Spectroscopic Studies of Metal Complexes Containing π-Delocalized Sulfur Ligands. Mössbauer and Kinetic Studies of Iron(II) and Iron(III) Complexes of the Antitumor Agent 2-Formylpyridine Thiosemicarbazone

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Abstract

Mössbauer studies on the Fe(II) and Fe(III) complexes of 2-formylpyridine thiosemicarbazone (FPT) showed the complexes to be low spin and provided evidence of the reducing character of the ligand. In addition, kinetic measurements of the Fe(III) complex reduction by the ligand gave an expression for the initial rate of the type $v_i = k'A + k''AB$, in which A stands for the concentration of the Fe(III) complex and B for the concentration of the reducing agent. From this equation one may infer the coexistence of two mechanisms, one expressed by the first term and occurring even in the absence of FPT. For the second term we propose an inner sphere mechanism in which the ligand is the reducing agent.

Introduction

The α(n)-heterocyclic carboxaldehyde thiosemicarbazones form a class of synthetic compounds possessing antitumor activity. As well established on the basis of experimental findings, these drugs inhibit DNA synthesis through the blockage of the enzyme ribonucleoside diphosphate reductase (RDR), which catalyses the conversion of ribonucleosides into desoxyribonucleosides [1].

French and Blanz have shown that the conjugate N^N^S tridentate system, as well as the heterocyclic nitrogen, is necessary for antineoplastic activity [2]. The 2-formylpyridine thiosemicarbazone, hereafter labelled FPT, is the simplest compound of this series, for which marked inhibiting activity of RDR has been observed [2].

The tridentate ligand FPT is able to form tetragonal 1:1 complexes with Cu(II) and Zn(II), and octahedral 2:1 complexes with Fe(II), Fe(III) and Ni(II). In the former case the fourth position is occupied by another ligand, an oxygen of a water molecule for instance. In the latter, two ligands coordinate to the metal in orthogonal planes, as shown in Fig. 1 [3].

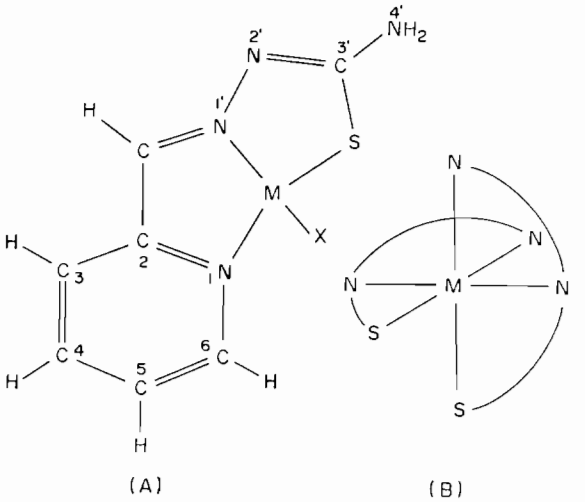

Fig. 1. General structure of (A) square planar and (B) octahedral complexes of 2-formylpyridine thiosemicarbazone.

Several investigations support the conclusion that the active form of the RDR inhibitor is its iron complex [1, 4, 5]. The target of the drug is the stable free radical present in the enzyme structure. However, the mechanism of action is not completely understood.

As reported previously, in aqueous solution, the Fe(II) complex exists in two forms: protonated and deprotonated [6]. Using resonance Raman spectroscopy we were able to show that up to pH 6.0 the protonated one, hereafter labelled $[Fe(II)(HFPT)_2]^{2+}$, contains thiono sulfur, the proton being located at the N(2′) level. The other form, hereafter

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labelled $[Fe(II)(FPT)_2]$, is present at higher pH and contains thiolate sulfur [7].

We have also undertaken a Mössbauer investigation on the Fe(II) and Fe(III) complexes in the solid state, as well as in aqueous and ethanol frozen solutions, the results of which were the subject of a previous communication [8]. In the course of this Mössbauer study, the reducing character of the ligand upon the metal was noticeable. As a consequence, a more complete investigation was undertaken, for the purpose of gaining further understanding of the process, particular emphasis being placed on kinetic studies.

