MAGNETICALLY DRIVABLE NANO-BIO-CONJUGATE MIMICKING FREE CURCUMIN REDOX BEHAVIOR

Massimiliano MAGRO\textsuperscript{a,c}, Rene CAMPOS\textsuperscript{b}, Davide BARATELLA\textsuperscript{a}, Giuseppina PACE PEREIRA LIMA\textsuperscript{b}, Radek ZBORIL\textsuperscript{c}, and Fabio VIANELLO\textsuperscript{a,c}

\textsuperscript{a} University of Padova, Padova, Italy, EU, fabio.vianello@unipd.it
\textsuperscript{b} Universidade Estadual Paulista (UNESP), Botucatu, São Paulo, Brazil
\textsuperscript{c} Palacky University in Olomouc, Olomouc, Czech Republic, EU

Abstract

Curcumin possesses wide-ranging anti-inflammatory and anti-cancer properties and its biological activity can be correlated to its potent antioxidant capacity. Novel maghemite (γ-Fe\textsubscript{3}O\textsubscript{4}) nanoparticles, characterized by a diameter of about 10 nm and possessing peculiar colloidal properties and surface interactions, called Surface Active Maghemite Nanoparticles (SAMN), were superficially modified with curcumin by simple incubation, due to the presence of under-coordinated Fe(III) atoms on nanoparticle surface. The resulting curcumin-modified SAMNs (SAMN@curcumin) were characterized by transmission electron microscopy (TEM), FTIR, Mossbauer, EPR and UV-Vis spectroscopy. The redox properties of bound curcumin were tested by electrochemistry. Finally, SAMN@curcumin was studied in the presence of different electroactive substances, namely hydroquinone, NADH and ferrocyanide, in order to assess its electrochemical behavior. Moreover, SAMN@curcumin was electrochemically tested in the presence of one of the most diffuse reactive oxygen specie, such as hydrogen peroxide, demonstrating its stability. SAMN@curcumin in which curcumin is firmly bound, but still retaining its redox features represents a feasible adduct: a magnetically drivable nano-bio-conjugate mimicking free Curcumin redox behavior. The proposed nanostructured material could be exploited as magnetic drivable curcumin vehicle for biomedical applications.

Keywords: curcumin, maghemite nanoparticles, nanomaterial electrocatalysis, hydrogen peroxide, NADH oxidation

1. INTRODUCTION

Curcumin [IUPAC name, (1E,6E)-1,7-bis-(4-hydroxy-3-methoxy-phenyl)-1,6-heptadien-3,5-dione; CAS number: 458-37-7] is a homodimer of feruloyl-methane, containing a methoxy group and a hydroxyl group, a heptadiene with two Michael acceptors, and an α,β-diketone [1] (see Fig. 1). Curcumin has shown significant efficacy as chemo-preventive in cell culture studies, as it elicits efficacy in various clinical studies [2, 3]. Its introduction into clinical settings is hindered mostly by its poor solubility and rapid metabolism, ultimately resulting in poor bioavailability upon administration [4]. Therefore, to circumvent these limitations and to ease its transition to clinics, alternative strategies should be explored. Drug delivery systems such as liposomes, microemulsions, polymeric implantable devices are emerging and, of course, nanoparticles as one of the viable alternatives that have been shown to deliver therapeutic concentrations of various potent chemopreventives, such as curcumin [5]. The research on iron oxide based nanoparticles has captured attention in the recent years as efficient drug delivery system and contrast agent in magnetic resonance imaging, with numerous biomolecule immobilization studies being reported [6]. In the present report, we exploited under-coordinated Fe(III) atoms on SAMN surface for curcumin conjugation. Redox events associated with the curcumin shell on maghemite core resulted reversible and reproducible, for at least 1 month, indicating that curcumin is stable and still electroactive even if firmly bound on SAMN surface, leading to an active curcumin adduct bound on magnetic drivable and tunable maghemite nanoparticles. These results are very informative for the construction of nanodevices for biomedical purposes.
2. METHODOLOGICAL BASES

2.1. Electrode preparation

A carbon paste electrodes (CPEs) were prepared by mixing graphite powder with silicon grease in a 70:30 weight-to-weight ratio. The resulting CPEs were inserted into the cavity of glass electrode holders (1.35 mm diameter, 0.01428 cm² geometric area). A copper wire had been inserted into the paste through the opposite side of the glass capillary to create the electrical contact with the potentiostat. Finally, the electrode surface was carefully smoothed on a weighting paper and rinsed with double distilled water before each experiment.

