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UV-Induced Reduction of Cu(II) in DNA Complex Studied by Cu-K-Edge XANES

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INTRODUCTION

DNA *in vivo* is known to contain copper ions that can react with the nitrogen bases and produce changes in the electronic spectrum of this molecule, which are mainly evident in the 300–320 nm range.[1]

One would expect that, because of the higher absorbance, the DNA-Cu(II) complex would suffer far more damage when irradiated with mid-uv light than Cu-free DNA.

Preliminary results[2] show that irradiation at $\lambda = 310$ nm of the DNA-Cu(II) complex causes a photoreduction of some of the copper sites: i.e., the DNA-Cu(II) complex is gradually transformed under irradiation to a DNA-Cu(I) complex.

It is known that divalent copper ions bound to DNA may also be reduced *in situ* by the addition of reductants such as ascorbic acid or borohydride.[3] Since copper reduction and oxidation may affect the local stability of double-stranded DNA, it has been suggested that this process is involved in the control of DNA as a primer.[3] However, information about copper reduction in the DNA complex has been based on indirect observations such as changes in the optical properties of the DNA molecule. It is therefore desirable to perform direct measurements that allow a more quantitative analysis of this process.

In this paper we show that high-resolution x-ray absorption spectroscopy is a suitable tool for this purpose. We have measured the percentage of copper sites that have been reduced by uv radiation via the intensity of the $1s^2\ 3d^{10}\ 4p^0 \rightarrow 1s^1\ 3d^{10}\ 4p^1$ localized atomiclike excitation that is characteristic of the Cu(I) ion. Evidence of a maximum of 35% reduced copper sites is given, suggesting the presence of a specific class of sites affected by uv radiation.

MATERIALS AND METHODS

Materials

DNA from calf thymus was purchased from Boehringer Mannheim GmbH. Overnight dialysis against $10^{-2}M$ NaCl with $2 \times 10^{-3}M$ EDTA (disodium salt) and subsequent dialysis against $10^{-2}M$ NaCl were performed. The DNA-Cu(II)

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complex was obtained by mixing a DNA solution with microliter quantities of a $CuCl_2$ solution for a ratio of 0.25 for the [Cu]/[nucleotide] concentration.

All the salts used were reagent grade, and deionized water (produced by a Millipore milliQ apparatus) was used throughout the experiment.

Nucleotide concentration was determined spectrophotometrically using the extinction coefficient ϵ $(P) = 6600$ mol^{-1} cm^{-1}.[4] The sample concentration used for the spectrophotometric measurements corresponded to $A_{260} = 1$ and that used for x-ray spectroscopy to $A_{260} = 40$. All the experiments were made with an unbuffered solution, pH about 6, to avoid chelation of Cu(II) by buffer components.

UV Irradiation

The DNA sample contained in a 1-cm path quartz cuvette, was irradiated with monochromatic light at 310 $\pm$ 10 nm using a Hilger & Watts D96 quartz prism monochromator equipped with an Oriel high pressure Xe-Hg 1000 W lamp. The samples were stirred during the irradiation to ensure a uniform distribution of uv photons. The average fluence was 750 kJ/m^2, which corresponds to the maximum observed effect. The fluence was measured with a thermopile-nanovoltmeter calibrated system.

Spectrophotometric Measurements

All the absorption spectra were measured at 20°C by a double-beam Cary 118 spectrophotometer equipped with an external thermostat device.

The modifications induced by uv radiation in the absorption spectrum of the DNA-Cu(II) complex were followed by a differential technique, where the sample was the irradiated DNA-Cu solution contained in the cuvette used for irradiation, and the reference was the nonirradiated DNA-Cu(II) solution.

RESULTS AND DISCUSSION

In Fig. 1 we report the x-ray absorption spectra of DNA-Cu(II) and of the uv-irradiated sample. The pre-edge background has been subtracted and the absorption coefficient α has been normalized to the high-energy atomic absorption $\alpha_{A\infty}$ value at ~100 eV above the jump.

