



Electroanalytical study of the interaction between dsDNA and curcumin in the presence of copper(II)

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ABSTRACT

As a result of the reaction between curcumin (CC) and copper(II) the characteristic peak of curcumin at -1.0 V significantly increased, and the peak at -1.6 V disappeared. Curcumin forms complex with copper(II).

The interaction between double stranded (ds) calf-thymus DNA and curcumin in the presence of Cu(II) was studied in solution, by differential pulse adsorptive transfer voltammetry using carbon paste electrode (CPE) and hanging mercury drop electrode (HMDE). Cu(II)–CC complex generated changes in calf-thymus DNA. The characteristic peak of dsDNA, due to the oxidation of guanine residues, decreased. The increased DNA damage by Cu(II)–CC complex was observed in the presence of various concentrations of the transition metal ions, copper(II).

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1. Introduction

Curcumin, a yellow spice and pigment from *Curcuma longa* L. (Zingiberaceae), is by far known for its anti-oxidant [1–4], anti-inflammatory [4,5] and anti-cancer [3,4,6,7] activities. Curcumin has proved not to have toxic, genotoxic or teratogenic properties [8–10], so this safe phytonutrient has been widely implied in pre-clinical and clinical studies [4]. It has been reported that curcumin directly binds to both synthetic and genomic nucleic acids [11]. Also, curcumin interacts strongly with biomacromolecules and the non-covalent interactions are known to play a decisive role in its mechanism of action [12].

Curcumin and its derivatives have shown the ability of being free-radical scavenger, interacting with oxidative cascade, quenching oxygen and chelating and disarming oxidative properties of metal ions [13–15]. Biological activity of curcumin has been attributed to the hydroxyl group substituted on the benzene rings and also to the diketonic structure [16]. The β -diketo moiety of curcumin undergoes a keto-enol tautomerism. Crystal studies [17–19] have shown that the symmetric structure of curcumin leads to a statistically even distribution of the enol proton between the two oxygen atoms. A growing body of evidence indicates that various metals act as catalysts in the oxidative deterioration of biological macromolecules, and therefore, the toxicity associated with these metals may be due at least in part to their ability to gener-

ate free radicals [20]. The strong chelating ability of diketones has been widely investigated towards a great number of metal ions; therefore, curcumin could be of great importance in the chelating treatment of metal intoxication and overload. Curcumin forms complexes of the type 1:1 and 1:2 with copper, iron, and other transition metals. This property of binding of curcumin to metals like iron and copper is considered as one of the useful requirements for the treatment of Alzheimer's disease [21] and it is interesting to note that among the Indian populations, regular usage of turmeric is one of the reasons responsible for the reduced risk of Alzheimer's disease [21].

Recently there have been many reports in the literature on the metal-chelating properties of curcumin, employing techniques like potentiometry and absorption spectroscopy [21–27]. Electrochemistry has been used with much success in metal–ligand studies [28,29]. Limson et al. [28] used electrochemistry to show metal–ligand interactions between the pineal hormone melatonin and a range of metals. Changes in current response and potential are good indicators of alterations in the chemistry of a target compound, such as that which occurs during an exchange of electrons in metal–ligand bond formation. Adsorptive stripping voltammetry exploits the natural tendency of analytes to pre-concentrate at an electrode and is a useful technique for investigation of metal–ligand interactions [30].

These considerations as well as all the other information about curcumin on the clinical and chemistry aspects have already been given in our previous paper [31] prompted us to electrochemically explore the interaction between calf-thymus DNA and curcumin in the presence of Cu(II). Our previous study [31] has explored

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the electrochemical properties of curcumin by cyclic and differential voltammetry. The present study is focused on the behavior of DNA exposed to curcumin and copper, based on the development of an electrochemical biosensor, a carbon paste electrode. As yet has been no literature report for the voltammetric detection of this interaction, by using differential pulse voltammetry.

2. Experimental

2.1. Reagents

All the chemicals used were of analytical reagents grade. Double stranded calf-thymus DNA (D-1501, highly polymerized) was purchased from Sigma. Graphite powder was purchased from Fluka (50870), p.a. purity (99.9%) and particle size <0.1 mm. A stock solution of curcumin ($5.00 \times 10^{-3} \text{ mol L}^{-1}$) was prepared just before use by dissolving CC in ethanol, and then diluted with ethanol as the working solutions. Copper standard solution ($5.00 \times 10^{-2} \text{ mol L}^{-1}$) was prepared by dissolving $\text{Cu}(\text{CH}_3\text{COO})_2 \times \text{H}_2\text{O}$ in doubly distilled water, purchased from Merck. The working solutions of copper(II) were prepared by dilution of the standard solution with doubly distilled water. A stock solution of curcumin ($5.00 \times 10^{-3} \text{ M}$) was prepared just before use by dissolving CC in ethanol, purchased from Sigma.

The supporting electrolytes were 50 mM sodium phosphate buffer solution + 0.3 M NaCl (pH 8.5) for measurement by hanging mercury drop electrode [32] and 0.2 M sodium acetate buffer solution + 20 mM NaCl (pH 5.0) for measurement by carbon paste electrode.