2.2. Surface coating of SAMN with curcumin

SAMN colloidal dispersion (200 mg/L) were incubated with curcumin (200 mg/L), for 1 hour under end-over-end mixing at 4.0 °C. After the incubation period, nanoparticles were separated by the application of an external magnetic field and the presence of the molecule in the supernatant was checked by spectrophotometry. The amount of bound curcumin was calculated from the disappearance of curcumin absorbance at 420 nm in the supernatant ($\epsilon = 3.33 \times 10^4$ M⁻¹cm⁻¹). SAMN@curcumin complex was magnetically isolated and washed several times with water. Molecule coverage on SAMNs was stable, without any loss in solution of curcumin checked by spectrophotometry.

3. RESULTS

UV-Vis spectroscopy was used to study SAMN@curcumin complex. The interaction of curcumin with SAMN surface produced an alteration of nanoparticle optical properties, both the shape and the position of maximum absorption were altered, indicating the influence of SAMN surface properties on optical characteristics. The electronic absorption spectrum of bare SAMNs, acquired in water, shows a wide band with a maximum at about 400 nm (Fig. 2) characterized by an extinction coefficient of 1520 M⁻¹cm⁻¹, expressed as Fe₂O₃ molar concentration. Differently, SAMN@curcumin spectrum is characterized by two peaks, at 436 and 494 nm (see Fig. 2), the red shift and change of shape, with respect to underivatized SAMN, are in good agreement with the same phenomenon observed with the binding of other molecules on nanoparticle surface [7].
The redox properties of SAMN@curcumin and free curcumin were investigated by electrochemistry. The comparison of CV curves of SAMN-CPE and SAMN@curcumin-CPE showed that the presence of curcumin on SAMN surface leads to an amplification of the capacitive current, suggesting an increase of the electrocatalytic available surface (see Fig. 3). The resulting cyclic voltammograms are characterized by the occurrence of reduction-oxidation peaks at 0.2 V and at 0.33 V vs. SCE, respectively (see Fig. 3). The cathodic and anodic peaks observed with the CP electrode modified with SAMN@curcumin were slightly shifted with respect to the free polyphenol CPE. These electrochemical features were not present with SAMN/CPE (see Fig. 3). In addition, the position of cathodic and anodic peaks depended on pH, both for SAMN@curcumin/CPE and curcumin/CPE. In particular, peaks were shifted to negative values as the pH increased and the potential difference between cathodic and anodic peaks appeared lower for SAMN@curcumin/CPE than curcumin/CPE, indicating a more reversible behavior of curcumin bound on iron oxide nanoparticle surface. Furthermore, at pH 7.0, which is the most common experimental condition used for biological samples, the potential difference between the anodic and cathodic peaks is close to 30 mV, suggesting a reversible 2 electron redox reaction. These findings are in good agreement with literature [8], notwithstanding Manaia and colleagues noted the appearance of two consecutive anodic and cathodic processes, at high potentials, attributed to irreversible redox reactions. In the present case, curcumin, free or immobilized on SAMNs, showed the same reversible electrochemical behavior, possibly because they are embedded in a carbon paste matrix. Interestingly, reversible redox peaks are well conserved after hundred cyclic voltammograms, indicating that curcumin is electrochemically active and still stable when firmly bound on nanoparticles surface.
Moreover, SAMN@curcumin/CP electrodes were tested in the presence of electroactive substances of biological interest, namely hydrogen peroxide and NADH, showing a remarkable electrocatalytic ability. The electrode response, following the addition of increasing concentrations of analytes was measured (see Table 1). The current response of SAMN@curcumin/CPE increased linearly with electroactive substances concentration in the ranges reported in Table 1, and compared with the response of bare SAMN/CPE. Interestingly, the presence of bound curcumin on SAMNs was responsible of an opposite effect on electrode performances. In the case of H$_2$O$_2$, the sensitivity was increased of about the 40% with respect to unmodified SAMNs, while, as regards NADH detection, the electrode sensitivity decreased by 75%. We attribute the different behavior to the reductant vocation of curcumin, which is more efficient in the electrocatalysis of H$_2$O$_2$ reduction, than towards NADH oxidation, further confirming the interaction of curcumin with SAMN surface, enhancing SAMN electrocatalytical properties. It should be noted that the sensitivity toward H$_2$O$_2$ electro-reduction of SAMN@curcumin/CPE was higher than that of bare SAMNs and that control experiments performed with CP electrodes prepared with free curcumin did not show any electrocatalytic activity on H$_2$O$_2$, suggesting a synergic effect between the molecule and metal oxide surface.

![Graph of current vs. potential](image)

**Table 1.** Analytical performances SAMN@curcumin on various electroactive substances of biological interest. Measurements on hydrogen peroxide were carried out in 50 mM sodium acetate, pH 5.0, containing 50 mM KCl, at an applied potential of -0.1 V vs. SCE; measurements on NADH were carried out in 50 mM Tris-HCl, pH 8.5, containing 100 mM KCl, at an applied potential of 0.4 V vs. SCE.

