EXAFS studies of the novel iron(III) complexes with an N/S(Se) chromophore simulating ligand environment of the active site of nitrile hydratase

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Novel metal chelates with an N/S(Se) chromophore have been synthesized and structurally characterized to model the iron site of the hydrolytic metalloenzyme nitrile hydratase. Iron- sulfur bond lengths Fe-S (2.24 Å) estimated from EXAFS are similar to that reported (Huang et al., 1997) for nitrile hydratase (2.21 - 2.23Å). Iron-nitrogen bond lengths Fe-N 2.00Å are also identical to those in the enzyme (1.99Å). Another investigated metal complex with $Fe(III)N_2S_2Cl$ coordination contains a potentially labile terminal chloride ligand which may be substituted by solvent molecules and can be useful as possible analogues of the substrate - bound form of the enzyme.

Keywords: EXAFS; metal chelates; nitrile hydratase.

1. Introduction

There is an increasing interest in the metal active centers of non-heme metalloproteins in biology. An example of a metalloenzyme with an iron-thiolate active site that does not belong to the heme or Fe-S clusters families is bacterial nitrile hydratase, which catalyzes the conversion of nitriles to corresponding amides (Kobayashi et al., 1992; Yamada H., Kobayashi M., 1996). Combined physicochemical results provided evidence for a presence of the low-spin (S=1/2) $Fe^{III}N_3S_2$ chromophore with a sixth coordination site occupied by a water molecule or hydroxide ion, which is probably replaced by nitrile in the catalytic cycle (Scarrow et al., 1987; Sugiura et al., 1987; Scarrow et al., 1996). A recent X-ray structural study (Huang et al., 1997) revealed a $FeS_3N_2(H_2O)$ site containing two deprotonated carboxamido nitrogens. Following the synthesis of several model compounds to imic a N/S ligand environment of the nitrile hydratase active site and some of its spectroscopic properties (Nivorozkin et al., 1997) we report here an EXAFS study of those and new chelate complexes of Schiff-base ligands

with the supplementary coordinating groups I-IV. A series of complexes I-IV represents the only available set of isoligand $Fe(III)N_xX_y$ species with variable donor atoms X=O, S, Se. Complex V contains a potentially labile terminal chloride ligand which may be substituted by solvent molecules and thus has a potential of a suitable starting point to pursue an ultimate goal of nitrile hydrolysis.
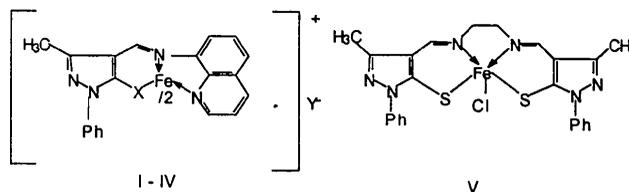
I: X=S, Y= $FeCl_4^-$; II: X=S, Y= ClO_4^- ; III: X=O ; Y= ClO_4^- ;
IV: X=Se, Y= ClO_4^-

2. Experimental section

Complexes I-V were synthesized according to ref. (Huang et al., 1997; Nivorozkin et al., 1997; Uraev et al., 1997) by a reaction of the ligands H_2L, [N,N'bis(5-mercapto-3-methyl-1-phenylpyrozol-4-ylmethylene)ethylenediamine) and HL, ['5-mercapto-1-phenyl-3-methyl-4-ylmethylene(8-amino)-quinoline] with $Fe(ClO_4)_3 \cdot nH_2O$ or $FeCl_3 \cdot 6H_2O$ in ethanol. The samples for XAS were ground to fine powders and mixed with apiezon grease. The mixtures were formed into flat samples of the appropriate thickness ($\mu x \approx 2.7$) for EXAFS measurements in transmission mode. X-ray absorption data were obtained using a laboratory XAFS spectrometer with a focussing crystal-monochromator (Shuvaev et al., 1988). A Mo X-ray tube (U=17 kV, I=25 mA) and cylindrically curved quartz (1340) crystal-monochromator of Iohan type in Rowland geometry were used. The energy resolution was determined using the width of the characteristic lines (~ 2 eV in the region of the FeK-edge).

The EXAFS data were weighted by k^3, and truncated at k=3 and k=12 Å^{-1}, following background substraction. The square filter window between 1.4 and 3.5 Å isolated the significant Fourier transform (FT) peaks, which were then back-transformed into k-dependent data. The best-fit distances (R), coordination numbers (N), and Debye-Waller (DW) factors were obtained from multishell fits to the Fourier-filtered data using empirical phase and amplitude functions.The empirical phase and amplitude functions were derived from FEFF5 (Rehr et al.,1991) of a crystallographically characterized complex I.

3. Results

The EXAFS and the $k^2\chi$ Fourier transforms (FT) magnitude for the complexes I-V and the corresponding data fit are shown in Fig. 1. The principal features of FT of the compounds I,II,V include a large peak at r= 1.69-1.73Å corresponding to the first coordination sphere, and smaller peaks at r=2.5-3.6 Å. The smaller FT peaks in III are similar to those in I,II,V whereas the large peak occurs at r= 1.49 Å due to the absence of the Fe-S/Cl scatterers in the molecule. The splitting of the largest feature in the FT of IV into two peaks is presumably due to the presence of the long Fe-Se distances.