For the study of the electrochemical behaviour of dsDNA on the CPE surface, the stock solutions (140 mg L^{-1}) was prepared in 10 mM Tris-HCl and 1 mM EDTA at pH 8.0 [32].

2.2. Apparatus

Differential pulse voltammetric measurements was performed with a PalmSens potentiostat purchased from IVIUM Technologies (The Netherlands, www.iviium.nl) and PalmSensPC software. The working electrodes were a hanging mercury drop electrode (EA 290 Metrohm, www.metrohm.com, type 6.0335.000, surface area 2.22 mm^2) and a carbon paste electrode with 3 mm inner and 9 mm outer diameter of the PTFE sleeve. The reference electrode was a saturated Ag/AgCl and the counter electrode was a platinum wire.

2.3. Preparation of working electrodes

2.3.1. Carbon paste electrode

The carbon paste was prepared in the usual way by hand-mixing graphite powder and nujol oil to form homogeneous mixture. The ratio of graphite powder to nujol oil was 75:25. The resulting paste was packed tightly into a PTFE sleeve and the electrode surface was polished to a smooth finish on a piece of weighing paper. Electrical contact was established with stainless steel screw. The constructed electrode was washed with distilled water and then was transferred to supporting electrolyte solution. The electrode was pre-treated by applying a potential at +1.7 V for 1 min without stirring prior to the accumulation step. The electrochemical pre-treatment produces a more hydrophilic surface state and a concomitant removal of organic layers [33].

2.3.2. Hanging mercury drop electrode

The hanging mercury drop electrode (HMDE) was transferred to de-aerated supporting electrolyte solution. Ultra pure argon was used to bubble the solutions of dissolved oxygen. Initially the solution was bubbled with argon for 5 min. Additionally the blank

background buffer was de-aerated for 1 min before each measurement.

2.4. Procedures

2.4.1. Differential pulse voltammetric study of the mixture of CC and Cu(II) solutions

The study was performed in phosphate buffer pH 8.5, when HMDE was used. The conditions were $E_{\text{step}} = 0.005 \text{ V}$, $E_{\text{pulse}} = 0.025 \text{ V}$ and scan rate = 0.050 V s^{-1} . In the case of CPE, acetate buffer pH 5.0 was used. The conditions were $E_{\text{step}} = 0.005 \text{ V}$, $E_{\text{pulse}} = 0.025 \text{ V}$ and scan rate = 0.050 V s^{-1} . Stock CC and Cu(II) solutions were added to the proper buffer to produce the required concentrations and the mixture was left to stand to equilibrate for 10 min.

2.4.2. Treatment of dsDNA with CC and Cu(II) in solution and immobilization at the CPE surface

The analysis of solution-phase DNA with CC and Cu(II) consisted of mixing the three components, followed by accumulation and measurement of the peak current of guanine residues, by transfer voltammetry, using differential pulse as the stripping mode. The electrode was rinsed for 5 s prior to each medium exchange. Stock CC and Cu(II) solutions were added to 0.2 M acetate buffer to produce the required concentrations and the mixture was left to stand for 10 min. Thereafter, stock DNA (140 mg L^{-1}) solution was added to the mixture and a new mixture was left to stand for 5 min.

A freshly polished carbon paste electrode, after the electrode pretreatment, was immersed into the solution. The accumulation of the mixture (DNA, CC and Cu(II)) was performed by applying a potential of +0.5 V for 5 min. The modified electrode was then removed from the solution and washed twice with doubly distilled water and with the background electrolyte solution. The washed electrode was then placed into a voltammetric cell. The DP curve was recorded in the blank acetate buffer solution, from +0.1 V to positive/negative potential values direction. The conditions were $E_{\text{step}} = 0.005 \text{ V}$, $E_{\text{pulse}} = 0.025 \text{ V}$ and scan rate = 0.050 V s^{-1} .

3. Results and discussion

3.1. Curcumin–Cu(II) interaction

In our previous paper [31] it has been studied the electrochemical properties of curcumin using voltammetric techniques and CPE. It has been shown that optimum deposition potential is 0.30 V and deposition time is 120 s for the reduction peak at 0.3 V. In the same condition, in this article, it has been conformed making complex between curcumin and Cu(II) [34], what is obvious from Fig. 1. The presence of $2.00 \times 10^{-5} \text{ M}$ Cu(II), at pH 5.0 affects the differential pulse voltammetric curves observed for CC. The characteristic peak of curcumin at 0.3 V significantly decreased, and a new peak appeared at 0.0 V as observed for the Cu(II)–CC system.

In addition, the voltammogram of curcumin at HMDE in the cathodic scan [31] is shown that optimum deposition potential is -0.8 V and deposition time is 60 s for reduction peak at -1.0 V and -1.6 V . Using the same conditions and appropriate concentrations of the metal and curcumin, reaction between those was followed. In the presence of an appropriate amount of Cu(II), the DPV signals differ from those of CC. As a result of the reaction between curcumin and Cu(II) (Fig. 2) the characteristic peak of curcumin at -1.0 V significantly decreased, and the peak at -1.6 V disappeared. The results show that there is an interaction between curcumin and copper(II), with the possible formation of a complex between the metal and this ligand, Cu(II)–CC complex.

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