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EXAFS INVESTIGATIONS ON AN NH_2-TERMINAL FRAGMENT OF HUMAN TRANSFERRIN CONTAINING A SINGLE IRON BINDING SITE

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Transferrins are a group of glycoproteins from different sources, characterized by the ability to bind specifically and reversibly iron(III) at two distinct sites with somewhat different affinities. Human serum transferrin (MW 80,000) is responsible for iron transport from sites of absorption to sites of storage and utilization.

The nature, the number and the geometry of the ligands around iron have been the subject of much debate. The presence of a synergistic anion (physiologically HCO_3^- or CO_3^{2-})directly bound to the metal has been confirmed by EXAFS measurements on ovotransferrin[1] and on the isolated C-terminal and N-terminal fragments of ovotransferrin which show the metal atoms to have coordination numbers consistent with a six-coordinate environment[1]. Spectroscopic studies have shown that the remaining ligands are probably three oxygen atoms from two tyrosine residues and a water molecule and two nitrogen atoms from histidine imidazole groups[2].

By limited proteolysis of the iron saturated protein with thermolysin, a single iron binding fragment of MW 35,000 can be obtained, corresponding to the N-terminal site. The possibility of investiganting by EXAFS a single iron binding site and avoiding superposition of the contributions from both sites to the X-ray absorption spectrum, has prompted this investigation.

The protein N-terminal fragment was obtained according to the procedure reported by Lineback-Zins and Brew[3]. EXAFS spectra have been recorded by the fluorescence technique at the PULS X-ray beam line at the Frascati SR facility in the energy range 6950-7950 eV. Mn filter on the scintillation detector has been used to reject the scattering noise. The sample was 1.5 mM in transferrin N-terminal fragment, buffer Tris-HCl, pH 8.5. Several spectra of the protein fragment were run under the same

conditions and averaged.

$Na[Fe(ehpg)]4H_2O$, $Fe(acac)_3$ and $[Fe(phen)_3](ClO_4)_3$ were prepared according to published procedures and used as model compounds. Their EXAFS spectra have been collected in the absorption mode on finely ground powders.

TF/2N = N-terminal fragment of human serum transferrin;
ehpg = ethylene-bis-(o-hydroxyphenylglycine);
acac = acetyilacetonate - phen = phenantroline.

Fig. 1 shows the shape of the EXAFS spectrum for the protein fragment and the model compound $Fe(acac)_3$. Similar amplitudes would be expected if approximately the same scattering atoms were present in the first coordination shell of the iron atoms. Close resemblance is observed between the protein and the $Fe(acac)_3$ EXAFS both in the overall shape and in the phases suggesting similar iron binding sites for the two compounds. However a difference exists in the amplitude.

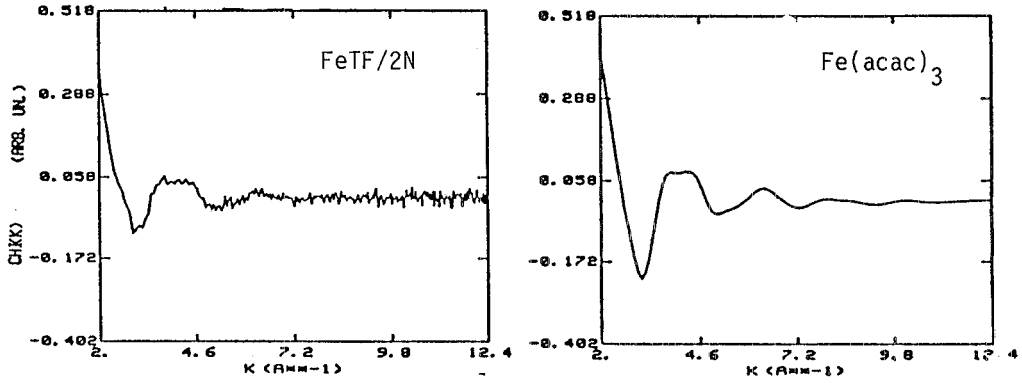

Fig. 1

Fourier transforms of the EXAFS spectra are reported in Fig. 2. The radial distribution for the protein fragment shows two major features. The larger peak is due to backscattering from atoms in the first coordination shell and the second arises from atoms up to approximately 4.0 Å from the iron ion.

The contribution of the first coordination shell to the EXAFS spectrum can be determined by a backtransform in the k space of the isolated corresponding peak in the Fourier transform. Comparison of the function with the analogous one from model compounds $Fe(acac)_3$ and $Fe(ehpg)^-$ of known structure gives a distance of 1.95 Å for 6 oxygen (nitrogen) atoms around the iron center in the protein fragment. A more precise analysis can be done fitting the backtransformed $\chi(k)$ function using amplitudes and phases from a model complex. This has been accomplished in a single shell fit using data from $Fe(acac)_3$ in which the iron is coordinated by

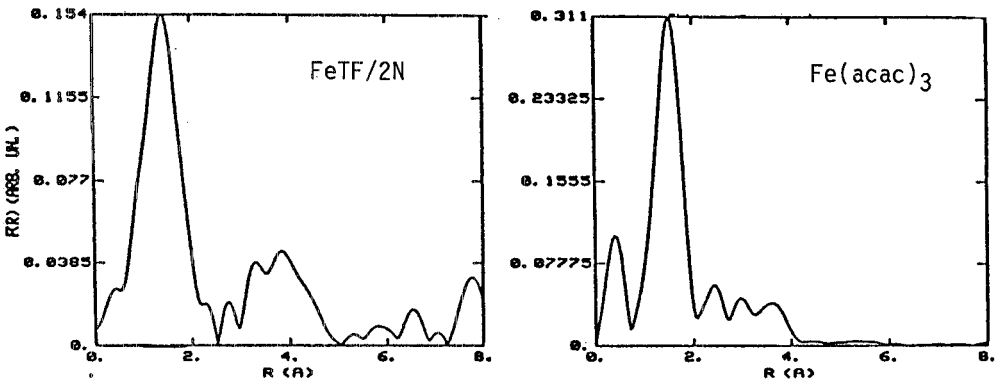

Fig. 2

six oxygen atoms in a regular octahedral arrangement. The best fit gives a first shell of 5.6 oxygen atoms at 1.94 Å with a fit index R=0.0199. The single shell fit does not completely describe the EXAFS of the protein fragment suggesting a distribution of distances in the first coordination shell and the need to include contributions from further shells of atoms for a satisfactory fit of the experimental data.

In conclusion our EXAFS data on NH_2-terminal fragment of human serum transferrin provide evidence for a first coordination shell of six oxygen-nitrogen atoms at an average distance of 1.95 Å from the Fe(III) ion and indicate an irregular arrangement of the ligand atoms sitting at slightly different distances around the metal center. Data analysis to obtain a more detailed picture of the iron binding site is in progress.

References:

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