EXAFS analysis of the zinc-binding domain of boar spermatidal transition protein 2

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Boar Spermatidal Transition Protein 2 (TP2; 137 amino acid residues) is supposed to play an important role in initiation of chromatin condensation and cessation of transcriptional activity during mammalian spermiogenesis. Boar TP2 has three potential zinc finger motifs and binds three atoms of zinc per molecule. However the structure of the zinc-binding domain of boar TP2 has not been completely determined. To elucidate the local structure around the zinc atoms of boar TP2, we performed an X-ray absorption fine structure (XAFS) measurement on the zinc-binding domain of TP2(TP2Z)(residues 1-103) in the fluorescence mode. By EXAFS analyses we have demonstrated that each of the three zinc atoms is coordinated by approximately two sulfur and two nitrogen atoms on average. The average Zn-S and Zn-N distances were found to be 2.36 Å and 2.01 Å, respectively. The sulfur and nitrogen atoms are attributed to cysteine and histidine residues, respectively, from comparison of the EXAFS spectra with model compounds ZnS and ZnTPP(zinc(II) tetraphenylporphyrin).

Keywords: Transition Protein 2; **zinc finger**; **fluorescence XAFS.**

1. Introduction

The chromatin structure undergoes extensive modification during mammalian spermatogenesis. Nucleosomal histones are transiently replaced by small basic proteins called transition proteins (TP1-4), and finally, by protamines. At the time, transformation of the nucleosomal-type chromatin into a highly compact and condensed chromatin fiber, initiation of chromatin condensation, and cessation of transcription occur. By comparison of the amino acid sequence of boar TP2 with the sequences of rat and mouse TP2 (Keim *et al.*, 1992 and Meetei *et al.*, 1996), it has been proposed that boar TP2 has three zinc finger motifs in the N-terminal 3/4 region and binds three atoms of zinc per molecule of the protein as shown in Fig. 1 (Sato *et al.*, 1999). Zinc finger domains are known to be of great importance in a number of proteins that are involved in nucleic acid binding and transcriptional control. Although it has been shown that boar TP2 binds three zinc atoms from atomic absorption and CD spectroscopies (Akama *et al.*, 1997), there is no direct evidence that boar TP2 has zinc finger structures at present. Therefore it is very important to study its structure by some direct methods to clarify the properties and functions of boar TP2.

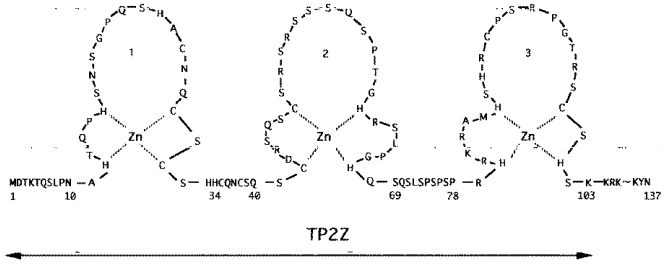

Figure 1
A scheme of boar TP2 with three potential zinc finger motifs and its zinc-binding domain (TP2Z).

Recently, we have developed an expression system for isolating the zinc-binding domain of boar TP2 (TP2Z) (Sato *et al.*, 1999), which enables us to prepare enough protein for an extended X-ray absorption fine structure (EXAFS) experiment. Atomic absorption and CD spectroscopy show that TP2Z binds three atoms of zinc per molecule of the protein and undergoes a zinc-dependent conformational change in a manner similar to that for intact TP2. TP2Z recognizes a CpG island sequence more preferentially than intact TP2, and its specificity is dependent on zinc (Sato *et al.*, 1999).

In this paper, we report the results of EXAFS analyses that directly probe the local environment around the zinc atoms of boar TP2Z. The zinc atoms of TP2Z are coordinated by two sulfur atoms with an average Zn-S distance of 2.36 Å and by two nitrogen atoms with an average Zn-N distance of 2.01 Å. Their atoms are attributed to cysteine and histidine residues, respectively.

2. Materials and methods

2.1. Preparation of boar TP2Z

Boar TP2Z was expressed in *Escherichia coli*, isolated and refolded as described previously (Sato *et al.*, 1999). The freshly refolded TP2Z was used in the experiments.

