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Antioxidant activity and electrochemical elucidation of the enigmatic redox behavior of curcumin and its structurally modified analogues

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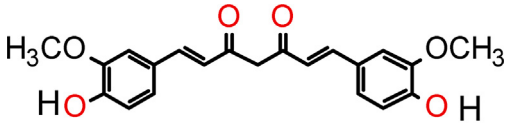
ABSTRACT

Here, we report studies on the antioxidant activity and redox behavior of curcumin and its structurally modified synthetic analogues. We have synthesized a number of analogues of curcumin which abrogate its keto-enol tautomerism or substitute the methylene group at the centre of its heptadione moiety implicated in the hydride transfer and studied their redox property. From cyclic voltammetric studies, it is demonstrated that H- atom transfer from CH_2 group at the center of the heptadione link also plays an important role in the antioxidant properties of curcumin along with that of its phenolic –OH group. In addition, we also show that the conversion of 1, 3- dicarbonyl moiety of curcumin to an isosteric heterocycle as in pyrazole curcumin, which decreases its rotational freedom, leads to an improvement of its redox properties as well as its antioxidant activity.

1. Introduction

Curcumin, a yellow colored phenolic compound isolated from the roots and rhizomes of *Curcuma longa* has been used since centuries as a spice, dietary pigment and traditional medicine in India and China [1–3]. It displays a wide spectrum of medicinal properties ranging from anti–bacterial, anti-viral, anti-protozoal, anti-fungal and anti–inflammatory to anti-cancer activity [4–7]. The ability of curcumin to neutralize chemical carcinogens such as superoxide, peroxyl, hydroxyl radical and nitric oxide radical constitutes a major subject of interest. While mode of its action is still under exploration, it is proposed to act through a number of targets and metabolic pathways involving transcription, cell growth, apoptosis etc [6,7]. Despite its invocation of a number of biologic targets it is practically non-cytotoxic to normal human cells but cytotoxic for cancer cells. It inhibits oncogenic cell proliferation by arresting cell cycle progression and induction of apoptosis [6,7]. However curcumin's low solubility, high rate of metabolism and decomposition, poor bioavailability and pharmacokinetics are responsible for its limited efficacy [8–10].

Chemically curcumin is (1 E, 6 E)-1,7-Bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-dienne-3,5-dione.



Curcumin

Curcumin is a multifunctional molecule and it has three functional groups that can contribute to its biologic activity, namely an aromatic *o*-methoxy phenolic group, α, β-unsaturated β-diketo moiety and a seven carbon linker. Its redox behavior is expectedly more complex and has led to conflicting views on the contribution of its key constituents [11–19].

The redox behavior of curcumin has led to proposal of two divergent mechanisms. Jovonoic et al., have proposed that its antioxidant mechanism involves H-atom abstraction from CH_2 group of the heptadione link and the H- atom donation from the phenol group makes merely 15% contribution towards it [13,14]. On the other hand, Barclay et al's proposal that it's a chain breaking antioxidant, donating H-atom from its phenolic group which discounts a role of CH_2 group of the heptadione link in its redox properties has received a much wider acceptance [15]. Litwinienko and Ingold later observed that curcumin donates hydrogen atom from the phenolic or enolic hydroxyl group via sequential proton loss electron transfer (SPLET) or hydrogen atom transfer (HAT) and hence, reconciled the earlier differing opinions [18,19]. Currently

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accepted mechanism of the antioxidant action of curcumin lays much of its emphasis on the chain breaking ability of its methoxyphenol group (s) that donates one H-atom and discounts a significant role of its methylene group in the process.

Here we have explored voltammetric analysis to understand the redox behavior and its correlation to phenolic O-H and methylene hydrogen attached to β-diketone of curcumin and its various synthesized analogues. These studies have investigated for the first time, systematically the role of the methylene radical at the center of curcumin's has crucial involvement in its redox behavior as its Knoevenagel condensate (*viz.* 4-(4-Hydroxy-3-methoxybenzylidene)) which lacks the ability of hydride transfer exhibits redox potential at a significantly higher potential (*viz.* decreased antioxidant ability) despite possessing an additional *ortho*-methoxy phenyl group. This in turn implies a significant role for the heptadione methylene group in manifesting the redox potential of its *ortho*-methoxy phenol group at a lower potential and higher intensity. That the redox behavior of curcumin emanates from an H-shift from its central methylene group was confirmed further by the diminution in the antioxidant abilities of butylidene and benzylidene derivatives of this methylene moiety.

2. Experimental

2.1. Chemicals

3-(3,4-Dihydroxyphenyl) prop-2-enoic acid (Caffeic acid) and (3-(3-hydroxy-4-methoxyphenyl) prop–2-enoic acid (ferulic acid), vanillin, isobutyraldehyde, benzaldehyde, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer and others reagent were purchased from Sigma-Aldrich. All solvents used were of spectral grade or distilled prior to use.

