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Studies of Fe(II) and Fe(III)-DNA complexes by XANES spectroscopy

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The composition of the ligands around the iron centers in Fe(II) and Fe(III)-DNA complexes has been investigated by X-ray Absorption Near Edge Structure (XANES) spectroscopy at the Fe-K-edge. The spectra are compared to the models $FePO_4$ and $Fe(dadipy)_2$, each one bearing only one of the ligands expected in the Fe-DNA interaction, namely oxygen (from phosphate groups, H_2O or bases) and nitrogen (from bases), respectively. The spectrum of Fe(II)-DNA exhibits a higher pre-edge peak and a more prominent shoulder at 7145 eV than Fe(III)-DNA. These spectral features indicate a distortion associated with the the presence of nitrogen in the first coordination sphere Fe(II)-DNA. These spectral features indicate a distortion associated with the presence of nitrogen in the first coordenation sphere of Fe(II)-DNA. Similarities between Fe(III)-DNA and $FePO_4$ are consistent with the possibility that only oxygen atoms are coordinated to the Fe(III) center.

Keywords: Iron-DNA complexes, $FePO_4$, $Fe(dadipy)_2$, X-ray absorption

1. Introduction

The presence of iron in the nucleus of mammalian cells has been associated with cancer and aging because iron can bind to DNA and catalyze free radical reactions, which are responsible for several pre-mutagenic DNA modifications (Meneghini et al., 1995). However, the nature of the interaction between iron and DNA is still ill defined. In a previous study of DNA damage it was observed that the DNA modification 3'-phosphogycolate is formed at much lower levels in cytosine-nucleotides than in other DNA residues of cells undergoing oxidative stress (Bertoncini & Meneghini, 1995). This effect has been attributed to a non-randomic Fe-DNA binding, since the very reactive free radicals, generated at the iron center, attack a nearby DNA residue to produce the DNA damage. An older study of iron-DNA binding by pulsed NMR spectroscopy suggested the participation of both phosphate and base moieties of DNA in iron chelation (Eisinger et al., 1962). However, these data can not be directly extended to physiological systems, because the work was performed under conditions favoring DNA denaturation and saturation of the iron binding DNA sites. This is, to our knowledge, the first work using XAS spectroscopy addressed to the structure of Fe-DNA complexes.

2. Materials and Methods

The Fe(III)-DNA and Fe(II)-DNA complexes were prepared in 0,01M Tris/Cl buffer (pH=7,4) by addition of ferric or ferrous ammonium sulfate to aqueous solution of 6mM calf thymus DNA. The mixtures were stirred for two days. Fe(II)-DNA was prepared under N_2 atmosphere. The separation of free iron from the DNA bound iron by Sephadex G25 columns was performed as previously described (Neto et al, 1991). DNA and iron concentration in the eluted fractions were measured by reading A_{260} and A_{520} (Bralet et al., 1992), respectively. $Fe(dadipy)_2$ was synthesized and characterized as described by Toma and Chaves-Gil, 1997.

All the Fe K edge spectra were recorded in the transmission mode at room temperature at the XAS beam line of the LNLS light source (Campinas, Brazil), running at 1.37 GeV and 120 mA. Fe-DNA samples consisted of 1 mg of purified solid complexes containing 1 iron per 10 nucleotides. The data were collected with a Si(220) double-crystal monochromator, with 0.4 eV steps.

3. Results and Discussion

The experimental XANES spectra of Fe(II) and Fe(III)-DNA are shown in Fig 1. The spectra exhibit some absorption features similar to that of carbonyl-iron-porphyrins octahedral reported by Cartier et al., 1992. We are, thus, following their notations to indicate the main features of our spectra, which are the pre-edge transition P, a main edge peak, C1, followed by a defined shoulder, D and a pronounced shoulder, C2. There are few, but relevant differences between Fe(II)-DNA and Fe(III)-DNA spectra: Fe(II)-DNA exhibits a more intense pre-edge peak P, lower C1 and a more prominent shoulder C2 than Fe(III)-DNA. These features indicate that the Fe(II) is in a more distorted octahedral than Fe(III).

Figure 2 illustrates the comparison between spectra of Iron-DNA complexes and inorganic compounds $FePO_4$ and $Fe(II)(dadipy)_2$, (dadipy = diacethylimino-piridine) Similarities are observed along the whole spectra of Fe(III)-DNA and $FePO_4$. However, the C2 shoulder is very pronounced in $Fe(dadipy)_2$ compared to Fe(II)-DNA. In a previous work of FEFF calculation of Cobalamins and related compounds spectra, it was considered that the shoulder C2 is determined by a resonance identified to the multiple scattering paths involving the nitrogen first neighbors through the Co central atom (Mimouni et al., 1997). Probably, the same interpretation can be applied to $Fe(dadipy)_2$ complex. However, similar structure is unlikely in Fe(II)-DNA, if we take into account the large size of DNA molecule and the stereochemical difficulties for more than one nitrogen to be involved in iron chelation. A possible explanation for the shoulder C2 in Fe(II)-DNA spectrum is the contribution of multiple scattering resonances involving multiple scattering pathways within the purinic or pyrimidinic base moieties, in the case of a ring nitrogen atom to be directly coordinated to Fe(II). The peak D has been considered as a 1s-4px,y transition, which becomes stronger in a more distorted octahedral. Generally, this distortion is imposed by stereochemical constraint. This does not seem to be the case for the Fe-DNA complexes, since peak D is barely defined in their spectra.

The pre-edge spectra of all compounds are illustrated by the amplification of the correspondent region in the Figure 3.

