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The characterisation of structural and antioxidant properties of isoflavone metal chelates

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ABSTRACT

Isoflavone metal chelates are of interest as isoflavones act as oestrogen mimics. Metal interactions may enhance isoflavones biological properties so understanding isoflavone metal chelation is important for the commercial application of isoflavones. This work aimed to determine if isoflavones, daidzein (4',7-dihydroxyisoflavone) and genistein (4',5,7-trihydroxyisoflavone) could chelate with metals as isoflavone chelates. Biochanin A (4'-methoxy-5,7-dihydroxyisoflavone) was also examined for it's ability to chelate with Cu(II) and Fe(III). This study found daidzein does not chelate with Cu(II) and Fe(III) but genistein and biochanin A chelate with a 1:2 M/L stoichiometry. The copper and iron chelates were synthesised and characterised by elemental analysis, FTIR, thermogravimetric analysis (TGA) and electrospray ionisation mass spectrometry (ESI-MS). These studies indicated a 1:2 M/L stoichiometry and suggested the isoflavones bind with the metals at the 4-keto and the 5-OH site. 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition assays showed that copper isoflavone chelates have higher antioxidant activity than free isoflavones while the iron isoflavone chelates showed pro-oxidant activity compared to the free isoflavone. Synergistic DPPH studies with 0.02 mM ascorbic acid revealed copper chelates exhibit reduced antioxidant activity versus free isoflavones whereas the iron chelates showed lower pro-oxidant activity except at 1.0 mM.

1. Introduction

Flavonoids are weak polybasic acids that are polyphenolic in nature and have a number of hydroxyl groups that can be subjected to protonation or deprotonation depending on their pKa. Flavonoids have the ability to help mediate ailments such as iron induced lipid peroxidation [1] and some types can act as anti-cancer agents [2]. Flavonoids are reported to behave as antioxidants primarily because of their polyphenolic nature and are reported that the greater the number of hydroxyl groups present on the flavonoid structure, the greater the antioxidant capacity of the flavonoid [3].

Isoflavones are a subclass of flavonoids, an isomerised form of flavones, with key structural features such as a 4-keto group and a C—C bridge between the 1′ carbon on the B ring and the 3 carbon on the C ring (Fig. 1) [4]. They are normally found in foods that are leguminous in nature such as soya products or nuts [5]. Certain isoflavones, such as genistein, can act as tyrosine kinase inhibitors making them potential agents for the treatment of hormonally related cancers such as breast or prostate cancer. This is due to the chromen-4-one component present in the isoflavone structure (Fig. 1) [2]. Isoflavones also show antioxidant qualities but have weaker antioxidant capacity relative to flavonoids like quercetin or kaempferol which possess more hydroxyl groups [3]. The knowledge of how isoflavones interact with metals is of interest as Cu(II) and Fe(III) are common metal species present in the human body and knowledge of these interactions can give information as whether these metal interactions will lead to health benefits such as phytosoya supplements [6,7]. This could be useful for using isoflavones as natural chelating agents [8] such as iron induced lipid peroxidation [9] in the human body.

Flavonoids have the ability to chelate with a variety of metals such as Cu(II), Fe(III) and Al(III). Their chelation depends on the presence of certain chelation sites in the structure of the flavonoid as well as the solvent type and pH conditions [10]. In the case of the isoflavones, the most likely metal chelation site would be from the 4-keto and 5-hydroxy groups [11]. Successful chelation has been found with transition metal species such as Cu(II) and Ni(II) to biochanin A (4′-methoxy-5,7-dihydroxy-isoflavone) by Chen et al. [12]. Chelation of isoflavones, daidzein and genistein, with Cu(II) and Fe(III) were attempted by Mira et al. but revealed no chelation in their spectrophotometric chelation studies [10]. Other studies have been found to be inconclusive as regards isoflavone metal chelation often looking at indirect factors such as cadmium excretion levels being reduced by daidzein [13] or inhibition of metal ions with genistein on α-glucosidase [14]. The research presented here broadens the knowledge of other isoflavone ligand species that can chelate with transition metal species and represents the first direct evidence for

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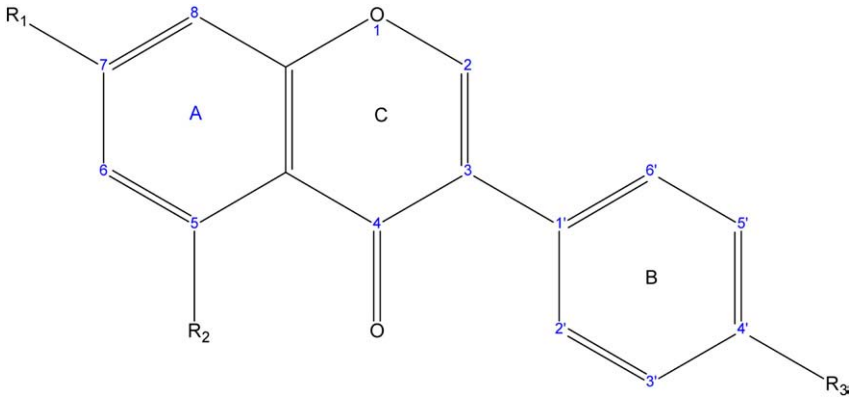

Fig. 1. Basic isoflavone structure for genistein ($R_1 = OH$, $R_2 = OH$ and $R_3 = OH$), biochanin A ($R_1 = OH$, $R_2 = OH$ and $R_3 = OCH_3$) and daidzein ($R_1 = OH$, $R_2 = H$ and $R_3 = OH$).

isoflavone metal chelation between Cu(II) with genistein and Fe(III) with biochanin A and genistein. Chelation, stoichiometry studies, synthesis and characterisation of these chelates was carried out using FTIR, TGA, elemental analysis and ESI-MS.