<table>
<thead>
<tr>
<th>SAMN modification</th>
<th>Hydrogen peroxide</th>
<th>NADH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no curcumin</td>
<td>no curcumin</td>
</tr>
<tr>
<td>Analyte</td>
<td>Sensitivity (µA mM$^{-1}$ cm$^{-2}$)</td>
<td>Detection limit (µM)</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>25.44</td>
<td>32.05</td>
</tr>
<tr>
<td>NADH</td>
<td>35.47</td>
<td>8.53</td>
</tr>
</tbody>
</table>

Previous electrochemical study on curcumin and other polyphenols identified in hydroxyl groups on on the polyphenol aromatic rings the redox active centers of curcumin [8]. The preservation of electroactivity of curcumin bound on SAMN surface could be due to the β-diketo molecule moiety, which assures the stability of the binding with the undercoordinated iron(III) sites. Meanwhile, aromatic hydroxyls of curcumin are available for oxidation-reduction reactions with ROS. The electron delocalization through curcumin molecule, involving the β-diketo moiety and aromatic rings (see Fig. 1) and, as consequence, nanoparticle iron(III) electroactive sites, could explain the enhanced sensitivity of the SAMN@curcumin complex towards H$_2$O$_2$ electro-reduction, with respect to bare SAMNs and free curcumin.
4. DISCUSSION

In the present paper, we reported on the development of a stable nanomaterial constituted of maghemite nanoparticles, characterized by the presence of surface under-coordinated iron, which were coated with curcumin, obtaining a core-shell curcumin derivatized maghemite nanoparticles (SAMN@curcumin). The nano-bio-conjugate material was easily prepared by simple incubation in water. Furthermore, the nanomaterial was structurally characterized by UV-Vis, FTIR and Mossbauer spectroscopies, evidencing that curcumin is firmly bound on nanoparticles surface and TEM images demonstrated the formation of a continuous shell around SAMNs.

It has been already demonstrated that SAMNs can serve as a promising contrast agent for future noninvasive in vivo tracking by MRI [9]. Furthermore, an extensive study on nanoparticle-cell interaction was carried out, and several techniques and methods were used to characterize SAMN toxicity and to explore their biocompatibility. A negligible effect on cell metabolic activity, up to 100 µg mL⁻¹, has been demonstrated [10]. In this sense, these nanoparticles are promising candidates for diagnostic and therapeutic applications.

Considering that reactive oxygen species (ROS), such as superoxide anion and hydroxyl radical, play a pivotal role in numerous human pathologies and are also thought to be involved in carcinogenesis. Consequently, the control of ROS concentration represents an important mechanism invoked in the prevention of several diseases and cancer. Curcumin is currently used as anti-oxidant to reduce ROS damage to tissues and organs. More specifically, curcumin has been shown to scavenge superoxide anion radicals and hydroxyl radicals [11, 12]. However, studies over the past three decades related to the absorption, distribution, metabolism and excretion of curcumin have revealed its poor absorption and rapid metabolism, severely impairing its bioavailability (Anand et al., 2007). At the same time, nanoparticles were proposed as one of the viable alternatives as drug delivery systems, and iron oxide nanoparticles have been shown to deliver therapeutic concentrations of various potent chemopreventives. SAMNs represent a suitable and friendly environment for curcumin, preserving its redox properties, as demonstrated by the electrochemical the SAMN@curcumin adduct. Cyclic voltammetry enabled the rapid screening of the redox properties of curcumin immobilized on iron oxide nanoparticles, evidencing both the high stability and reversibility of redox reactions. The present study demonstrated that SAMN@curcumin possess a remarkable electrocatalytic activity toward the reduction H₂O₂, which is one of main biologically important ROS (Reactive Oxygen Species).

5. CONCLUSION

Curcumin immobilization on SAMNs hampers its rapid metabolization and elimination, which are considered the most important drawbacks during in vivo curcumin treatment. The nano-bio-conjugate proposed is magnetic drivable and targetable to specific organ by an external magnetic field. SAMN@curcumin system widens the applicative possibilities of curcumin in future therapeutic treatments.

ACKNOWLEDGEMENTS

The present experimental work was partially funded by Italian Institutional Ministry grants cod. 60A06-7411 and 60A06-8055. Authors gratefully acknowledge the support by the Operational Program Research and Development for Innovations - European Regional Development Fund (project CZ.1.05/2.1.00/03.0058) and by the Operational Program Education for Competitiveness - European Social Fund (project CZ.1.07/2.3.00/20.0155) of the Ministry of Education, Youth and Sports of the Czech Republic and the Grant Agency of the Academy of Sciences of the Czech Republic (KAN115600801; KAN200380801).
LITERATURE