The structure of I was determined by X-ray crystallography (Nivorozkin et al., 1997) and served as a

reference to obtain EXAFS parameters used in subsequent fits of all the samples. The results of the curve fitting analysis are summarized in Table 1.
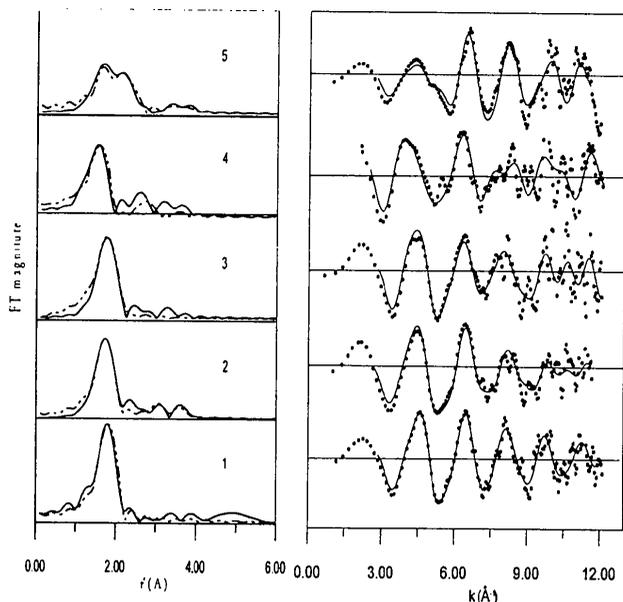
Figure 1

The left panel shows the magnitude of Fourier transforms of $k^2\chi$ for compounds: I - (1), II - (2), III - (4), IV - (5), V - (3). The solid line is the experimental data and the dashed line is the theoretical fit. The right panel shows EXAFS data (dotes) and fits (solid line) using parameters presented in Table 1.

The molecular structure of I indicates that the iron center is octahedrally coordinated by the two azomethine, and two quinoline nitrogens and two thiolate sulfur atoms in cis-positions. Iron- sulfur bond lengths, Fe-S_1=2.227(2) Å and Fe-S_2= 2.230(2) Å, are similar to that reported for nitrile hidratase (Fe-S= 2.21 Å from the EXAFS data (Scarrow et al., 1996). Iron-nitrogen bond lengths in I, Fe-N_{av}=1.99Å, are also identical to those in the enzyme. The EXAFS results on I agree with the X-ray diffraction data. Complexes I-IV are apparently isostructural. While I, II and IV contain low-spin (S=1/2) Fe(III)N_3X_3 iron centers, III is a high-spin species (S=5/2). S=1/2→S=5/2 spin transition is usually accompanied by a certain elongation of the metal-ligand bonds (Sim et al., 1981). This trend could be also revealed by a perusal of the Table 1.

Acknowledgment

Table 1

Results of multishell fits of the EXAFS data for compounds I-V and Fe(acac)₃ (R-average distance, N- number of scattering atoms, σ^2 - Debye-Waller factors, Q-parameter of quality of fit)[a].

Compd/parm	R, Å	N	σ^2, Å²	atom	Q,%
I[b]	2.00	4	0.0020	N	
	(1.969, 2.005)				
	2.24	2	0.0037	S	1.6
	(2.227, 2.230)				
	2.18	4	0.0030	Cl[c]	
II	2.03	4	0.0035	N	
	2.24	2	0.0030	S	2.8
III	1.97	2	0.0026	O	
	2.05	4	0.0043	N	
	3.12	8	0.0031	C	2.0
	3.52	6	0.0040	C	
IV	2.04	4	0.0020	N	
	2.43	2	0.0040	Se	3.0
	2.37	1	0.0030	Fe[d]	
	1.95	1	0.0040	C[d]	4.0
V	2.00	2	0.0030	N	
	2.24	2	0.0040	S	2.0
	2.28	1	0.0030	Cl	
Fe(acac)₃[e]	2.02	6	0.0027	O	2.0
	(1.998)				

[a]The first-shell Fe-N/O/S distances are typically accurate to ±0.02 Å, second and third shell to ±0.05 Å. Coordination numbers are generally considered accurate to ±20-25 %. The estimates are determined from comparisons between EXAFS and crystallography of distances and coordination numbers. [b]The coordination numbers for the compound I given in the table are for the [FeN₄S₂] centre, as well as [FeCl₄]⁻ anion. For simulations were used average of these coordination numbers. [c] Distance of the Fe-Cl in FeCl₄ anion. The crystallographic distances for I are given in parentheses (Nivorozkin et al., 1997). [d] R and N were obtained from Se K-edge EXAFS spectra.[e]Distance in parentheses is from (Scarrow et al., 1987).

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