Experimental

2-Formylpyridine thiosemicarbazone (FPT) was obtained following the method of Anderson *et al.* [9]. The Fe(II) complex was prepared by the method of Ablov and Belichuk [6].

The Fe(III) complex was prepared by adding 106 mg (0.6 mmol) of FPT to a hot alcoholic solution of iron chloride (65 mg of $FeCl_3 \cdot 6H_2O$ in 30 ml of ethanol) under oxygen flow. After cooling, the brown precipitate was washed with ethanol and ether (yield 59%). The complex was analysed for carbon and sulfur contents. *Anal.* Found: C, 39.0; S, 14.6. Calc. for $C_{14}H_{16}N_8S_2Fe$: C, 40.4; S, 15.4%.

Chemicals of the best reagent grade and demineralized distilled water, previously freed of organic contaminants, were used throughout.

Absorption measurements at 62 °C were made on a Cary 17D spectrometer. Mössbauer spectra were obtained using a conventional constant acceleration spectrometer, moving a Co–Rh source at room temperature. All spectra were computer-fitted assuming Lorentzian line shapes.

Results and Discussion

Mössbauer Studies

Mössbauer measurements were performed at 298 and 85 K for all complexes in the solid state, as well as in aqueous and ethanol frozen solutions. In this section, only a brief discussion on the main results in the solid state is presented. More details can be found in our previous report [8].

The values of the isomer shift (δ) relative to metallic iron and those of the quadrupole splitting (Δ) obtained throughout this work are characteristic of Fe(II) and Fe(III) low spin states.

For the complexes $[Fe(II)(HFPT)_2]^{2+}$ and $[Fe(II)(FPT)_2]$ we found practically the same δ value (δ = 0.24 mm s^{-1} at 298 K). This result makes clear that within experimental error, the isomer shift, and consequently the total s-electron density at the iron nucleus for both forms of the Fe(II) complex are fairly insensitive to the nature of the sulfur ligand (thiono or thiolate).

On the other hand, the quadrupole splitting, measured at 298 K, increases appreciably in going from $[Fe(II)(HFPT)_2]^{2+}$ ($\Delta = 0.48 \pm 0.02$ mm s^{-1}) to $[Fe(II)(FPT)_2]$ ($\Delta = 0.67 \pm 0.02$ mm s^{-1}) showing that this parameter is considerably affected by the nature of the sulfur ligand. This effect is probably due to the ligand inequivalencies, arising from differences in bond order when one goes from thiono to thiolate sulfur.

As mentioned in 'Experimental', we prepared Fe(III) complexes using an Fe(III) salt. The Mössbauer spectrum of the product thus obtained, measured at 85 K, in the solid state is illustrated in Fig. 2(A). It consists of two well resolved doublets. The large one, with $\delta = 0.14 \pm 0.02$ mm s^{-1} and $\Delta = 2.47 \pm 0.02$ mm s^{-1}, is characteristic of Fe(III) low spin and corresponds to $[Fe(III)(HFPT)_2]^{3+}$. The small doublet, with $\delta = 0.32 \pm 0.02$ mm s^{-1} and $\Delta = 0.66 \pm 0.02$ mm s^{-1}, is characteristic of Fe(II) low spin and corresponds to $[Fe(II)(FPT)_2]$. By means of the Mössbauer area, the percentual amounts of the Fe(III) and Fe(II) complexes were estimated to be 80 and 20% respectively.