2.2. EXAFS studies

The experiments were performed using the laboratory XAFS measurement system EXAC820 (Technos Co., Ltd.) which is equipped with a rotating-anode X-ray source and a curved-crystal monochromator. Typically, the X-ray source was operated at 17 kV, 100mA in the measurements. We measured zinc K-edge X-ray absorption spectra of boar TP2Z in fluorescence mode using a Ge(220) crystal monochromator. Data were collected in 5 eV increments in the EXAFS region. The measurement time was 60 seconds per point. The sample used in the measurement was a 400 μl solution which contained 1.0 mM of TP2Z (3.0 mM of zinc). We used a sample cell with a 10 μm polypropylene window. Measurements were also carried out on ZnS and ZnTPP (zinc(II) tetraphenylporphyrin) (Scheidt *et al.*, 1986) as model compounds of the boar TP2Z in transmission mode using a Ge(400) crystal monochromator. To optimize edge jumps, a powder of these compounds was diluted with graphite and formed into disks with 13 mm diameter. In fluorescence mode, the incident and fluorescent X-ray intensities, I_0 and F, were monitored by a Ne-gas proportional counter and a three-element Ge detector. In transmission mode, the incident and transmitted X-ray intensities, I_0 and I, were monitored by an Ar-gas proportional counter and a Ge solid state detector. Typically, data were collected in the energy range $h\nu$ =

9360 - 10400 eV. All measurements were carried out at room temperature. To determine the local structure parameters, a computer code was developed and employed for the data analysis. X-ray absorption is calculated as $\mu t = \ln(I_0/I)$ in transmission mode and F/I_0 in fluorescence mode. The self absorption effect is negligible for this zinc concentration. A smoothed atomic absorption spectrum μ_0 was estimated by the smoothing spline method (Cook *et al.*, 1981). The spectra were converted to $\chi(k)$ space and multiplied by k^3, which were Fourier-filtered and analyzed using the single-scattering EXAFS formula. Amplitude factor and phase shifts were calculated using FEFF 8 (Rehr *et al.*, 1992).

3. Results and discussion

3.1. Analysis using model compounds

The fluorescence EXAFS spectrum of boar TP2Z is shown in Fig. 2. Figure 3 shows the EXAFS oscillation $\chi(k)k^3$ obtained by subtracting the smooth atomic absorption from the above spectrum.

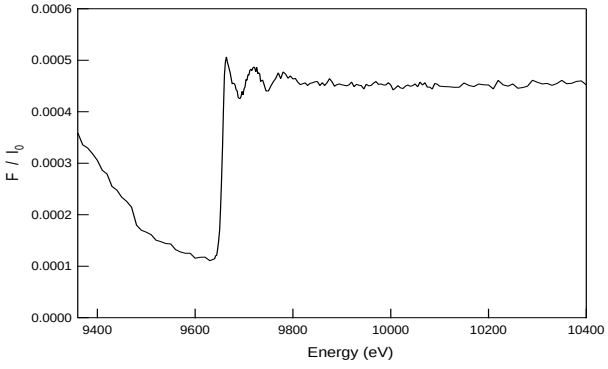

Figure 2
Fluorescent EXAFS spectrum of boar TP2Z (3.0 mM in Zinc). The X-ray fluorescence divided by incident X-ray intensity is plotted.

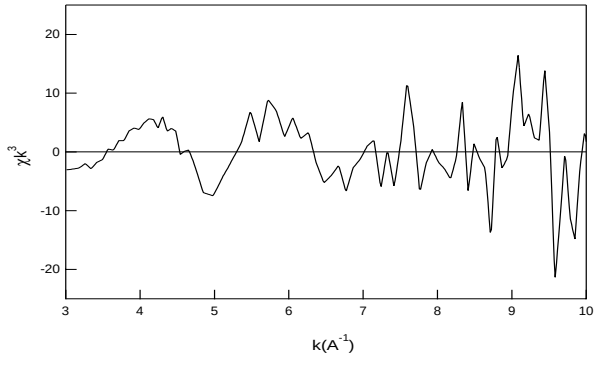

Figure 3
$\chi(k)k^3$ data from boar TP2Z spectrum (fig. 2). The χ data are multiplied by k^3 to enhance the oscillations at high k values, so that the low k values have a small influence in the nonlinear-least-squares fitting. The background removal (or isolated atom subtraction) was accomplished by using the smoothing-spline method. Windows from 3.0 to 11 Å^{-1} were used to generate the FT data of Fig. 4.

