/AgCl for at least 4 h) after the addition of the liposome suspension to the electrochemical cell. After the formation of the lipid bilayer on gold electrode, the remaining liposome suspension in the cell was replaced by buffer and water for further experiments.

Radical treatment

Reactive oxygen and nitrogen species were generated either by a cold physical plasma source, the kINPen® (neoplas), or by UV photolysis (Metrohm). The plasma jet consists of a

central pin-type electrode powered by a voltage of 2–6 kV with a frequency of 1 MHz. Argon was used as feed gas (3 standard l/min) [29]. For all experiments with the kINPen®, a direct treatment regimen was chosen and achieved by exposing 10 mL of water-covered solid-supported lipid bilayer to the plasma effluent at a distance of 9 mm to the aqueous surface. Radical exposure of SAM-modified electrodes was performed in quartz test tubes (Metrohm) filled with 10 mL of 1 mM H_2O_2 . Radicals were generated by photolysis of H_2O_2 by UV light radiation (705 UV Digester, Metrohm). The electrodes were exposed to the formed radicals for treatment intervals of 30 s.

Electrochemical measurements

All electrolytes were deaerated before electrochemical measurements (600-s nitrogen purging prior to measurement). The voltammetric measurements were performed with a 663 VA stand in combination with an Autolab PGSTAT 20 and Eco Chemie IME 303 (Metrohm). The modified gold electrode served as the working electrode, a platinum electrode as the counter electrode, and an Ag/AgCl (3 M KCl) electrode as the reference. Data were recorded with NOVA 2.0 software (Metrohm). Data analysis was performed with OriginPro 2016 (OriginLab Cooperation). As electrolyte, either 10 mM solution of $\text{K}_4[\text{Fe}(\text{CN})_6]$ in 50 mM phosphate buffer (pH 7.4) or 1 mM $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$ in 0.1 M acetate buffer (pH = 4.7) was used. Cyclic and differential pulse voltammograms of $\text{K}_4[\text{Fe}(\text{CN})_6]$ were recorded in the potential range from -0.3 to $+0.7 \text{ V}$ vs. Ag/AgCl and 50 mV s^{-1} as a scan rate. Differential pulse voltammograms of $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$ were recorded in the potential range from $+0.3$ to -0.7 V vs. Ag/AgCl.

Results and discussion

Gold electrodes covered with a 1-decanethiol SAM showed a complete suppression of the reduction signal of the redox probe $\text{Ru}(\text{NH}_3)_6^{3+}$, as it is shown in Fig. 1. We used the $\text{Ru}(\text{NH}_3)_6^{3+}/\text{Ru}(\text{NH}_3)_6^{2+}$ as the redox probe in these experiments, since it undergoes a simple charge transfer process with a high rate constant, as was shown by Wilke et al. [30]. A treatment with ROS produced via UV photolysis of H_2O_2 altered the SAM structure in a way that the redox probe can penetrate the SAM, and the reduction signal is regained. However, the initial current maximum of the bare gold electrode was not regained after ROS treatment up to 50 min (Fig. 1). Previous experiments with hexacyanoferrate(II) as redox probe and hexanethiol, C_6SH , demonstrated that a complete removal of the SAM with hydroxyl radical generated by Fenton reaction could not be achieved. Since the ROS

Fig. 1 DPV measurements in 1 mM $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$ solution in 0.1 M acetate buffer (pH = 4.7). Black line: bare gold electrode before SAM preparation; red line: gold electrode covered with C_{10}SH SAM; blue, purple, green, and orange line: gold electrode covered with C_{10}SH SAM after 30-s, 150-s, 300-s, and 600-s exposure time to photolytically produced ROS

concentrations in our experiments were even lower, we assumed that complete SAM removal by the generated ROS cannot be expected. Therefore, the active electrode surface remains below the initial surface area resulting in a lower maximum reduction current measured at the electrodes after 10-min ROS treatment. Nonetheless, until this time point a gradual increase of the reduction signal correlating with the ROS treatment time is recorded at the electrodes (Fig. 1). This suggests that ROS induced damages to the SAM, which might lead to the removal of thiol molecules from the gold surface as well as rearrangements of the alkyl chains, are irreversible and that even though reorganization events of the remaining thiol molecules on the gold surface might occur, the extent of exposed active gold sites is proportional to the time of ROS exposure. Previous studies with AFM have shown that thiol molecules are removed from the surface resulting in holes within the layer. However, we cannot prove that the Au-S bond specifically is broken [28].