In Fig. 1(a) we identify the characteristic pre-edge dipole-forbidden 1s$\rightarrow$3d transition p_2, and the main multiple scattering resonance A for excitations of the photoelectron of p symmetry in the continuum.

The XANES spectrum of DNA-Cu(II) shows the A, B, C, and D features at higher energies due to multiple scattering similar to those found for the Cu(II) in solution. The transition to the atomic continuum can be simulated in a first approximation with an arctan function, as shown in the figure. The spectrum of the reduced sample is shown in Fig. 1(b). The intensity decrease of the main multiple scattering resonance A and the overall variation of the XANES spectrum indicate a change of the Cu site structure.

A new feature P_1 appears at threshold after irradiation. We have fixed the zero of the energy scale E_0 at the energy of the 1s$\rightarrow$3d weak pre-peak P_2 of the DNA-Cu(II) complex. In this energy scale the feature P_1 is at ~3 eV. This feature transition appears in the polarized XANES spectra of single crystals only in the directions where there are no neighbor atoms.[5] We assign this transition to an atomiclike $1s^2\ 3d^{10}\ 4p^0 \rightarrow 1s^1\ 3d^{10}\ 4p^1$ excitation characteristic of Cu(I) compounds,[6] corresponding to a quasi-atomic bound state 4p due to

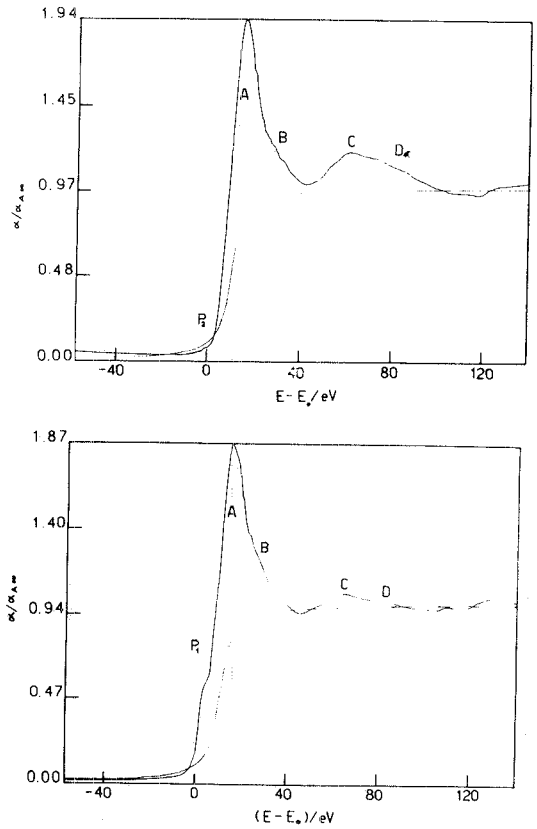

Fig. 1. X-ray absorption near the edge spectrum of DNA-Cu(II) (panel a); the same after uv irradiation, showing the P_1 feature characteristic of Cu(I) (panel b). We report the relative absorption coefficient, $\alpha/\alpha_{A\infty}$ where $\alpha_{A\infty}$ is the atomic absorption at high energy. The zero of the energy scale has been fixed at the pre-edge peak P_2 of DNA-Cu(II).

an asymmetric cluster potential that is atomiclike in the direction where there are no ligands. Therefore this excitation is an atomic probe of Cu(I) ions, and it can be well identified since the absorption cross-section for Cu(II) ions is small at the same energy.

