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Original Contribution

Evaluation of a new copper(II)–curcumin complex as superoxide dismutase mimic and its free radical reactions

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Abstract

A mononuclear (1:1) copper complex of curcumin, a phytochemical from turmeric, was synthesized and examined for its superoxide dismutase (SOD) activity. The complex was characterized by elemental analysis, IR, NMR, UV-VIS, EPR, mass spectroscopic methods and TG-DTA, from which it was found that a copper atom is coordinated through the keto-enol group of curcumin along with one acetate group and one water molecule. Cyclic voltammetric studies of the complex showed a reversible Cu^{2+}/Cu^{+} couple with a potential of 0.402 V vs NHE. The Cu(II)–curcumin complex is soluble in lipids and DMSO, and insoluble in water. It scavenges superoxide radicals with a rate constant of $1.97 \times 10^5\ M^{-1}\ s^{-1}$ in DMSO determined by stopped-flow spectrometer. Subsequent to the reaction with superoxide radicals, the complex was found to be regenerated completely, indicating catalytic activity in neutralizing superoxide radicals. Complete regeneration of the complex was observed, even when the stoichiometry of superoxide radicals was 10 times more than that of the complex. This was further confirmed by EPR monitoring of superoxide radicals. The SOD mimicking activity of the complex was determined by xanthine/xanthine oxidase assay, from which it has been found that 5 μg of the complex is equivalent to 1 unit of SOD. The complex inhibits radiation-induced lipid peroxidation and shows radical-scavenging ability. It reacts with DPPH radicals with rate constant 10 times less than that of curcumin. Pulse radiolysis-induced one-electron oxidation of the complex by azide radicals in TX-100 micellar solutions produced strongly absorbing (~500 nm) phenoxyl radicals, indicating that the phenolic moiety of curcumin remained intact on complexation with copper. The results confirm that the new Cu(II)–curcumin complex possesses SOD activity, free radical neutralizing ability, and antioxidant potential. Quantum chemical calculations with density functional theory have been performed to support the experimental observations.

Keywords: Cu(II)–curcumin complex; Curcumin; SOD mimics; Superoxide radicals; Free radicals; Antioxidant; Density-functional theory

Introduction

Superoxide radicals ($O_2^{\bullet -}$, also known as superoxide anion) are produced in the body during aerobic respiration, enzymatic reactions, and drug metabolism [1–4]. The electron transport chain of mitochondria, which assists in transferring four electrons to oxygen to form water, sometimes leaks a single electron to form superoxide radicals [1]. Although a superoxide radical itself is not so reactive to biomolecules, it helps in generation of more powerful

Abbreviations: SOD, superoxide dismutase; DMSO, dimethyl sulfoxide; SCE, standard calomel electrode; NHE, normal hydrogen electrode; EPR, electron paramagnetic resonance; TG-DTA, thermogravimetry and differential thermal analysis; NBT, nitroblue tetrazolium; DPPH, 2,2′-diphenyl-1-picryl hydrazyl; TEAP, tetraethyl ammonium perchlorate; DFT, density-functional theory; ECP, effective core potential; TE, total electronic energy; TD-DFT, time-dependent density-functional theory; SCRF, self-consistent reaction field; PCM, polarized continuum model.

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hydroxyl radicals through the Haber-Weiss reaction [3]. The toxicity of superoxide radicals and its role in deleterious processes in biology are well established. Superoxide dismuatase (SOD) is one of the most important antioxidant enzymes that catalyzes superoxide neutralization by converting it to hydrogen peroxide and oxygen [4,5]. SODs are being explored as useful pharmacological agents [6,7]. The native enzyme, however, has several limitations, such as the short shelf life, low lipid solubility, and low penetration into cells [5]. Metals are known to participate in reversible redox reactions, but free metals can be highly toxic [3]. Several copper and manganese complexes of important compounds have been shown to exhibit the ability to efficiently catalyze superoxide dismutation [8–17]. It has been suggested that metal complexes of antioxidants and other inflammatory drugs can be a better alternative to act as SOD mimics [16].