It should be mentioned that any attempt to obtain pure Fe(III) complexes using Fe(III) salts and performing reactions in air, following the method developed by Ablov and Belichuk for the Fe(II) species, was unsuccessful [6]. In all cases, always a small amount (10–20%) of the Fe(II) complex was formed together with the Fe(III) species, as evidenced

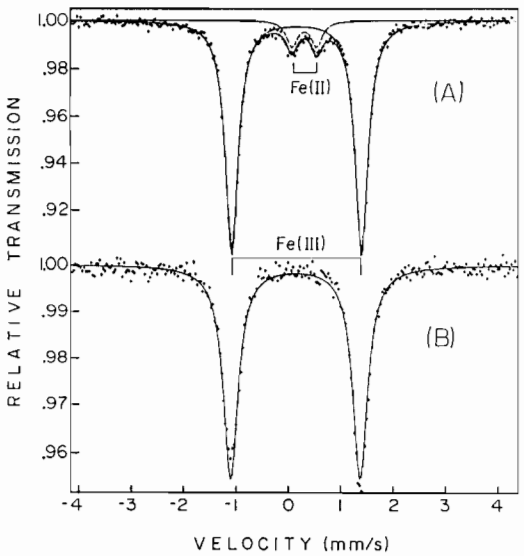


Fig. 2. Mössbauer spectra at 85 K of iron complexes of 2-formylpyridine thiosemicarbazone synthesized with iron-(III) salts: (A) without bubbling oxygen during the reaction; (B) with bubbling oxygen during the reaction.

by Mössbauer spectroscopy. By permanently bubbling the solution with oxygen as the reaction takes place we succeeded in preparing pure Fe(III) complexes using Fe(III) salts. The Mössbauer spectrum of the product obtained under such conditions, measured at 298 K in the solid state, is shown in Fig. 2(B). It consists of a unique doublet characteristic of $[Fe(III)(HFPT)_2]^{3+}$, with $\delta = 0.08 \pm 0.02$ mm s^{-1} and $\Delta = 2.45 \pm 0.02$ mm s^{-1}.

Although the reducing properties of the ligand were evidenced, it was not evident whether the reduction of Fe(III) to Fe(II) was previous to complexation, concomitant or even internal, therefore a kinetic study was undertaken.

Kinetic Studies

In order to know the reducing effect of the ligand on the Fe(III) already coordinated to FPT, the kinetics of the $[Fe(III)(HFPT)_2]^{3+}$ reduction process in solution by an excess of FPT was investigated using absorbance variations as a function of time to measure velocities. As the reaction is very slow, the initial rate, $v_i = \Delta A/\Delta t$ can be accurately determined using a time interval during which the reduction has just started.

Measurements were carried out in buffer solutions at pH 4.0 and pH 6.3. Under such conditions the Fe(III) complex is reduced to the Fe(II) species either in the protonated (thiono) or deprotonated (thiolate) form. The absorption spectrum of the Fe(II) thiono complex exhibits a band with maximum at 580 nm that shifts to 600 nm upon deprotonation. Therefore absorbances were measured either at 580 or 600 nm depending on pH conditions.

At each pH two sets of measurements were made: (i) keeping the reducing agent concentration constant, the Fe(III) complex concentration was varied; (ii) conversely, keeping the complex concentration constant whereas that of the reducing agent was varied. In Fig. 3 the initial rate is plotted as a function of either the concentration of FPT (A) or the Fe(III) complex (B), both at pH 4.0.

The experimental data were fitted using a least-squares programme, to a linear relation of the type $v_i = k'A + k''AB$ where $A = [FPT]$ and $B = [|Fe(III)(HFPT)_2|]$. For the sake of simplicity charges are omitted. The best fitted parameters gave $k' = 8 \times 10^{-6}$ s^{-1} and $k'' = 5 \times 10^{-3}$ M^{-1} s^{-1}.

As can be noticed in Fig. 3(A), the intercept indicates that a reduction of the Fe(III) complex occurs even in the absence of the free ligand, suggesting an internal transfer of electrons between ligand and metal. The parameters obtained from Fig. 3(B) are compatible with the k' and k'' values found in Fig. 3(A).