Hilgemann et al. [25] employed the electrochemical assay to measure the radical scavenging capacity of plant extracts. For data evaluation, they plotted the logarithm of the standardized DPV peak currents against the radical treatment time ($\ln(1 - [i_t/i_0])$ vs. t ; i_0 is the peak current for the bare gold electrode). For the resulting function, they obtained two distinct linear sections describing the exponential decay of the curve: a fast decay from 0 to 5 min and a slower decay from 5 to 30 min. The kinetic constant of the second decay decreases in the presence of antioxidants and the evaluation of this kinetic constant can be used to estimate antioxidant capacity. Figure 2 shows the logarithmic increase of the $\text{Ru}(\text{NH}_3)_6^{3+}$ reduction signal in dependence of the treatment time under

Fig. 2 Kinetics of the logarithmic inverse of the increase of the $\text{Ru}(\text{NH}_3)_6^{3+}$ reduction signal (i) at C_{10}SH SAM-covered gold electrodes in dependence of the exposure time towards photolytically generated ROS from 1 mM H_2O_2

the experimental settings described, here. The mode of ROS generation, the applied thiol to form the SAM, the redox probe, and the employed H_2O_2 concentration used to generate ROS differed from the parameters reported by Hilgemann et al. [31]. We generated ROS photolytically instead of Fenton reaction to avoid additional elements (e.g., iron) in our experimental set-up and used 1 mM instead of 500 mM H_2O_2 to better mimic biologically relevant conditions. Therefore, the obtained SAM degradation curve differed slightly from the data reported before. For this low H_2O_2 concentration, the increase of the measured peak currents was low in the first 30 s, followed by a time period with a faster increase of the peak currents. At longer treatment times, the increase of the peak currents with time slowed down again. We assume that the increase of currents measured within the first 30 s is lower because ROS mainly attacks the alkyl chains without exposing active gold sites. Then, the film degradation can take place as described by Hilgeman et al. for SAMs with shorter alkyl chains and higher ROS concentrations. However, using the linear part of the function $\ln[1 - (i_t/i_0)]$ vs. t (see red line in Fig. 2) allows the comparison of the ROS scavenging potential of individual substances. For that purpose, the slope representing the SAM destruction rate in the presence of an antioxidant (k_{Aox}) is compared with the rate of destruction in the absence of the antioxidant substance (k):

$$\text{scavenging}[\%] = \left[\frac{(k - k_{\text{Aox}})}{k} \right] \times 100$$

In Fig. 3, the impact of different concentrations of ascorbic acid on the regain of the $\text{Ru}(\text{NH}_3)_6^{3+}$ reduction signal at C_{10}SH SAM-covered gold electrodes is shown. Ascorbic acid can slow down the degradation of the SAM by photolytically generated ROS ($c(\text{H}_2\text{O}_2) = 1 \text{ mM}$) in a concentration-dependent manner, which is mirrored in the prolongation of

Fig. 3 **a** DPV currents (I) measured at $C_{10}SH$ SAM-covered gold electrode in the presence of different concentrations of ascorbic acid plotted against the exposure times towards photolytically generated ROS from 1 mM H_2O_2 . **b** Radical scavenging activity of different substances at a

concentration of 100 μM determined by their potential to decrease SAM degradation by ROS. AA ascorbic acid, CA caffeic acid, FA ferulic acid, EGCG epigallocatechin gallate

the first plateau phase and decreased slopes of the curves in the linear region (Fig. 3a). The ability to scavenge ROS in this experimental setting differs between different substances (Fig. 3b). At a concentration of 100 μM , five different substances showed the following scavenging activities: ascorbic acid (AA) $82 \pm 3\%$; caffeic acid (CA) 97%; ferulic acid (FA) $75 \pm 4\%$; epigallocatechin gallate (EGCG) 94%; Trolox 99% (Fig. 3a). Therefore, the ranking of the antioxidant activities is as follows: Trolox > caffeic acid (CA) > epigallocatechin gallate (EGCG) > ascorbic acid (AA) > ferulic acid (FA), (Fig. 3b).

