Journal of Inorganic Biochemistry 132 (2014) 52–58

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Improved separation of the curcuminoids, syntheses of their rare earth complexes, and studies of potential antiosteoporotic activity

Yasmin Mawani, Chris Orvig *

Medicinal Inorganic Chemistry Group, Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, British Columbia V6T 1Z1, Canada

ARTICLE INFO

Article history:
Received 23 April 2013
Received in revised form 9 December 2013
Accepted 13 December 2013
Available online 21 December 2013

Keywords:
Rare earths
Lanthanides
Curcumin
Toxicity
Antiosteoporotic agents

ABSTRACT

The first reported homogenous rare earth curcumin (H**Curc**; ((1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione)) complexes with the formula M**L**$_3$, where M^{3+} is Eu(III), Gd(III) or Lu(III), were synthesized and characterized by mass spectrometry, infrared spectroscopy and, in the case of the lutetium complex, ^{1}H NMR spectroscopy. Most importantly an improved separation of the three curcuminoids, H**Curc**, H**DMC** ((1*E*,6*E*)-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione) and H**BDMC** ((1*E*,6*E*)-1,7-bis(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione) was realized using a combination of normal-phase column and phosphate-impregnated preparative-thin layer chromatographies. The toxicities of the metal curcumin complexes and ligands were investigated in MG-63 cells, an osteoblast-like cell line, for potential activity as antiosteoporotic agents.

1. Introduction

Turmeric, derived from the rhizome of the *Curcuma longa* plant, is a vibrant yellow spice that is used not only to flavor mustards and curries, but also for its medicinal properties in the eastern medicinal practice of Ayurveda [1]. The bright color is due to the curcuminoids, the active isolate, which make up 2–9% of turmeric powder [2,3]. The major curcuminoids found in turmeric are curcumin (H**Curc**, 77%), demethoxycurcumin (H**DMC**, 17%) and bisdemethoxycurcumin (H**BDMC**, 3%), shown in Fig. 1 [2,3].

Curcumin was first isolated by Vogel in 1842, characterized by Lampe and co-workers in 1910 and first synthesized by Lampe and Milobedeska in 1913 [1]. Curcumin has a surprising number of medicinal properties including anti-inflammatory, antioxidant, anticoagulant, anti-fertility, antifungal, antiviral and chemotherapeutic activities, in addition to many other effects [1,3,4]. Many of these properties stem from the ability of curcumin to act as a free-radical scavenger and hydrogen donor. Curcumin has been shown to be a powerful natural chelating agent, due to the β-diketone moiety. As such, it has been coordinated to a variety of metal ions, particularly those of iron and copper, and as a result has been suggested for the treatment of Alzheimer's disease [3,5]. Previous work in our group has demonstrated that curcumin can be coordinated to vanadyl (VO^{2+}) [6], gallium (Ga^{3+}) and indium (In^{3+}) [7] by the diketone functionality, forming neutral *bis*- and *tris*-ligand complexes with the respective metal ions.

Due to the abundance of metabolic iron disorders, and the fact that only slight disturbances in iron uptake and loss can lead to deficiency and overload [8], Fe^{3+}-curcumin binding has been studied. Saladini and co-workers [9] have determined the stability constants for Fe^{3+} coordinated to curcumin using potentiometric techniques, while Bernabé-Pineda et al. [10] studied the formation of H**Curc** complexes with both Fe^{3+} and Fe^{2+} ions, spectrophotometrically and by cyclic voltammetry. Beck and co-workers reported curcumin complexes of Fe^{3+} possessing the formula Fe(**Curc**)$_3$ [11]. Syntheses of complexes of Cu^{2+} with one or two equivalents of H**Curc** have also been reported [5,11,12]. Beck and co-workers [11] have also successfully synthesized curcumin complexes of Pd(**Curc**)$_2$, and mixed ligand and curcumin complexes of Pd, Rh, Ir, Pt, Co and Ru.

