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IR, NMR and EPR investigation of iron recognizing molecule - desferrioxamine B

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IR, NMR and EPR spectroscopies were used for the structural investigation of the chelator desferrioxamine B and its Fe(III) complex, ferrioxamine B. By comparing the FT-IR absorptions of desferrioxamine B and ferrioxamine B, the structural changes due to the Fe(III) chelation were clearly deduced. Thus a resonance process in the O=C-N bond with the stabilization of C=N and C-O bonds were evidenced by chelating the Fe(III) ion. This fact is confirmed by the appearance of two new bands at 1577 cm^{-1} and 1043 cm^{-1} in the IR spectra. The homogenization of the electronic charge of the six oxygen atoms situated around the Fe^{3+} ion in the octahedral ferric chelate is thus achieved. ^1H and ^{13}C NMR spectra show that the high stability of octahedral DFO-Fe chelate is due to the high coordinative energy of hydroxamic acid for Fe^{3+} and also to the number and spacing of the coordinative groups. EPR spectra suggest that DFOB has the same ability to chelate also Mn^{2+} ions which have a similar electronic configuration ($3d^5$,$^6S_{5/2}$) as Fe^{3+} ions.

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1. Introduction

The recognition properties of some molecules are very important for many biological processes and generally for life [1-8]. Molecular recognition can be carried out by supermolecules which are molecules whose covalent bonds are saturated and which are held together by intermolecular forces.

Iron is an essential element for the growth of almost all forms of life. Iron in its most common form, Fe(III) hydroxide, is not a readily available nutrient since its solubility is limited, typical concentrations at neutral pH are about 10^{-18} M. The low environmental concentration of soluble Fe(III) has forced the microorganisms and higher plants to synthesize and secrete siderophores, that can chelate Fe(III) and carry it into the cell via specific high-affinity uptake receptors.[1] The mechanism for Fe acquisition by siderophores such as desferrioxamine B, (Fig. 1), relies on the selectivity of these ligands for Fe(III) to form very stable complexes.

In order to improve the understanding of the mechanism by which siderophore-mediated iron transport occurs, the interaction of desferrioxamine B with iron was studied in detail[2-6].

According to the crystal structure of ferrioxamine B[2] the chelate molecule consists of two closed loops and an open chain containing a protonated amine (Fig. 1a). The six hydroxamate oxygen atoms coordinated to the Fe(III) center form a distorted octahedral geometry around the metal center (Fig. 1b).

A recent study has investigated the desferrioxamine B and ferrioxamine B molecules sorbed on activated Sepharose gels[3].

Ludwig and Dzung[7] give also a wide review about calixarene – type macrocycles used for different metal cation recognition selectivity. A new infrared sensing scheme based on an evanescent wave was proposed by Huang and Yang [8] for selective detection of copper ion in aqueous solutions.

In order to improve the understanding of the mechanism by which desferrioxamine B – mediated Fe^{3+} ions transport, new structural data obtained by IR, NMR and EPR spectroscopies concerning the interaction of desferrioxamine B with iron are given by us in this paper.

2. Experimental

All the employed chemicals were reagent grade or better. Desferrioxamine B methanesulphonate (Desferal) was purchased from Ciba Geigy, Basel, Switzerland.

The FT-IR spectra of desferrioxamine B and ferrioxamine B were obtained for a wavenumber range of 400-3500 cm^{-1} with a Bruker Equinox 55 spectrometer with a resolution of 2 cm^{-1}. Samples for analysis were prepared by mixing and powdering 300 mg potassium bromide with 0.8 mg of the tested material and then compressing the mixture into pellets. To obtain the FT-IR spectra 32 scans were collected.

^1H and ^{13}C solid-state NMR spectra were recorded using a Bruker DSX-500 spectrometer operating at 500.45 MHz for ^1H and 125.72 MHz for ^{13}C. The data were collected at room temperature under spinning conditions at 10 kHz. For the proton measurements the length of the $\pi/2$ pulse was about 6 μs and the dwell time was set to 2.5 μs. The recycle delay was 5 s for the measurement of desferrioxamine B and 1 s for ferrioxamine B. The ^{13}C

measurement for desferrioxamine B was made using cross-polarization with a contact time of 3 ms. In the case of ferrioxamine B, the measurement was made applying a 90^0 pulse on the carbon channel and with decoupling of the proton during the data acquisition.

