

EXAFS Investigation of the Iron Center in the AlkB Protein

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Introduction

The *Escherichia coli* AlkB protein has been known to play an important role in alkylated DNA damage repair [1, 2]; however, the exact function of AlkB was only recently discovered. From using sequence profile analysis, AlkB was predicted to be a member of the 2-ketoglutarate- and iron-dependent dioxygenase superfamily that contains a metal-ion-binding site [3]. Subsequent biochemical studies revealed that the repair of the cytotoxic 1-methyladenine and 3-methylcytosine (Fig. 1A) in single- and double-stranded DNA by *E. coli* AlkB was dependent on the addition of dioxygen, 2-ketoglutarate, and iron (II). The DNA repair function of AlkB represents a new type of activity that directly removes DNA-alkylation damage through a metal-mediated mechanism. A novel oxidative demethylation mechanism was proposed, as shown in Fig. 1B [4, 5]. Human homologues of AlkB were found to exhibit similar repair reactivities [6].

Despite breakthroughs in identifying possible cellular targets for AlkB, the exact repair mechanism of AlkB is still unknown. We have recently identified the active site of this protein through a chemical cross-linking study [7]; however, the presence of an iron atom in this active site has not been established unambiguously. Our recent effort has resulted in isolation of iron-containing AlkB directly from *E. coli*.

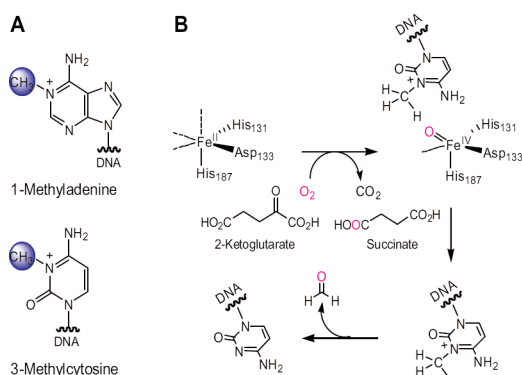


FIG. 1. A shows 1-methyladenine and 3-methylcytosine lesions in DNA. B shows proposed oxidative demethylation of 1-methyladenine and 3-methylcytosine lesions in DNA by AlkB in a putative iron (II)-containing active site.

Methods and Materials

The current study aims to probe the local structure of the active-site iron in AlkB in the absence and presence of DNA by x-ray absorption spectroscopy (XAS) studies. The iron-containing protein is quite unstable. The active-site iron (II) was found to be oxidized after being stored for several days at -80°C and overnight at 4°C . The close proximity of the APS not only provides a brilliant x-ray source but also provides prompt access for investigating proteins with limited stability. After several trials, we found that the best way to ensure the quality of this protein is to have it purified in the morning and immediately transported to the APS for spectroscopic measurement.

Results and Discussion

Good-quality spectra of iron (II)-AlkB and of iron (II)-AlkB bound to a single-stranded DNA were obtained from the measurements performed in July and November 2003 (Fig. 2). The presence of iron (II) in the protein was confirmed from the study. The coordination environment was revealed for the first time for this class of very important DNA repair proteins. The potential perturbation of the iron geometry caused by DNA

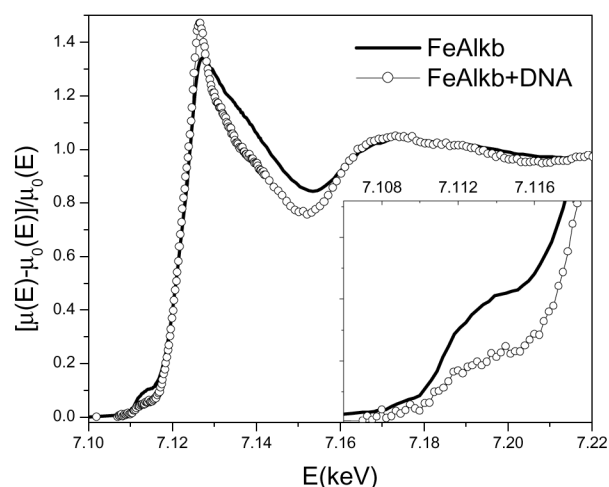


FIG. 2. XANES spectra of FeAlkb and FeAlkb+DNA, where the change in the pre-edge peak indicates increased central symmetry around the Fe(II) site due to the binding, and the change in the white light peak intensity suggests an increase in the coordination number of Fe(II) due to the binding.

binding can be investigated. The preliminary data indicate that as the protein binds the DNA, the coordination number increases, and the oxidation state of iron remains the same. Further detailed data analysis is in progress. These results are very important and will lead to publications in the near future. We believe that in the future, a series of such studies on the protein substrates and its inhibitors will greatly help in understanding the mechanism and function of this protein family.

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