Fourier transformed (FT) spectra of boar TP2Z and the model compounds are shown in Fig. 4. The FT data are quite interesting, since they reflect the coordination around the zinc atom. The boar TP2Z FT data can be seen as a combination of nearest-neighbor contributions of the ZnTPP and ZnS spectra. It has two peaks at 1.5 Å and 2.0 Å, where the first-shell peak of ZnTPP and ZnS spectra is located at 1.6 Å corresponding to the nearest-neighbor nitrogen atoms, and 2.0 Å corresponding to the nearest-neighbor sulfur atoms, respectively. In addition, ZnTPP data clearly show the TPP macrocycle contributions (α carbons at 2.6 Å and β carbons at 3.8 Å). The ZnS data show the next-nearest zinc atoms at about 3.5 Å. The fact that the position of the main peak (2.0 Å) of boar TP2Z spectrum is about the same as that of ZnS indicates that the zinc atoms of boar TP2Z bind sulfur atoms. The other peaks(at 1.5, 2.6, 3.2 and 4.0 Å) of the boar TP2Z spectrum has similar structures to that of ZnTPP. This strongly indicates that the zinc atoms of boar TP2Z bind the nitrogen atoms of the histidine residues. The zinc atoms in ZnS are coordinated by four sulfur atoms, and in ZnTPP by four nitrogen atoms.

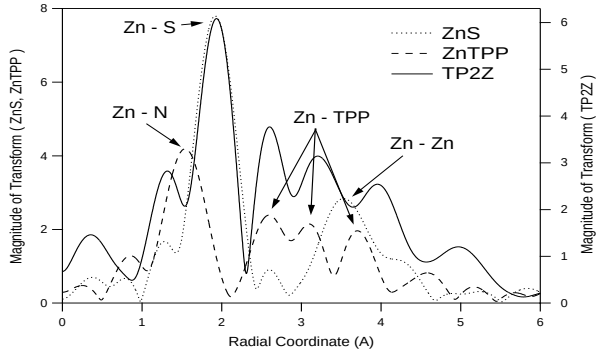

Figure 4
Fourier transforms of χk^3 for ZnTPP, ZnS and TP2Z. The transform magnitude is plotted versus the relative radial distance from the central atom in Å. The ZnTPP (dashed line, left ordinate) shows a main peak from the four coordinated nitrogen at 1.8 Å and TPP macrocycle contributions at 2.6, 3.1, and 3.8 Å. The ZnS (dotted line, left ordinate) shows a main peak from the coordinated sulfur atoms at 2.0 Å and the distant zinc atoms at 3.5 Å. The ZnTPP (solid line, right ordinate) shows a main peak at 1.8 Å and three subpeaks at 2.6, 3.2, and 4.0 Å

From Fig. 4, it can be seen that the relative peak height at 1.5 Å and 2.0 Å in boar TP2Z spectra is roughly the same as that of the main peaks of ZnTPP and ZnS. It can be inferred from this that in boar TP2Z, the number of sulfur atoms around a zinc atom is roughly the same as the number of nitrogen atoms. These observations are consistent with the proposed picture (Fig. 1), namely that three zinc atoms in boar TP2Z are coordinated by sulfur and nitrogen atoms which are attributed to cysteine and histidine residues, respectively.

3.2. Curve fitting

Shown in Fig. 5 is the Fourier-filtered spectrum of boar TP2Z. We determined distances, Debye-Waller factors and edge energies by nonlinear least-squares fitting. In the forward Fourier-transform we used the data from 1.5 to 12 Å^{-1} and from 1.0 to 2.6 Å in the Fourier-filtering. With these windows sufficient degrees of freedom are available in a two-atom type fit. The data range used for the fitting was 3-10 Å^{-1}. Variations in the individual Zn-S distance are not resolved by this procedure; with this k-range we cannot resolve contributions that are separated by ≤ 0.1Å.

