

THE IRON BINDING-SITES OF CHICKEN OVOTRANSFERRIN

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Abstract - We have shown previously that the EXAFS spectrum of diferric chicken ovotransferrin (Fe₂COT) can only be adequately simulated assuming a split first shell co-ordination [1]. EXAFS and XANES spectra of Fe₂COT measured in solution and as a freeze-dried powder provide evidence for perturbation of the iron-binding sites on freeze-drying which involves the loss of one of the long (~2.04Å) first shell ligands (presumably water). Measurement of the XANES of the C-terminal monoferric COT and a C-terminal domain fragment suggests that the metal binding site remains largely unperturbed by the fragmentation process. The possibility of site interaction is briefly discussed.

Introduction

The role of chicken ovotransferrin (COT) in egg white is presumed to be as a bacteriostatic agent acting via its iron-sequestering capability. Evolutionarily, it is related to serum transferrin, the principal iron transport protein of vertebrate plasma.

The transferrins are monomeric glycoproteins of Mr ~80,000 capable of specifically binding a maximum of two ferric ions concomitant with 2 (bi)carbonate anions at homologous iron-binding sites, (for review see [2]). X-Ray diffraction [3] and molecular fragmentation studies have shown the molecule to have a two domain structure. Spectroscopic and chemical modification evidence suggests the presence of histidine and tyrosine ligands to the iron atoms as well as a (bi)carbonate anion and/or water molecule (see [2]). We have shown previously using EXAFS [1] that the first shell atoms are split into two distances involving 2 light atoms (O/N) at ~1.85Å and a further four light atoms at ~2.04Å.

On the basis of model compounds we assign the shorter distance to the phenolic oxygens of 2 tyrosine residues. A summary of the first shell distances for Fe₂COT and its N- and C- domain iron-binding fragments are given in Table 1.

Materials and Methods

Chicken ovotransferrin and its iron-binding fragments were isolated as described previously [1]. Protein was made iron-saturated by addition of excess Fe(III) nitrilotriacetate and desalted on Sephadex G25. Freeze-dried Fe₂COT was made by

shell freezing a solution of iron-saturated protein in 50mM NH_4CO_3 using liquid nitrogen and lyophilisation at $\sim 5 \times 10^{-2}$ Torr. C-Monoferric COT was prepared by addition of 0.03 vol of 0.6M sodium citrate, pH 5.0, to a solution (20 mg/ml) of Fe_2COT in water. After the extinction at 470 nm had fallen to 30% of its original value a theoretical threefold excess of desferrioxamine was added and the resulting iron-desferrioxamine complex removed by gel filtration on G-50. The monoferric protein was freeze-dried prior to use. All solution EXAFS and XANES were recorded in 0.1M NaHCO_3 .

X-Ray absorption spectra were recorded on Station 8.1 of the SERC Daresbury SRS, using a double focusing Si (220) monochromator. During data collection the SRS beam energy was 2 GeV and maintained an average circulating current of 220 mA. Simulation of the EXAFS region have involved an approach described elsewhere [4].

Table 1 First shell EXAFS parameters used in the simulation of spectra from Fe_2COT and its N- and C- terminal single domain fragments.

Atom	No	N- Fragment		C- Fragment		Fe_2COT	
		R(Å)	$\sigma(\text{Å}^2)$	R(Å)	$\sigma(\text{Å}^2)$	R(Å)	$\sigma(\text{Å}^2)$
O	2	1.83	0.005	1.87	0.005	1.85	0.006
N	4	2.04	0.002	2.06	0.005	2.04	0.003

Results and Discussion

Fig 1 shows a comparison of the EXAFS of Fe_2COT recorded in 0.1M NaHCO_3 buffer and as a freeze-dried powder. The amplitude and frequency of the major shell contribution is reduced in the freeze-dried sample suggesting the loss of a ligand from the first co-ordination sphere. This is evident in the Fourier transform (inset) where the first shell is shifted to lower R and is of reduced intensity. Rearrangement of some of the outer shells is also apparent from the 3Å region of the Fourier transform.

The enhancement of features a and b (Fig 2) in the edge structure of the freeze-dried protein confirm that geometrical changes have occurred as a consequence of freeze-drying. Some evidence [5] has been provided for a negative correlation between the intensity of the pre-edge feature a and the co-ordination number. The increased intensity of this transition in the freeze-dried spectrum is therefore consistent with the loss of a ligand from the metal binding-sites.

Fig 3a shows the experimental spectrum for the freeze-dried protein superimposed upon the theoretical simulation for the solution sample [1]. Removal of one ligand from the 2.04Å shell (Fig 3b) without refinement produces an improvement in the fit index from 0.5 to 0.3. Refinement of this model reduces the fit index to 0.16 (Fig 3c). We suggest the most probable explanation for these effects is that a co-ordinated water molecule is lost on freeze-drying which results in minor ligand rearrangement including the shortening of the 'long' first shell distance to 2.02Å. E.p.r. measurements at 77K (not presented here) confirm the observed perturbation at the iron sites as a consequence of freeze-drying.