Similar results were obtained for the reaction at pH 6.3, indicating that the initial rate does not depend on pH in the pH range studied. Thus, one

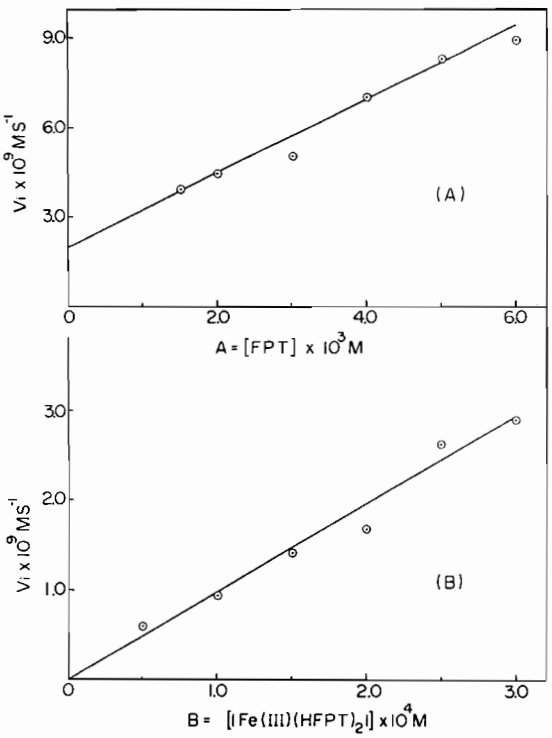


Fig. 3. Initial rate as a function of the concentration of (A) FPT and (B) the Fe(III) complex, both at pH 4.0.

may infer that the same mechanism takes place either for the protonated or the deprotonated forms.

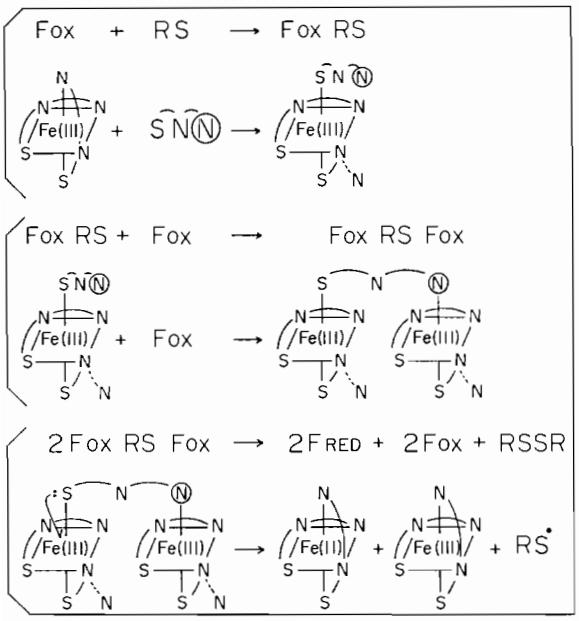
Presumably this mechanism involves oxidation of either two thiols (RSH) or two thiolates (RS^-) to a disulfide by transferring one electron from each, either thiol or thiolate, to a Fe(III) of the complex through an inner sphere process. We may then postulate the net following processes:

$$2Fox + 2RS \longrightarrow$$

$$2Fred + RSSR\ (+2H^+ \text{ for thiol case}) \quad (1)$$

in which Fox stands for the Fe(III) complex and Fred for the Fe(II) one, both at either pH, and RS stands for either thiol or thiolate, depending on pH conditions.

As resonance Raman data strongly suggests, the pyridine nitrogen is loosely bound to the metal ion and can exchange rapidly with other ligands present in the solution [7]. Therefore one may assume an inner sphere process in which a ligand molecule binds to the central atom of one Fe(III) complex through the sulfur end. In a second step this new entity, hereafter called Fox RS, binds through its pyridine extremity to another Fe(III) complex, forming the binuclear Fox RS Fox intermediate, in which FPT acts as a bridging ligand between two iron atoms. In a final step, by collision between two entities of the type Fox RS Fox, two electrons are transferred



Scheme 1. Proposed mechanism for the reduction of $[Fe(III)-(HFPT)_2]^{3+}$ by an excess of ligand (FPT).

from two ligands to two Fe(III) ions, forming two $RS^{\cdot}$ radicals which are released and bind together to form a disulfide RSSR. Simultaneously the pyridines coordinate to the metal again (see Scheme 1).