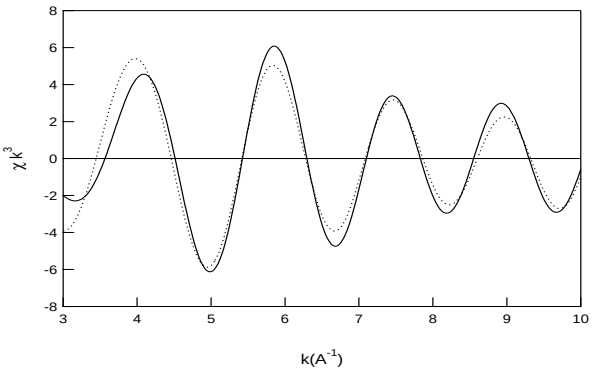

Figure 5
Fourier-filtered data from boar TP2Z (solid line) and nonlinear least-squares fitting data (dashed line). The former results from a window of 1.0-2.3 Å applied to FT data of boar TP2Z in Fig. 4. The latter involves two shell fitting (2:2 S:N) using the single scattering EXAFS equation.

Table 1
Nonlinear least-squares fitting results of boar TP2Z. Coordination numbers are held fixed to resolve the Zn-S and Zn-N contributions to the data. The 2:2 S:N coordination numbers provide a good fit.

Ratio of S to N ligands (S:N)	Model	Distance (Å)	ΔE_0 (eV)	$\Delta\sigma^2$ ($Å^2$)	χ^2
4 : 0	Zn-S	2.33	23.64	0.00946	70.35
3 : 1	Zn-S	2.33	23.11	0.00636	64.35
	Zn-N	1.89	-19.37	0.00333	
2 : 2	Zn-S	2.36	7.82	0.00288	54.13
	Zn-N	2.01	-1.15	0.00739	
1 : 3	Zn-S	2.38	1.78	-0.00232	61.10
	Zn-N	2.08	6.76	0.00590	
4 : 0	Zn-N	2.20	16.56	0.00216	220.24

The results of curve fitting with fixed coordination numbers are listed in Table 1. Using the single-scattering EXAFS formula, we obtained the distances, Debye-Waller factors, and edge energies for boar TP2Z along with the sum-of-residuals squared (χ^2). We compared fits with several different ratios of sulfur and nitrogen coordination numbers (S:N) held fixed in the fitting procedure. In table 1, the S:N = 2:2 fit has the best χ^2, with an average Zn-N distance of 2.01 Å and an average Zn-S distance of 2.36 Å. The Zn-ligand distances obtained are reasonable on the basis of surveys of crystal structure data. In this case the individual Zn-S distances cannot be distinguished because the resolution of the data, i. e. the ability to distinguish distances differing by less than 0.1 Å, is limited by the data range. However, the difference between the Zn-S and Zn-N distances can be safely resolved. The S:N = 3:1 and 1:3 fits have a χ^2 value only slightly higher than the 2:2 fit. However these fits have unreasonable values of edge energy (-19.7 eV) or Debye-Waller factor ($-2.32 \times 10^{-3} Å^2$). Also the other fits are poor; 4:0 and 0:4 fits have χ^2 values outside the reasonable range. Present EXAFS data exclusively suggests two sulfur ligands and two nitrogen ligands. The EXAFS data reflects an average coordination number of the three zinc atoms in TP2Z. Present result is consistent with the S:N ratio of 1.7:2.3 suggested by the scheme in Fig. 1.

4. Conclusions

We have measured zinc K-edge XAFS spectra of boar TP2Z, ZnS and ZnTPP. From comparison of the EXAFS spectra with model compounds ZnS and ZnTPP it was found that three zinc atoms in boar TP2Z were coordinated by the sulfur and nitrogen atoms attributed to cysteine and histidine residues. From the two shell curve fitting, the zinc atoms of boar TP2Z were coordinated by two sulfur and two nitrogen atoms on average, with average Zn-S distance of 2.36 Å and average Zn-N distance 2.01 Å.

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