The near edge structure for Fe_2COT , the N- and C- terminal single domain fragments and the C- monoferric intact molecule (in which the N- site is unoccupied) are shown in Fig 4. The similarity of the C- terminal fragment and its monoferric counterpart suggests that the iron site remains largely unperturbed by the fragmentation process.

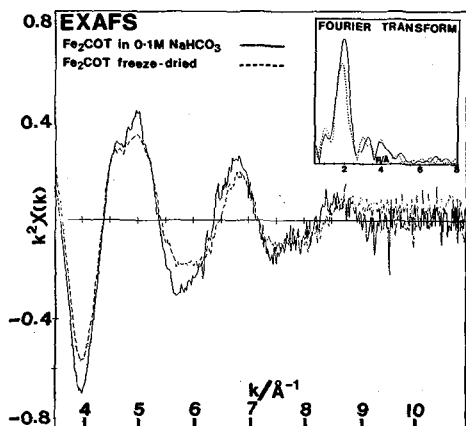


Figure 1 EXAFS and Fourier transforms (inset) of Fe_2COT recorded in 0.1M NaHCO_3 (—) and as a freeze-dried powder (---).

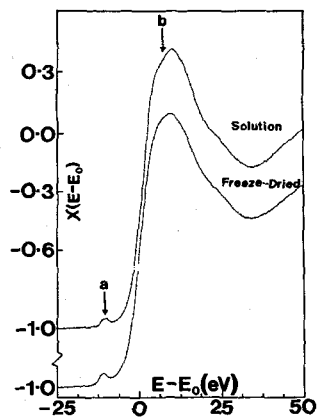


Figure 2 XANES spectra of Fe_2COT in 0.01M NaHCO_3 solution (above) and as a freeze-dried powder (below).

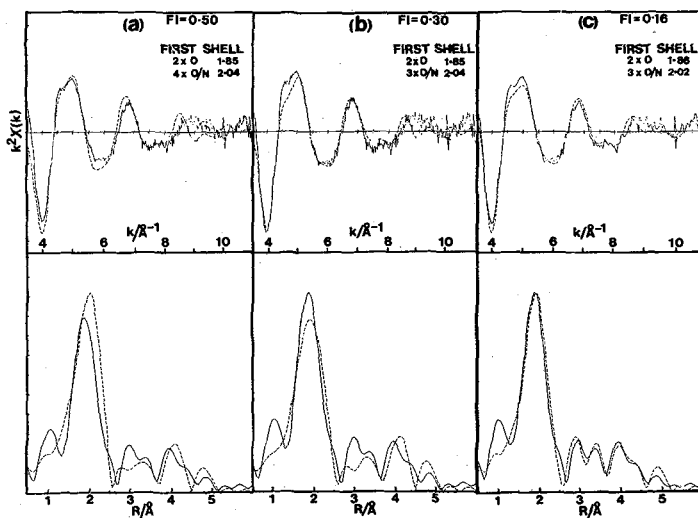


Figure 3 Simulation of the freeze-dried Fe_2COT EXAFS experiment (—), theory (---). FI is the fit index (a) parameters are those for Fe_2COT in solution [1], (b) assuming a 2/3 split first shell, (c) as in (b) after refinement.

The similarity of all spectra shown in Fig 4 means that it is not possible to definitely determine any structural interaction between the sites of chicken ovotransferrin, despite the fact that interdomain interactions are known to occur [6] and that the protein shows cooperativity for iron binding [7]. However, recently collected data on human serum transferrin which is reported elsewhere [8] has demonstrated that for the human protein at least, such intersite communication does result in significant geometrical changes at the iron sites which are observed in the edge structure.

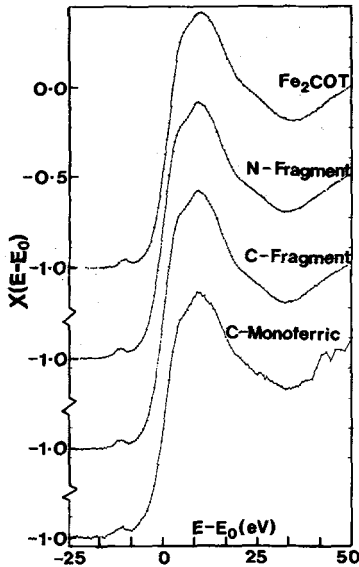


Figure 4 XANES spectra of Fe_2COT , the N- and C- terminal single domain fragments and the C- monoferric intact molecule.

Acknowledgements

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