While there are many reports of the curcuminoids bound to transition metals, there are only three examples where this ligand set has been coordinated to members of the rare earth series, all of which involve heterogenous complexes. Seltzer et al. [13] reported the first example of curcumin coordinated to a lanthanide; they synthesized, and studied the near-infrared fluorescent properties of, the Nd^{3+} and Yb^{3+} curcumin complexes coordinated to three H**Curc** ligands and one 1,10-phenanthroline-5,6-dione, yielding neutrally charged complexes. Song et al. [14] synthesized the Eu^{3+}, Sm^{3+} and Dy^{3+} analogs and studied their antibacterial properties. More recently Hassain et al. synthesized heterogenous curcumin terpyridyl Ln(III) complexes and studied them for their potential photocytoxicity [15].

The lanthanide series is of particular interest in our group due to their potential use in the treatment of bone density disorders such as osteoporosis [16,17]. For decades it has been known that lanthanide ions have a high affinity for bone [18]. Lanthanides have been shown to inhibit formation of osteoclasts [19] (the cells responsible for bone resorption) and to have a proliferative effect on osteoblasts [20] (the cells responsible for bone formation).

* Corresponding author. Fax: +1 604 822 2847.
E-mail address: orvig@chem.ubc.ca (C. Orvig).

0162-0134/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jinorgbio.2013.12.004

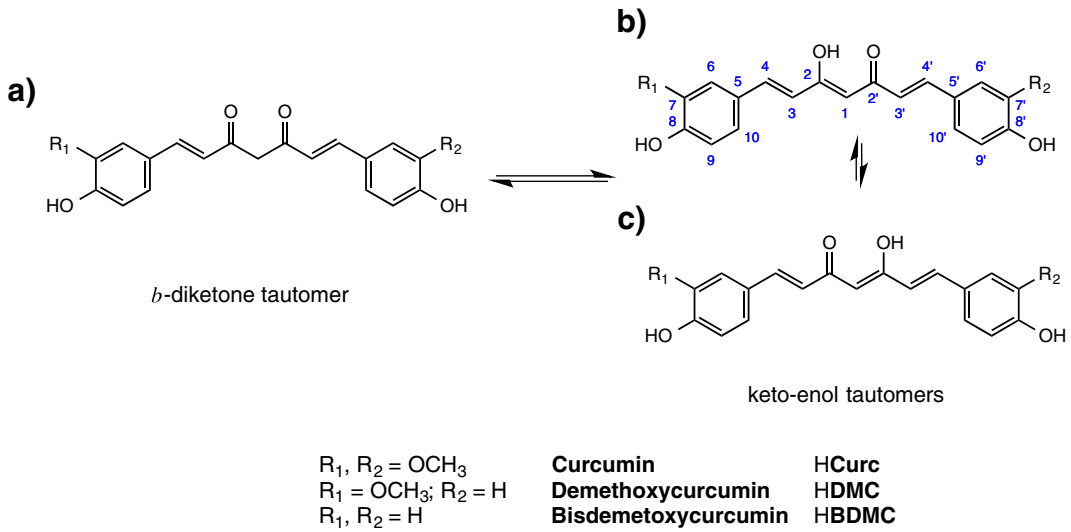

Fig. 1. The three main curcuminoids isolated from turmeric: curcumin ((1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione), demethoxycurcumin ((1*E*,6*E*)-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione), bisdemethoxycurcumin ((1*E*,6*E*)-1,7-bis(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione) are shown along with the numbering system used for ^{1}H NMR characterization. The β-diketone (a) and keto–enol tautomers (b and c) are all shown.