Curcumin is an important natural phytochemical found in the rhizomes of *Curcuma longa* or turmeric, which has been known for its medicinal properties since ancient times. Curcumin shows remarkable pharmacological activity including anti-inflammatory, anticarcinogenic, and antioxidant activity [18–24] with almost no side effects. It has been found to be nontoxic to humans up to the dose of 10 g/day [18]. It is considered as a potential chemo-preventive agent and the clinical trials in this direction are in different stages [18,23]. It is a diferuloyl methane having two *o*-methoxy phenolic OH groups attached to the α, β-unsaturated β-diketone (heptadiene-dione) moiety, which undergoes keto-enol tautomerism, where the diketone moiety can form metal chelates. Recently there have been many reports in the literature on the metal-chelating properties of curcumin, employing techniques like potentiometry and absorption spectroscopy [24–30]. 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 [24,31] 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 [24]. There also appears to be some correlation between drugs for Alzhiemer's disease and the metal complexes acting as SOD mimics [24,31]. With this background it is felt that the metal-chelating ability of curcumin can be of use in developing new SOD mimics.

In this paper a new mononuclear copper complex of curcumin was synthesized, purified, and characterized by UV-VIS, IR, mass, and EPR spectroscopic methods. Supportive evidence for this has been obtained from TG-DTA analysis. The redox potential of the complex has been evaluated by cyclic voltammetry. The superoxide radical reactions of the complex were determined by a stopped-flow spectrometer and the SOD equivalents of the complex were estimated by comparing its ability to inhibit xanthine and xanthine oxidase activity. The ability of the complex to scavenge free radicals and in vitro antioxidant activity of the complex were also examined in model systems.

Materials and methods

Experimental part

Curcumin, potassium superoxide (KO_2), dicyclohexano-18-crown-6-ether, xanthine, xanthine oxidase, nitroblue tetrazolium dichloride (NBT^{2+}), superoxide dismutase, DPPH, and TX-100 were purchased from Sigma/Aldrich (USA). Spectrograde dimethyl sulfoxide (DMSO) from Spectro Chem. India, Mumbai, was used as received. Superoxide–crown ether complex was prepared according to the procedure given in Refs. [17,21]. KO_2 was weighed in a previously weighed sealed tube and was quickly added to equivalent moles of 18-crown-6-ether in DMSO solvent. It was stirred under sealed conditions for 30 min. In order to minimize the effects of light on these solutions, all the experiments were carried out in the dark. The concentration of superoxide was determined by using the extinction coefficient of 2690 M^{-1} cm^{-1} at 250 nm [32]. Absorption studies were carried out using JASCO V–530 spectrophotometer.

Stopped-flow studies were carried out using a SX-18 MV stopped-flow spectrometer from Applied Photophysics, UK, in the single mixing mode. Here the two mixing syringes contain DMSO solutions of superoxide–crown ether complex and Cu(II)–curcumin complex separately and the two solutions were mixed in the stopped-flow cell. The absorption changes either at 420 nm or at 560 nm after the mixing were monitored as a function of time. The dead time of the instrument is 1.3 ms. Similarly DPPH in DMSO was mixed with the complex in the stopped-flow cell and the absorbance change at 517 nm [33,34] was monitored as a function of time, in the presence of different concentrations of the complex.

EPR spectra were recorded on Bruker ESP 300 spectrometer operated at X-band frequency (9–10 GHz) using 100-kHz field modulation. DPPH sample was used as a field marker. The spectra were recorded at 77 K using a liquid nitrogen Dewar insert. The *g* values of the Cu(II)–curcumin complex were determined relative to DPPH as standard having a *g* value of 2.0036.

^{1}H NMR spectra were recorded with a Bruker 200 MHz spectrometer. Spectra were referenced to residual DMSO. IR spectra were recorded with a Nicolet Impact 410 spectrometer in KBr discs (peaks are reported in cm^{-1}).

Elemental analysis was carried out by using C, H, and N analyzer and copper was estimated by an atomic absorption spectrometer.

The mass spectrum of the complex was recorded using indigenously developed matrix-assisted laser desorption and ionization (MALDI) time of flight mass spectrometer using sinnapinic acid matrix [35].