The radical scavenging activity depends on the ability to protect the SAM from structural damages that result in the loss of its insulating properties. This can either be achieved by direct interaction of the molecules with the UV light or radicals (hydroxyl radicals, superoxide) generated by the UV light or by interaction with secondary radical products that result from the first generation of radicals. Experiments were undertaken in aqueous media, while the $C_{10}SH$ SAM presents a more hydrophobic surface. In comparison with a lipid bilayer, the SAM lacks the hydrophilic head groups as well as embedded proteins that might alter its hydrophobicity. The antioxidant that performed best in this system was the water-soluble vitamin E derivative Trolox. In comparison with vitamin E, Trolox possesses the chroman head and the hydroxyl group that is associated with its radical scavenging activity but lacks the hydrocarbon tail. Therefore, similar radical scavenging activities have been reported for both molecules in aqueous media [31]. Since the radicals are produced within the aqueous phase, Trolox performs well in our system. Caffeic acid and EGCG have a high radical scavenging impact on the SAM degradation rate, as well. All three substances are also soluble in organic solvents [32–34]. On the other hand, the

radical scavenging activity of ascorbic acid and ferulic acid, which are only soluble in water, is lower. This indicates that the assay might also assess to some extent the interaction of the substances with the lipophilic thiol molecules of the SAM. It has been reported before that Trolox is capable to interact with lipophilic structures [31] in a way that potentially also enables radical scavenging directly at the interface SAM/electrolyte solution. The results further indicate that direct absorption of UV light, which would also add to the measured radical scavenging activity, seems negligible for the investigated substances. If direct UV-light absorption would contribute significantly, we would expect more homogeneous scavenging activities for all investigated substances.

Effect of reactive species on lipid bilayer

Cyclic voltammetry was used to verify lipid bilayer formation at the gold electrode. $K_4[Fe(CN)_6]$ was chosen as the redox probe because it is a surface-sensitive probe and it has better passivation properties at the surface of the electrode after lipid bilayer formation compared with $[Ru(NH_3)_6]Cl_3$ [30]. Moreover, the electron transfer between the solution species and the electrode must take place through bilayer defects [35]. For this purpose, cyclic voltammograms of 10 mM $K_4[Fe(CN)_6]$ were measured before and after the formation of the bilayer on the gold electrode. According to the voltammogram (Fig. 4), oxidation and reduction peaks of the redox probe can be observed for the bare gold electrode and are lost after the formation of the lipid bilayer, indicating that it completely covers the electrode. After exposing the lipid bilayer to cold physical plasma-generated reactive species, their impact was monitored after subsequent replacement of the treated liquid by the redox probe solution. The results of the

Fig. 4 Cyclic voltammograms of 10 mM $K_4[Fe(CN)_6]$ in 50 mM phosphate buffer of gold electrode (GE), before (POPC) and after (treated POPC) 30-min plasma treatment of lipid bilayer

voltammetry (Fig. 4) showed the oxidative degradation of the lipid bilayer by restoring the initially present oxidation and reduction peaks of the redox system. However, similar to the SAM experiments, the original current of the bare gold electrode was not reached again after the treatment of the lipid bilayer.

Protection effect of lipid bilayer by antioxidants during plasma treatments

Differential pulse voltammetry (DPV) as a more sensitive voltammetry technique was used to study the protective effect of the lipid bilayer by different antioxidants during plasma treatments. Ascorbic acid (AA), caffeic acid (CA), epigallocatechin gallate (EGCG), and Trolox were used as antioxidants in this study. According to DPV voltammograms (Fig. 5), in the presence of antioxidants, an attenuated restoration of the oxidation peak was registered. 200 μ M EGCG showed a complete protection against CPP-derived reactive species, but at lower concentrations of EGCG (50 μ M) oxidative damage of the SAM recurred. As can be seen in Fig. 5, the passivation of the electrode surface is even better in the case of high concentrations of EGCG compared with the lipid bilayer. The same was found for Trolox at high concentrations. We assume that both antioxidants are distributed on the surface of the lipid bilayer, leading to an additional blocking of the electron transfer. In addition, the results showed a positive potential shift with increasing antioxidant activity in the system and a decrease in the peak current of the redox probe. The positive potential shift is due to less defect formation in the lipid bilayer and slow electron transfer between the redox probe and surface of electrode. The results also indicated that Trolox has maximum antioxidant activity in this study.

Fig. 5 Differential pulse voltammograms of 10 mM $K_4[Fe(CN)_6]$ in 50 mM phosphate buffer after 30-min plasma treatments of a lipid bilayer with and without different antioxidants. AA ascorbic acid, CA caffeic acid, EGCG epigallocatechin gallate, Trolox

In addition, the percentage of the protective effect of antioxidants was calculated using the following equation:

$$\% \text{Protection effect} = [(i_1 - i_2) / i_1] \times 100$$

where i_1 and i_2 are the electrochemical currents obtained after plasma treatment of lipid bilayer without and with antioxidants, respectively. As shown in (Fig. 6), a protective effect of $71 \pm 3\%$, $54 \pm 5\%$, $49 \pm 1\%$, 100%, and $32 \pm 4\%$ was observed for Trolox (50 μ M), caffeic acid (50 μ M), EGCG (50 μ M), EGCG (200 μ M), and ascorbic acid (200 μ M), respectively.