Similar mechanisms have already been proposed for other redox reactions of metal complexes involving the oxidation of dithiols to disulfides [10].

The expressions (2), (3) and (4) describe the elementary steps of the proposed mechanism

$$\text{Fox} + \text{RS} \xrightarrow[\text{fast}]{k_1} \text{Fox RS} \tag{2}$$

$$\text{Fox RS} + \text{Fox} \xrightarrow[\text{fast}]{k_2} \text{Fox RS Fox} \tag{3}$$

$$2\text{Fox RS Fox} \xrightarrow[\text{slow}]{k_3}$$

$$2\text{Fred} + 2\text{Fox} + \text{RSSR}\ (+2\text{H}^+ \text{ for thiol case}) \tag{4}$$

By means of eqns. (1) through (4) one obtains

$$v_i = \frac{d}{dt}|\text{Fred}| = \frac{2d}{dt}|\text{RSSR}| = -\frac{d}{dt}|\text{RS}| = -\frac{d}{dt}|\text{Fox}| \tag{5}$$

$$\frac{d}{dt}|\text{Fox}| = -k_1|\text{Fox}||\text{RS}| - k_2|\text{Fox}||\text{Fox RS}| + 2k_3|\text{Fox RS Fox}|^2 \tag{6}$$

$$\frac{d}{dt}|\text{RS}| = -k_1|\text{Fox}||\text{RS}| \tag{7}$$

Using the steady approximation for the intermediates Fox RS and Fox RS Fox the analytical form of the rate law may be obtained. Therefore

$$\frac{d}{dt}|\text{Fox RS}| = k_1|\text{Fox}||\text{RS}| - k_2|\text{Fox RS}||\text{Fox}| = 0$$

or

$$k_1|\text{Fox}||\text{RS}| = k_2|\text{Fox RS}||\text{Fox}| \tag{8}$$

and

$$\frac{d}{dt}|\text{Fox RS Fox}| = k_2|\text{Fox RS}||\text{Fox}| - 2k_3|\text{Fox RS Fox}| = 0$$

or

$$k_2|\text{Fox RS}||\text{Fox}| = 2k_3|\text{Fox RS Fox}| \tag{9}$$

By combining expressions (5), (6), (8) and (9) we have

$$v_i = -\frac{d}{dt}|\text{Fox}| = -\frac{d}{dt}|RS| = k_1|\text{Fox}||\text{RS}|$$

Therefore, the rate law derived from the proposed mechanism is of the type $v_i = kAB$, which corresponds to first order kinetics with respect to both concentrations of the complex and the reducing agent.

As already mentioned, experimental data lead to the expression $v_i = k'A + k''AB$, which indicates the coexistence of two mechanisms. In one of them, the reaction is first order with respect to the concentration of the complex and occurs through a pathway which is independent of the concentration of the excess of FPT.

Therefore one may assume the occurrence of internal reduction of the metal by the ligand already coordinated, which acts as a reducing agent. Further investigations will be undertaken in order to get additional information on this subject. As the concentration of FPT increases the reduction takes place preferably through the mechanism described in Scheme 1, in which the excess of ligand is the reducing agent.

Conclusions

It is already known that the active form of RDR contains a stable electron deficient tyrosile radical which disappears by inactivation of the enzyme [11]. Any species able to provide one electron to this

radical may in principle act as an enzyme inhibitor, as has been proposed in the case of hydroxyurea [12]. Our kinetic results suggest a similar mechanism, involving oxidation of the Fe(II) to the Fe(III) complex by releasing one electron to the free radical and the subsequent internal reduction of Fe(III) by the ligand.

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