Isoflavone metal chelates are of scientific interest as flavonoids have been shown, in previous studies of flavonoid metal chelates, to have exhibited modulation of biological activity such as enhancing anti-viral, anti-cancer and, of interest to this group, antioxidant activity [15]. Some interactions with metal ions showed increases in antioxidant activity while others showed a prooxidant effect on flavonoids [16,17]. The antioxidant properties of flavonoid metal chelates have been characterised using techniques such as a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay [18,19]. Using this antioxidant technique, it is possible to characterise antioxidant capacity and assess the modulating effect a metal has on a flavonoids' antioxidant activity i.e. whether it is prooxidatively or antioxidatively modulated when chelated with a metal. Additionally, synergistic studies can be performed with established antioxidants such as ascorbic acid and quercetin to see what effect these antioxidants have on the antioxidant capacity of free flavonoids and flavonoid metal chelates [18]. These types of synergistic studies were performed with isoflavone metal chelates and ascorbic acid and are reported in this paper. To the authors' knowledge, such studies have not been reported in the literature before for isoflavone metal chelates.

2. Materials and methods

2.1. Chemical and reagents

Genistein was obtained from LC LABS (Woburn, MA, USA). DPPH, daidzein and biochanin A were from Sigma-Aldrich Ireland (Dublin, Ireland). $[Fe(NO_3)_3]\cdot 9H_2O$, and $[Cu(NO_3)_2]\cdot 3H_2O$ metal salts were all of analytical grade and were purchased from Lennox Laboratory Supplies Ltd. (Dublin, Ireland). Methanol, hydrochloric acid and sodium hydroxide were of analytical grade and also obtained from Lennox Laboratory Supplies (Dublin, Ireland). All chemicals were used without further purification.

2.2. Chelation studies

A 50 μM quantity of each isoflavone was mixed with 50 μM quantities of Cu(II) and Fe(III) metals from metal nitrate salts all made up to 10 mL with methanol. All solutions were made up in triplicate. UV/Vis spectra were collected on a Varian Cary 50 UV/Vis spectrophotometer in the range 200–700 nm at a scan speed of 600 nm/min.

2.3. Stoichiometry studies

Stoichiometry studies used a combination of the mole ratio and Job plot techniques for confirmation of M/L ratio values. Mole ratio studies were performed with 50 μM isoflavone versus 6.25 to 200 μM Cu(II) and Fe(III). The Job plot used 100 μM isoflavone versus 100 μM metal solutions and varied from 1:9 to 9:1 metal volume to ligand volume ratios. UV/Vis spectra were performed on a Varian Cary 50 UV/Vis spectrophotometer from 200 to 700 nm at a scan speed of 600 nm/min. Spectra for the copper chelates were monitored at 376 nm and spectra for the iron chelates were monitored at 550 nm.

2.4. Synthesis of isoflavone metal chelates

Synthesis of the isoflavone metal chelates is based on a method modified from Bukhari et al. [20]. Stoichiometric amounts of isoflavone and metal nitrate salt were mixed together i.e. 1:2 M/L for Fe(III) isoflavone complex and 1:2 M/L for Cu(II) isoflavone complex. The isoflavone was dissolved in 10 mL of methanol and stirred for 15 min, followed by addition of the metal salt dissolved in another 10 mL of methanol. Additional stirring of the complex solution was done for 90 min. The sample was rotary evaporated to dryness, vacuum filtered on Millipore 47 mm media pads and washed three times with t-BuOH. The samples were vacuum dessicated for 6 h, foil wrapped and stored at −20 °C until needed.

2.5. FTIR analysis

The samples were ground in a 1:20 mix with KBr. The sample/KBr mix was compressed at a pressure of 5 tonnes for 4 min on a KBr press using 13 mm die. FTIR spectra were collected in the range of 4000 to 400 cm^{-1}, a resolution of 2 cm^{-1} and 40 scans per spectrum. Background correction was carried out. The FTIR was a Varian 660-IR Mid-IR with a DTGS detector.

2.6. TGA analysis

Approximately 5 mg of sample was analysed on an aluminium pan. The TGA was heated from 30 to 600 °C at a temperature ramp of 5 °C/min. The TA Instruments Q-50 series TGA was tared with the aluminium pan on the platinum pan without sample.

2.7. ESI-MS analysis

ESI-MS characterisation was carried out on a Bruker Daltonics electrospray mass spectrometer in positive ion mode for all samples. A 400–800 M/Z range was used for the copper chelates, 500–900 M/Z for the iron chelates and 200–500 M/Z range for the isoflavones. Samples were dissolved in MS grade methanol. Samples were analysed in Dublin City University, Dublin, Ireland.