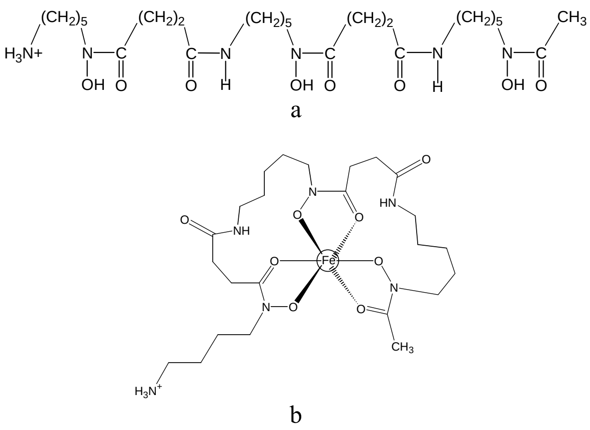


Fig. 1. A schematic drawing of desferrioxamine B (a) and ferrioxamine B (b).

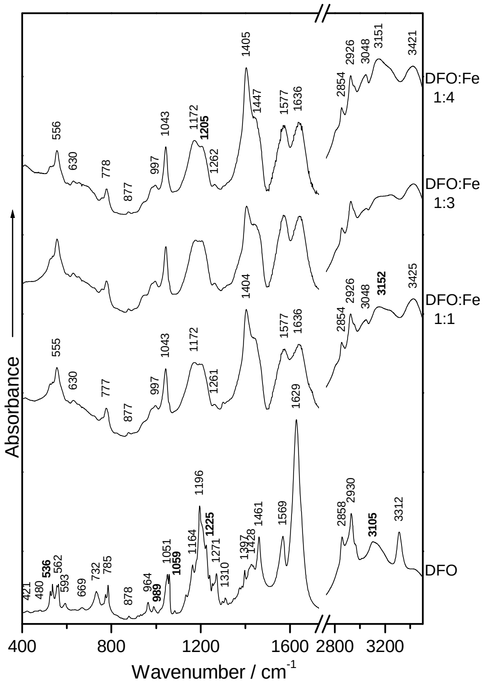


Fig. 2. FT-IR spectra of desferrioxamine B (DFO) and ferrioxamine B (DFO:Fe) with the stochiometric ratios as indicated.

EPR measurements were performed at room temperature with a SE/X2543 type spectrometer, in the X frequency band (9.4 GHz).

3. Results and discussion

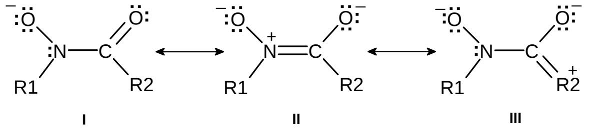
FT-IR spectroscopy. The FT-IR absorption spectrum of desferrioxamine B and the FT-IR spectra of desferrioxamine B Fe(III) complexes, prepared at the stoichiometric ratios 1:1, 1:3, 1:4 are shown in Fig. 2.

The FT-IR spectrum of desferrioxamine B is dominated by the strong amide I band of the C=O stretching vibrations, observed at 1629 cm^{-1} due to the internal hydrogen bonding, that reduced the frequency of the carbonyl stretching. An important contribution to this experimental band is expected also from the asymmetric bending vibrations of NH_3^+ group [9]. The absorption at 1569 cm^{-1} is an amide II band, due to the superposition of the N-H bending vibrations of the secondary amides and C-N stretching vibrations of the secondary amides and of the hydroxamate groups.These assignaments are in agreement with other studies on compounds containing similar functional groups [10-13]. The N-O stretching vibration of the hydroxamate groups is present as a medium peak at 1051 cm^{-1}. In the high wavenumber region, the N-H stretching vibrations at 3105 and 3312 cm^{-1} are overlapped by the broad O-H stretching band of the hydroxyl groups [10].

Desferrioxamine B is available as methanesulphonate salt. The symmetrical and asymmetrical SO_3 stretching vibrations are usually strong IR active and are therefore present in the spectrum at 1058 and 1164 cm^{-1}, respectively.

Comparing the ferrioxamine B FT-IR spectra from Fig. 2, the shapes of these spectra present similar features, consequently, independently of the used Fe(III) ratios, this meaning that desferrioxamine B chelates Fe(III) to a complex with a unique structure. The medium-strong band at 555 cm^{-1} in the FT-IR spectra of ferrioxamine B is attributed to the Fe-O stretching vibration. The amide I band of ferrioxamine B appears broadened and blue shifted at 1636 cm^{-1}, due to the overlapping of the C=O stretching vibrations of the secondary amides and the C=O stretching vibrations of the hydroxamate groups whose O atoms are involved in the Fe(III) bonding. Also, the intensity of the amide I band is significantly reduced due to a resonance O=C-N bond that arise after the Fe(III) chelation, as indicated by the X-ray diffraction [2]. The X-ray diffraction of crystallized ferrioxamine indicates an increase in the C-N double bond character on complexation to Fe(III), as shown by the resonance structure II in Scheme 1.

