UREA AND N-SUBSTITUTED UREA DERIVATIVES COMPOUNDS OF IRON (II) ARE SUITABLE INTERMEDIATES IN THE SYNTHESIS OF COMPLEXES MIMIKING THE NONHEME IRON-CONTAINING ENZYMES

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abstract: Four complexes of iron (II) having as ligands only urea (or N-substituted urea derivatives) and easily replaceable water molecules, having low molecular weight, an electrolytic nature and a reasonable stability in aqueous medium, were synthesised in order to be used as intermediates in the aqueous synthesis of other iron (II) compounds, with mixed ligands, mimicking anti-oxidant nonheme, iron-containing enzymes. The compounds were characterised by analytical procedures, measurement of some physical constants, IR and electronic spectroscopy and stability studies.

Introduction

A large class of nonheme iron-containing enzymes is essential for the biosynthesis of a diverse array of compounds, catabolism of selected bio-molecules, repair of alkylated DNA and RNA and oxygen sensing in cells [1-7].

In the past decade, protein structures of such several mononuclear non-heme iron(II) enzymes were solved, such as isopenicillin N synthase (IPNS), deacetoxycephalosporin C synthase (DAOCS), clavaminic acid synthase (CAS), proline 3-hydroxylase (P-3-H), taurine dioxygenase (TauD), and anthocyanidin synthase (ANS). This increased structural information caused a growing interest in these enzymes and several mimicking complexes have been investigated [8-14].

The high molecular mass of the large part of the natural antioxidants induces some difficulties in their pharmacological formulation and administration; consequently, the synthesis of low-molecular weight complexes mimicking the natural antioxidants is an area of intense current interest.

Urea, the most common nitrogen-containing final product of proteins catabolism, inhibits the oxidation of iron (II) and this way it may protect brain and liver tissues against lipid peroxidation; a recent study evaluated in vitro and ex vivo the potencies of urea and several urea derivatives as scavengers of oxygen reactive species, largely responsible for cardiac dysfunctions [15].

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In designing mimicking systems of the natural anti-oxidants, the compounds of iron (II) with urea (or urea derivatives) may represent potential antioxidants by themselves or intermediates in the synthesis of compounds with mixed ligands. We report here the synthesis of a series of such compounds of iron (II), having as ligands urea or N-substituted urea derivatives. Similar compounds of other metal centres with biological relevance, copper (II) and manganese (II), were reported previously [16, 17].

In this step, we were mainly interested to obtain pure, low-molecular weight, water-soluble compounds of iron (II) with urea (U) and some urea derivatives, respectively methyl-urea (MeU), ethyl-urea (EtU) and 1,3 dimethyl-urea (Me₂U), reasonably stable in order to allow their use as intermediates in the synthesis of some complexes with mixed ligands, mimicking nonheme iron-containing enzymes.

**Experimental**

**Apparatus and methods**

Chemical analysis of metal was performed using volumetric methods; sulphate was determined gravimetrically, nitrogen by combustion and water by thermal analysis.

The *melting points* were measured using a SMP-3 apparatus. *IR spectra* were recorded with a BIO RAD FTIR 135 spectrometer, using the potassium bromide technique, in the range 4000 – 400 cm⁻¹. *Electronic spectra* by diffuse reflectance technique, with MgO standard, were recorded in the range 380 - 1100 nm, on a VŠU2-P-Zeiss Jena spectrometer; absorption spectra were recorded in the range 200 - 1100 nm, on a Jasco V 530 spectrometer, in aqueous solutions, using aqueous NaCl solutions 0,9%. The molar conductivities were measured with a CONSORT C533 apparatus. The heating curves (TG, T, ADT and DTG) were recorded in a MOM (Budapest) derivatograph, type Paulik-Paulik-Erdely, in a static air atmosphere with a sample weight of 40-45 mg over the temperature range 20-1000°C, using various heating rates (2.5 – 10 k/min).

**Syntheses and physical constants**

All reagents were of commercial analytical quality and were used without further purification.

The urea compound was prepared by adding drop wise, on ice, a cold isopropyl alcohol solution of Fe(NH₄)₂(SO₄)₂·6H₂O to a concentrated aqueous urea solution (in 4:1molar ratio urea : Fe(II) and 50:1 volumes ratio isopropyl alcohol : H₂O). The mixture was allowed to stir on ice during 30 min. The (pale orange) water-soluble solid compound was washed with isopropyl alcohol and dried. The urea-derivatives compounds (pale orange or pale yellow) were prepared similarly, using appropriate alcoholic solvents for the ligands. The compositions that the elemental analyses indicate and some physical constants are:

\[ \text{Fe(U)₂SO₄(H₂O)₂} \]  found: Fe: 17,42 % N: 17,92%, SO₄²⁻: 32,05 %; requires for FeC₂O₅N₂SH₂: Fe: 18,12 % N: 18,18%, SO₄²⁻: 31,17 %; yield 76%, m.p. 426K, \( \Lambda_m \) (molar conductivity in \( 10^{-3} \text{M} \) aqueous solution): 120 Ω⁻¹ cm² mol⁻¹
Results and Discussions

The coordination modes of urea and urea derivatives in the synthesised iron (II) compounds were put into evidence by the IR spectra, interpreted by comparison with those of the corresponding ligands [18-20]. In sum, the IR spectra reveal two main coordination modes of these species: as bidentate ligands, both by nitrogen and oxygen atoms, forming by coordination four membered rings, or as unidentate ligands, through the oxygen atoms of the CO group. The presence of alkyl radicals in the N-substituted urea derivatives is supposed to increase the electronic density on the oxygen atom, favouring the function of these derivatives as unidentate ligands.