Amongst the many known and prospective applications of the curcuminoids in medicine, one can find indications that curcumin may be effective as a bone density disorder treatment [21–25]. Curcumin is a potent inhibitor of the transcription factors NF-κB (nuclear factor-κB) and activator protein-1 (AP-1), both of which have been implicated in the survival of osteoclasts [21–24]. The NF-κB inhibitors pyrrolidine dithiocarbamate, *N*-tosyl-L-phenylalanine chloromethyl ketone and gliotoxin have been shown to stimulate the apoptosis of rabbit osteoclasts [21]. Ozaki et al. [22] used mature rabbit osteoclasts to show that curcumin stimulates cell apoptosis in osteoclasts. Curcumin was also shown, *in vitro*, to inhibit dramatically osteoclastic bone resorption [22]. While the precise mechanism has not been elucidated, it is believed [22] that curcumin inhibits AP-1 and NF-κB transcription. In the presence of monocytes (RAW 264.7 cells), RANKL (receptor activator of NF-κB) induces NF-κB activation, leading to osteoclastogenesis, however this process is not well understood [22]. Bharti et al. [24] showed that pre-exposure to curcumin completely suppressed RANKL-induced NF-κB activation. Lastly, French and co-workers [25] demonstrated, using ovariectomized rats (OVX) as a model for postmenopausal osteoporosis, that curcumin increases both bone turnover and bone strength; curcumin administration at a dose of 15 mg/d over a period of 6 months led to an increase in femur size of the OVX rats.

As the curcuminoids possess the β-diketone functionality, and can be coordinated to the trivalent metal ions, aluminum, gallium and indium [7], as well as lanthanide ions [13,14], it may be possible to form a bifunctional anti-osteoporotic agent by coordinating a lanthanide ion with curcumin. Herein, an improved separation of the three curcuminoids, and the syntheses of Ln(**Curc**)$_3$ (Ln = Eu^{3+}, Gd^{3+}, and Lu^{3+}) are described, along with preliminary relevant toxicity studies in MG-63 cells.

2. Experimental

2.1. Materials

All solvents were HPLC grade and purchased from Fisher Scientific. Water was purified using an Elgastat Maxima HPLC reverse osmosis and deionization system or a PureLab Ultra system (Elga, Bucks, England). All water used was type 1, 18.2 MΩ cm, purified by full spectrum UV to control bacterial levels. Curcumin was purchased as a mixture (~70% H**Curc**) from Sigma-Aldrich, and purified into its three main components by chromatography as described below. Lanthanum nitrate, europium nitrate, gadolinium nitrate, lutetium nitrate and gallium nitrate were purchased from Sigma-Aldrich and Alfa Aesar as their hexahydrates and used without further purification. Vanadyl acetylacetonate was purchased from Alfa Aesar and used without further purification. Analytical thin layer chromatography (TLC) plates (which were alumina backed ultra pure silica gel 60 Å, 250 μm) and flash column silica gel (standard grade, 60 Å, 32–63 mm) were purchased from Silicycle.

For cell studies, MG-63 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD and Manassas, VA, U.S.A.). Media for the cells, minimum essential medium alpha (MEM-α), fetal bovine serum (FBS), 0.25% trypsin-EDTA, Penicillin–Streptomycin–Neomycin 100× (Pen–Strep) and phosphate buffered saline solution (PBS) were purchased from Life Technologies (Burlington, Ontario, Canada). T-75 culture flasks and 96-well plates were purchased from Corning-Costar (Cambridge, MA, U.S.A.). Sterile 15 mL and 50 mL centrifuge tubes were purchased from Fisher Scientific. Barrier pipette tips were purchased from Diamed (Mississauga, Ontario, Canada). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Alfa Aesar and *cis*-diamminedichloroplatinum(II) (cisplatin) was obtained from Acros Organics.

2.2. Instrumentation

^{1}H NMR spectra were recorded at room temperature using a Bruker AV-300 or AV-400 spectrometer. Low-resolution electrospray ionization mass spectra (ESI-MS) were obtained on a Bruker Esquire Ion Trap ESI-MS spectrometer. Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectra were obtained on a Bruker Biflex IV instrument. Fourier transform infrared (FTIR) spectra were obtained on a Nicolet 6700 FTIR equipped with a Smart Orbit diamond attenuated total reflectance attachment. A Labnet Orbit P4 Digital Shaker with platform was used to shake the plates for the MTT assay. A Beckman Coulter DTX 800/880 Series plate reader was used with a filter for 570 nm to read the absorbance values of the plates.

2.3. Separation of the curcuminoids

Curcumin was purchased as a mixture of H**Curc**, H**DMC** and H**BDMC**. These three components were isolated from one another, as follows. A