Furthermore, the electrochemical parameters of the redox system for plasma treatment of the lipid bilayer in the presence

Fig. 6 Comparison of the protective effect of different antioxidants during plasma treatment of lipid bilayer

Table 1 Electrochemical parameters of 10 mM $K_4[Fe(CN)_6]$ in 50 mM phosphate buffer at bare gold electrode, and for lipid bilayer modified gold electrode after 30-min plasma treatment in presence of different antioxidants

	I_{ox} (μA) $\pm$ SD	I_{red} (μA) $\pm$ SD	ΔE (mV) $\pm$ SD
200 μM ascorbic acid	21.6 ± 0.3	19.7 ± 0.3	173 ± 6
200 μM EGCG	-	-	-
200 μM caffeic acid	-	-	-
200 μM Trolox	-	-	-
50 μM EGCG	16.9 ± 2.0	16.5 ± 1.3	262 ± 39
50 μM caffeic acid	16.0 ± 0.7	10.5 ± 2.2	392 ± 15
50 μM Trolox	5.9 ± 1.1	4.3 ± 1.5	640 ± 45
Absence of antioxidants	28.9 ± 1.0	27.1 ± 1.0	148 ± 5
Gold electrode	39.1 ± 2.7	39.6 ± 2.0	142 ± 16

of antioxidants were investigated by means of cyclic voltammetry. The results showed (Table 1) an increase in peak separation and a decrease in peak currents of the redox system in the presence of antioxidants, confirming the slow electron transfer between the redox probe and the surface of the gold electrode, and the reduction of defect formation in the lipid bilayer in the presence of antioxidants. The results also confirm the DPV data, verifying Trolox to have the maximum antioxidant activity in this study. Therefore, the order of radical scavenging activity of the antioxidant to protect the lipid bilayer from reactive species is as follows: Trolox > CA > EGCG > AA. According to the results, Trolox showed the maximum protective effect in this study. As we discussed at the SAM part, the structure of Trolox consists of a carboxyl group connected to a chroman ring, and due to a similar structure of Trolox and vitamin E, several studies have reported that Trolox is able to partially diffuse into the lipid bilayer [36, 37]. Therefore, in addition to its hydrophilic antioxidant activity, it can also partially act as a lipophilic antioxidant. However, the diffusion of Trolox in the lipid bilayer depends on the structure of the bilayer and also on the presence of cholesterol and sphingomyelin lipids at the membrane [38]. In the current work, the lipid bilayer is neutral due to the positive charge of the choline group and the negative charge of the phosphate group. Under such conditions, the negative charge of the carboxyl group in the Trolox leads to electrostatic interactions with the choline group of the lipid bilayer. This distribution of the Trolox on the surface of the bilayer is sufficient to meet the reactive species and thus terminate the peroxidation process of the lipid bilayer [31].

Conclusions

In this work, a gold-based self-assembled monolayer was used to study the radical scavenging activity of various antioxidants against reactive species. The results were compared with the data obtained using a gold-supported lipid bilayer as a model for a cell membrane. In addition, photolysis of hydrogen

peroxide and plasma treatment of water was used to generate short-lived reactive species as cellular free radicals. DPV and CV measurements confirmed the oxidative damage at both sensors by reactive species and showed the ability of antioxidants as scavengers to protect SAM and lipid bilayers from free radicals. Interestingly, a similar order of radical scavenger activity was obtained for both electrochemical sensors. The protective effect depends on the structure of the antioxidants and also on the interaction between the antioxidant and the sensors. As Trolox has a maximum of antioxidant activity, this could be due to the structure of Trolox as a water-soluble analog of vitamin E. Because of the amphiphilic behavior, it can interact with the SAM and the lipid bilayer at the surface to the electrolyte, and it can also partially diffuse into the lipid bilayer to protect it from reactive species. In addition to the similarity of the SAM electrode to the POPC model membrane in the assessment of antioxidants, the stability, robustness, and simple fabrication of these sensors offer unique advantages. To conclude, the electrochemical assay based on SAM electrode is an alternative to similar antioxidants assessment based on electrochemical sensors, which often suffer from limited stability, high costs, and the inability to function outside their optimal conditions.

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