In Fig. 2 we report the XANES of DNA-Cu(II), uv-irradiated DNA-Cu and chemically reduced DNA-Cu with sodium borohydride. The continuum cross section, simulated with an arctan function shown in Fig. 1, has been subtracted. The intensity scale is normalized to the value of $\alpha_{A\infty}$. The P_2 peak $1s \rightarrow 3d$ is suppressed in the reduced DNA-Cu samples because of the $3d^{10}$ configuration of Cu(I). The intensity of the P_1 peak is a direct probe of the number of reduced Cu sites. In the case of the chemically reduced sample, the intensity of the P_1 feature is like that found in Cu(I) model compounds.[5,6] The intensity of the P_1 feature in the uv-irradiated sample has been used to calculate the percentage of Cu(I) sites formed by irradiation. The experimental spectrum has been fitted with a linear combination of the XANES spectra of DNA-Cu untreated and the $NaBH_4$ fully reduced complex. We have found that only 35% of the Cu sites are reduced.

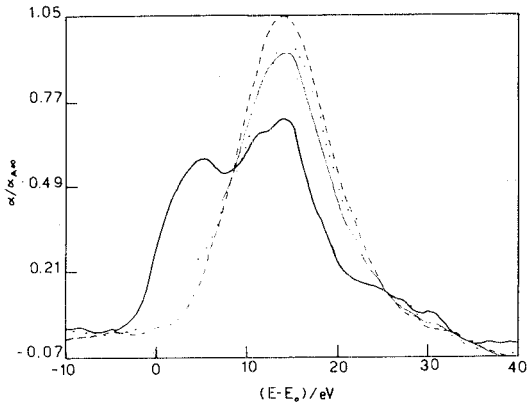

Fig. 2. Comparison of the near-edge structure of the DNA-Cu complex after subtraction of the arctan curve shown in Fig. 1: untreated (dashed line), uv irradiated (dotted line), $NaBH_4$ reduced (solid line), and linear combination [35% DNA Cu(I) + 65% DNA-Cu(II)] (light line).

We found that irradiation at $\lambda = 310 \pm 10$ nm of the DNA-Cu(II) complex also causes marked changes (fluence dependent) in the DNA absorption spectrum in the uv region, as shown in Fig. 3. We observe a decrease of extinction between 240 and 280 nm with a minimum at $\lambda = 257.5$ nm and an increase at $\lambda > 280$ nm with a maximum at $\lambda = 296.5$ nm. This difference spectrum is similar to that obtained by adding reducing agents, such as sodium borohydride, to a DNA solution containing Cu(II) ions.

The difference between the optical spectra of the chemically reduced and the uv-irradiated DNA, in the energy range around 260 nm, the DNA absorption band, can be assigned to local denaturation of irradiated DNA.[7] The maximum absorption change at 296.5 nm in the uv-irradiated sample, even

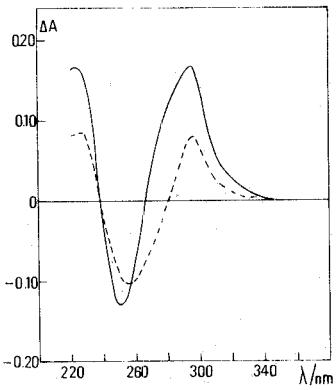

Fig. 3. Changes in the DNA-Cu(II) ($r = 0.25$) absorption spectrum as a result of irradiation at 310 nm or addition of sodium borohydride. The difference ΔA (in absorbance) between treated and untreated samples is plotted. DNA-Cu(II) irradiated at the fluence $F = 750$ kJ/m^2 (dashed line), DNA-Cu(II) reduced to DNA-Cu(I) by adding sodium borohydride ($[NaBH_4]/[CuCl_2] = 1$) (solid line).

after very large fluences, is only about 35–40% of the maximum change observed by adding sodium borohydride, thus indicating that uv irradiation results in a photoreduction of only some of the copper sites.

The photoreduction process is strictly connected with uv absorption of the DNA-Cu(II) complex, which exhibits a small absorption band, extending up to mid-uv, assigned to a charge transfer excitation. This excitation probably consists of a one-electron transfer from a ligand to the Cu(II) ion leading to a Cu(I) species and a radical.

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