2.2. General Procedures Used for the Synthesis and Characterization of Curcumin Analogues

Reaction progress was monitored by TLC using Merck silica gel 60 F_{254} with detection by UV. Column chromatography was performed using Merck silica gel 230–400 mesh. Melting points were determined in Pyrex capillary tube using Büchi Melting Point B-540 apparatus. ^{1}H NMR spectra was recorded on 300 and 400 MHz Bruker NMR spectrometers using tetramethylsilane as an internal standard and the chemical shifts are reported in (δ) units. Coupling constants are reported as a *J* value in Hertz (Hz). The sample concentration in each case was approximately 10 mg in chloroform-d/methanol-d_4/DMSO-d_6 (0.6 mL). Mass spectra were recorded on an Electrospray–MS (Bruker Daltonis) instrument.

2.3. General Procedure for the Preparation of Curcumin Pyrazole, N-(-Substituted) Phenyl Pyrazoles and Knoevengel Condensates of Curcumin

Curcumin pyrazole, and *N*-(substituted) phenyl pyrazoles derivatives of curcumin were prepared according to previously reported procedure by us and others with some modifications (Schemes 1 and 2) [1,20,21]. Curcumin (1 mmol) was dissolved in glacial acetic acid (5 mL), hydrazine hydrate and the various phenyl substituted hydrazine hydrochlorides (1.2 mmol) were added to the solution. The solution was refluxed for 8 –24 hr, and then the solvent was removed in vacuum. Residue was dissolved in ethyl acetate and washed with water. Organic portion was collected, dried over sodium sulfate, and concentrated in vacuum. Crude product was purified by column chromatography.

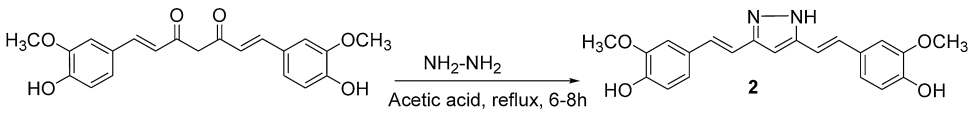
Knoevenagel condensates of curcumin was prepared by treatment of curcumin with aromatic aldehyde (4-hydroxy-3-methoxybenzaldehyde; vanillin) in presence of piperidine (as catalyst) and anhydrous DMF (as solvent) to yield 4-(4-Hydroxy-3-methoxybenzylidene) curcumin. Likewise, butylidiene curcumin and benzylidiene curcumin were prepared in good yields using the above procedure wherein isobutyraldehyde and benzyldehyde were used instead of (4- hydroxyl -3-methoxy benzaldehyde) (Scheme 3) [1,22–25]. Reaction mixture was diluted with ethyl acetate and washed with water and saturated brine. The organic phase was dried over Na_2SO_4 concentrated under vacuum, and purified by chromatography with EtOAC/hexanes mixtures to provide pure compound.

2.4. Electrochemical Studies

Voltammetric experiments were performed with a potentionstat PGSTAT 30, Autolab (ECO CHEMIE Ltd.-The Netherlands) driven with GPES software (Eco Chemie). A conventional three-electrode cell consisting of a gold electrode, a platinum wire as counter electrode and Ag/AgCl (saturated KCl) as a reference electrode were used. For characterization of gold electrode, we have used Ag/AgCl (saturated KCl) as a reference electrode. The gold (Au) electrode was carefully polished with 1.0, 0.3 and 0.05 μm Al_2O_3 slurry, and cleaned by brief ultrasonication. Cyclic voltammetry experiment was performed in 10 mM HEPES buffer, where Ag/AgCl has been used as a reference electrode. For electrochemical studies, a stock solution of 2×10^{-3} M curcumin was prepared in DMSO just before experiment and then these solutions were diluted with buffer to the convenient concentration after mixing with 10 mM HEPES as buffer supporting electrolyte. The 10 mM HEPES buffer solution has been made in 0.1 M KCl solution. The UV–vis absorption spectra of curcumin and its various analogues were collected after 15–20 min subsequent to its solubilization in buffer to estimate degradation of curcumin. Slight degradation of curcumin was noted at 15–20 min subsequent to dissolution. Pyrazole curcumin and its analog were stable at physiological pH and no significant degradation was observed even after 30 min subsequent to dissolution. Our observation is similar to finding of Griesser el.al. [26] and Soumyananda et al. [27]. The data were analyzed using origin 8 software for obtaining the value of current (I) and oxidation potential (V).

2.5. Evaluation of Antioxidant Activity

The antioxidant activities of curcumin analogues were determined in terms of radical scavenging activity using stable radical DPPH. Stock solution of each compound was diluted to variable concentration from 25–150 μM of each compound. 60 μM of freshly prepared methanolic solution DPPH solution (1 mM) was added to each sample solution to make final volume 1 ml. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. In the radical form, DPPH shows a maximum absorbance at 517 nm, but on reduction by an antioxidant, the absorption disappears and the pale yellow non



Scheme 1. Synthesis of Pyrazole derivative of curcumin.