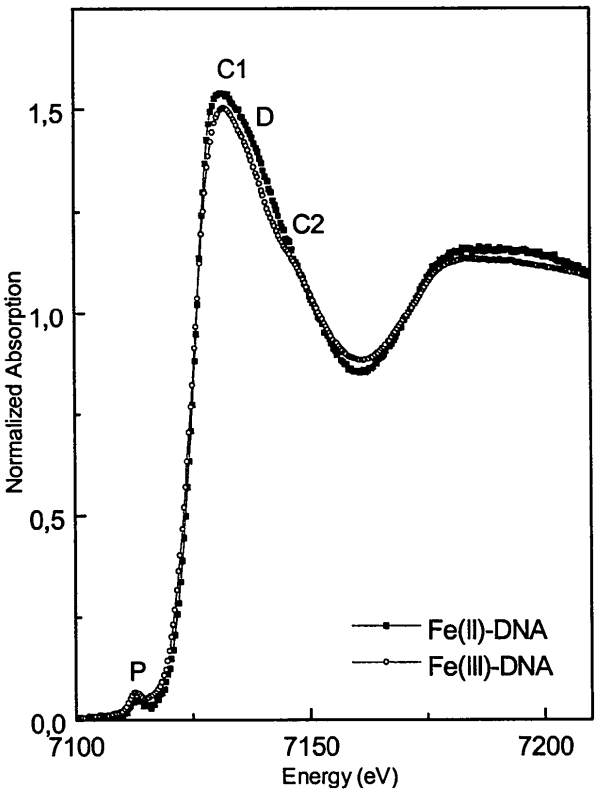

Figure 1
Fe K edge XANES spectra of Fe(II) and Fe(III)-DNA complexes.

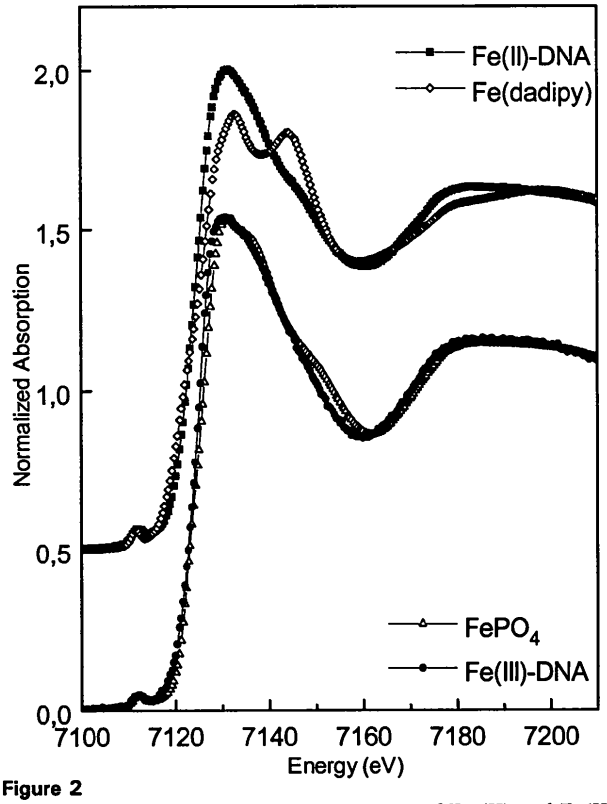

Figure 2
Comparison of Fe K edge XANES spectra of Fe (II) and Fe(III)-DNA and $FePO_4$ and $Fe(dadipy)_2$.

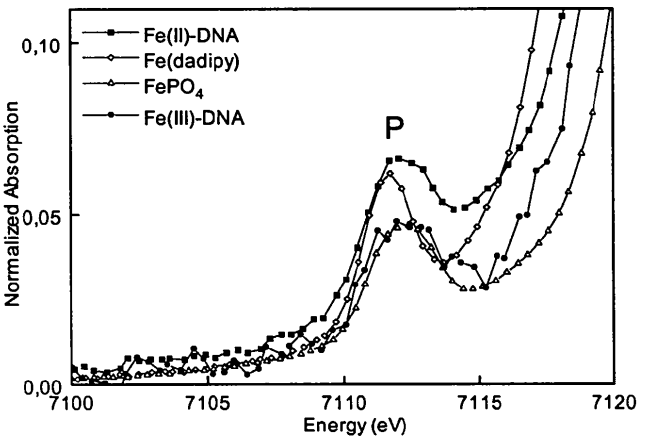

Figure 3
Fe K pre-edges spectra of the Fe(II) and Fe(III) compounds.

There are many similarities between Fe(III)-DNA and $FePO_4$ as well as between Fe(II)DNA and $Fe(dadipy)_2$. The latter exhibit higher peak P than the former two. It has been established that as a metal site distorts from a centrosymmetric environment, the intensity of peak P increases, because the dipole forbidden transition 1s-3d becomes partially allowed owing to mixing of the 3d-4p orbitals. Thence, we can deduce that iron centers have differences in hybridization and are more symmetric in Fe(III) than in Fe(II)-DNA. These results give support to the interpretation that only oxygen atoms participate in Fe(III) chelation to form a more symmetric core in Fe(III)-DNA.
It is possible that oxygen ligands from different moieties, such as phosphate, bases and H_20, are responsible for the distortion in Fe(II)-DNA. However, in view of the XANES spectra, specially the shoulder C2, we suggest that nitrogen is one of the ligands in an axial position of the first coordination sphere of Fe(II)-DNA. This may be an explanation for the specific Fe-DNA binding.

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