The recorded IR spectra reveal that in the compounds FeU$_2$(H$_2$O)$_2$SO$_4$ and Fe(MeU)$_2$(H$_2$O)$_4$SO$_4$, urea and methyl-urea act as bidentate ligands. By difference, in Fe(EtU)$_2$(H$_2$O)$_4$SO$_4$ and Fe(Me$_2$U)$_2$(H$_2$O)$_4$SO$_4$, ethyl-urea and 1, 3 dimethyl-urea act as unidentate ligands.

The spectra presented in Fig. 1 reveal these two coordination modes, for FeU$_2$(H$_2$O)$_2$SO$_4$ and Fe(Me$_2$U)$_2$(H$_2$O)$_4$SO$_4$, by comparison with the corresponding ligands. As Fig. 1 reveals, in the interval 3200 – 3500 cm$^{-1}$, characteristic for the N-H stretching vibration modes, the spectrum of the compound FeU$_2$(H$_2$O)$_2$SO$_4$, displays a significant multiplicity of the absorption bands (3461, 3381, 3358, 3344, 3300, 3277, 3257, 3214 cm$^{-1}$) by comparison with the spectrum of urea (3423, 3347, 3259 cm$^{-1}$). This indicates that only one of the two nitrogen atoms of the urea molecule participates at the coordination at the metallic centre. In the same interval, the spectrum of the compound with dimethyl-urea is practically similar to those of the ligand. The large bands recorded in this interval allow the supposition that water molecules are also present in the composition of the compounds.

Around 1600, both categories of complexes show significant differences by comparison to the corresponding ligands, as consequence of the implication of the oxygen atoms from the CO groups in coordination. The formation of the Fe-O bonds tends to decrease the double character of the C-O bond. For example, the corresponding absorption maxims are at 1615 cm$^{-1}$ for FeU$_2$(H$_2$O)$_2$SO$_4$, by comparison with 1667 cm$^{-1}$ in free urea, at 1667 cm$^{-1}$ for Fe(MeU)$_2$(H$_2$O)$_4$SO$_4$, by comparison with 1686 cm$^{-1}$ for methyl-urea, at 1651 cm$^{-1}$ for Fe(EtU)$_2$(H$_2$O)$_4$SO$_4$, by comparison with 1664 cm$^{-1}$ for ethyl-urea and at 1647 cm$^{-1}$ for Fe(Me$_2$U)$_2$(H$_2$O)$_4$SO$_4$, versus 1679 for dimethyl-urea.
The formation of the Fe-O bonds also produces different separations of the bands associated to the C-O and C-N stretching vibrations.

The spectra of all the compounds reveal the presence of the uncoordinated sulphate ions, by the intense bands at about 1100 cm\(^{-1}\) and the weaker bands around 625 cm\(^{-1}\).

The geometry of the Fe(II) centres environment in the solid state of the compounds, suggested by the diffuse reflectance electronic spectra, is a distorted octahedral one, as indicated by the maximums at around 12200 and 680 cm\(^{-1}\) that the spectra of all the compounds display; the spectra also display shoulders at around 19600 cm\(^{-1}\), attributable to charge transfer transitions [21].

Correlation of analytical and thermal analyses data with IR and diffuse reflectance electronic spectra suggests the implication of water molecules in the coordination.

The values of the molar conductivities in aqueous solutions, indicating an electrolytic nature of the compounds, also suggest that only urea (or urea derivatives) and water molecules assure the hexa-coordination of the metallic centres.

The absorption electronic spectra, recorded in 10\(^{-2}\) M aqueous solutions, in the range 200-1100 nm indicate the conservation of the metallic centres surrounding.

Stability studies were performed by absorption spectroscopy in aqueous solutions, in physiological-like conditions. The stability at pH 7.2 was studied using aqueous NaCl solutions 0.9%, the absorption spectra of the same sample being recorded immediately after dissolution (t =0), after 10, 20, 30, 40, 50, 60, 90, 120, 150 minutes.

The position of the absorption maxims and the values of the molar absorbance coefficient indicate that all the synthesised compounds have satisfactory stability in time. For
FeU$_2$(H$_2$O)$_4$SO$_4$ and Fe(EtU)$_2$(H$_2$O)$_4$SO$_4$ the absorption spectra remain practically unchanged in the studied time-interval; extremely slight modifications appear in the spectra of Fe(MeU)$_2$(H$_2$O)$_4$SO$_4$ and Fe(Me$_2$U)$_2$(H$_2$O)$_4$SO$_4$.

Fig. 2 illustrates a significant area of the absorption spectra of Fe(MeU)$_2$(H$_2$O)$_4$SO$_4$.

**Fig. 2:** Absorption spectra of aqueous solution of Fe(MeU)$_2$(H$_2$O)$_4$SO$_4$ at pH=7.2, in time.

**Conclusions**

The four complexes of iron (II) reported here may be characterised by the followings: they have as ligands only urea and N-substituted urea derivatives (with proved anti-oxidant activity by themselves) and easily replaceable water molecules; they have low molecular weight, an electrolytic nature and a reasonable stability in aqueous medium. For all these reasons, they are suitable intermediates in the aqueous synthesis of other iron (II) compounds, with mixed ligands, mimicking nonheme, anti-oxidant iron-containing enzymes.

**REFERENCES**