Consequently, new bands of C=N and C-O appear at wavenumbers of 1577 and at 1043 cm^{-1}, respectively.



Scheme 1. Major resonance structures for a hydroxamate anion.

An increase in C-N double bond character and the localization of a greater negative charge at the carbonyl oxygen results in a relatively strong Fe(III) complex [2].

The dissociation of the hydroxyl groups resulting from the coordinative bond to Fe(III) influences the N-H stretching vibrations at 3152 and 3425 cm^{-1}, being blue shifted. Bonding Fe(III) induces also modifications in the shape of the C-H deformation bands. Thus the band at 1404 cm^{-1} with a shoulder at 1447 cm^{-1} appears broadened, whereas the C-C skeletal stretching vibrations of the alkanes are shown by the intense bands, blue shifted at 1172 and 1205 cm^{-1}.

Solid-state NMR spectroscopy. The proton (^{1}H) and carbon (^{13}C) spectra of desferrioxamine B (Figs. 3a, 4a) show the presence of several peaks corresponding to the different functional groups.

The chemical shifts (ppm) of these lines and their correspondent Cross-polarization proton MAS spectra show typical rotational bands for whole multiples of the spinning frequency. The main chemical shifts and their correspondent functional groups assignment of the ^{1}H and ^{13}C NMR spectra of desferrioxamine B are given in Table 1.

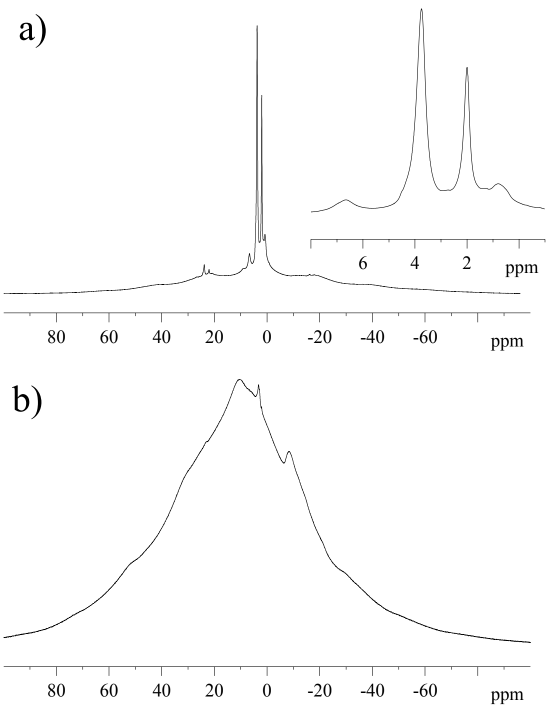


Fig. 3. ^{1}H spectra of desferrioxamine B (a) and ferrioxamine B (b).

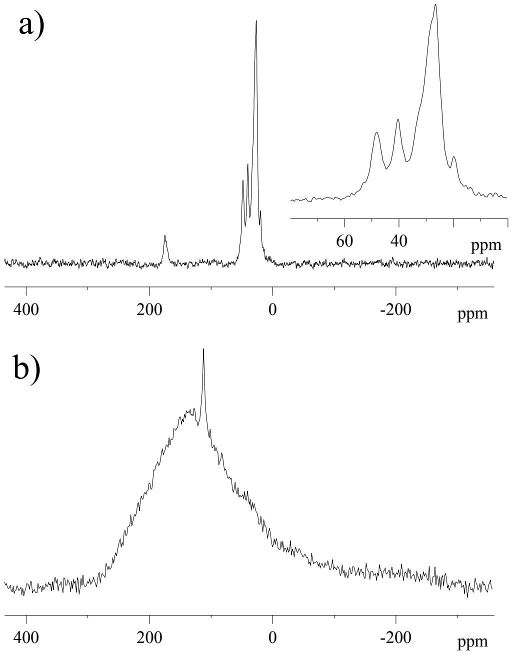


Fig. 4. ^{13}C spectra of desferrioxamine B (a) and ferrioxamine B (b).

DFOB contains three hydroxamic acid groups and a free amino group. It coordinates with Fe^{3+} through its three hydroxamates groups by forming an octahedral Fe chelate (Fig.1). The stability of the Fe^{3+} complex is very high (logK=31.4) [14] and this may be attributed not only to the high coordinative energy of the hydroxamic acid for Fe(III) but also to the number and spacing of the coordinative groups. Evaluation of these Fe scavengers in living organisms has shown that the spacing of hydroxamic acids adjusts so that the three hydroxamic acids of the siderophore precisely fit the required octahedral coordination sphere of Fe [15].

Table 1. Chemical shifts (ppm) and their correspondent functional groups assignment of desferrioxamine B.

^{1}H spectra chem. shift (ppm)	Assignment	^{13}C spectra chem. shift (ppm)	Assignment
0.7	-OH	20	$-CH_3$
2	$-CH_3$	27.5	$-CH_2$
4	$-CH_2$	40.5	$-CH_2-\overset{\overset{O}{\|}}{C}-$
6.7	-NH	48.5	$-CH_2-N-<$
		175	$-\underset{\underset{O}{\|}}{C}-N<$

In the case of ferrioxamine B, both ^{1}H and ^{13}C NMR spectra (Figs. 3b, 4b) are very broad, showing a strong dipolar interaction and a large distribution of the local magnetic fields due to the presence of paramagnetic Fe^{3+} ions characterized by a short T_1 relaxation time [16].

In the spectrum from Fig. 4b, over the large line, we can see that the peak corresponding to the CH_2 groups from ferrioxamine B at a chemical shift of 117 ppm is well resolved. The same thing can be seen in Fig. 3b for the peaks corresponding to CH_2 and CH_3 groups at 2 ppm and –9 ppm respectively. The rotation bands occur also in this spectrum. The peak with negative chemical shift is not a real peak, but is one of the so called spinning sidebads which appear due to the modelling of the dipolar Hamiltonian under MAS conditions [17].

EPR spectroscopy. The EPR spectrum of ferrioxamine B (Fig. 5) consists from two main absorptions centered at $g \approx 4.3$ and $g \approx 2.0$.

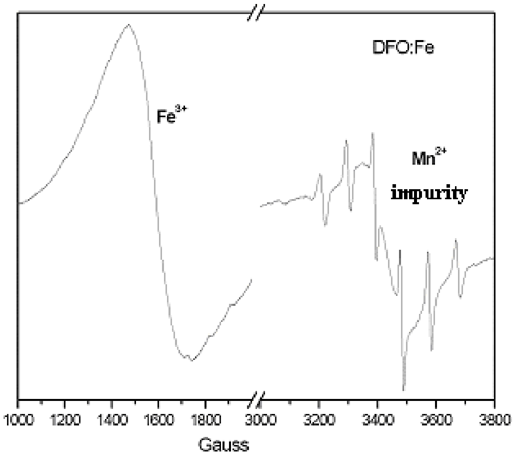


Fig. 5 EPR spectrum of ferrioxamine B.

The resonance line at $g \approx 4.3$ is due to the isolated Fe^{3+} ions ($3d^5$, $^6S_{5/2}$) situated in a hexacoordinated distorted octahedral symmetry (rhombic or tetragonal) subjected to strong crystal field effects [18]. The $g \approx 2.0$ line may be attributed either to Fe^{3+} species interacting by the dipole-dipole mode in sites of less distorted octahedral (tetragonal) field or to the superexchange coupled pairs (Fe^{3+}-Fe^{3+}) [18]. This fact is in agreement with the possible dimeric configurations of ferrioxamine B [19]. In the $g \approx 2.0$ region appear also six hyperfine lines due to the Mn^{2+} ($3d^5$, $^6S_{5/2}$) ions impurities. The presence of Mn^{2+} ions impurities in our EPR spectrum suggests the fact that DFOB manifests the same chelating properties for Mn^{2+} ions because they have a similar electronic configuration with Fe^{3+} ions.

4. Conclusions

The structural changes induced by the complex formation of desferrioxamine B with Fe(III) were evidenced by the FT-IR spectra, the Fe-O stretching absorption being observed at 555 cm^{-1}. By chelating the Fe(III) ion, a resonative process in the O=C-N bond of DFOB molecule was evidenced. Thus the new bonds of C=N and C-O appear in the IR spectra at 1577 cm^{-1} and 1043 cm^{-1}, respectively. This fact leads to the homogenization of the electronic charge of oxygen environment around ferric ion in octahedral Fe chelate.

Both ^{1}H and ^{13}C NMR spectra show that DFOB coordinates with Fe(III) through its three hydroxamates groups forming an octahedral Fe chelate. The high stability of this complex is attributed to the high coordinative energy of hydroxamic acid for Fe(III) and also to the number and spacing of the coordinative groups.

EPR spectra suggest also that Fe(III) ions are situated in a hexacoordinated distorted octahedral symmetry subjected to strong crystal field effects. The $g \approx 2.0$ line shows the presence in this compound of superexchange coupled pairs (Fe^{3+}- Fe^{3+}), too. The appearance of the six hyperfine lines characteristic for Mn^{2+} impurity (I=5/2 for ^{55}Mn) in the $g \approx 2.0$ region suggests that DFOB has the same ability to chelate Mn^{2+} ions because they have a similar electronic configuration as Fe^{3+} ions.

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