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Structural Characterization of Ethylene Binding Site in Ethylene Sensor Protein ETR1 by XAFS

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Introduction

The gaseous hormone ethylene (C_2H_4) is involved in the regulation of developmental processes and stress responses in higher plants, induces seed germination, seedling growth, leaf abscission, fruit ripening, organ senescence and pathogen responses. Ethylene responses in *Arabidopsis* are mediated by a small family of receptors, including ETR1. The ETR1 protein consists of three domains: a membrane-associated ethylene-binding domain, a histidine kinase domain, and a signal receiver domain. As far, the molecular mechanisms underlying ethylene perception and signal transduction have not been established. The three-dimensional structural information is essential for understanding the mechanisms. Although the crystal structure of the signal receiver domain is known, there is no structural information about the ethylene-binding domain. In our best knowledge, copper (I) ion associated with the ethylene-binding domain is required for high-affinity ethylene-binding activity. [1] To further delineate the structural information on ETR1, we have measured Cu K-edge XAFS spectra of the ultra-diluted solution (0.1mM) of the ethylene-binding domain with and without ethylene gas treatment.

Material and methods

Sample preparation

The ethylene-binding domain of ETR1 protein was expressed in *E. coli*. Addition of $CuSO_4$ in the presence of excess thiol reductant to the solubilized membrane and successive purification yield a complex with a copper/protein ratio of 0.5 to 0.7. The samples were concentrated to 0.1 mM at maximum. Further concentration caused aggregation. Sample of the ethylene-binding form was prepared by mean of incubation in sealed micro tube for 1 h in the presence of ethylene gas.

XAFS measurements

All XAFS measurements were performed at BL12C station. The Cu K-edge absorption spectra were recorded in the fluorescence mode with 19 elements Ge SSD detector at 77 K.

Results and discussion

Figure 1 show the Cu K-edge XANES spectra for the ultra-diluted ETR1 solution (0.1mM). Although the considerable oxidation state for ethylene-binding is Cu (I), the spectra we have obtained first showed typical edge feature for Cu (II) (**B** in Figure 1), indicating the oxidation of copper, as is usually observed in mononuclear Cu (I) coordination complexes. Proteins that stabilize mononuclear Cu (I) form a constrained His_2Cys coordination environment as in blue copper protein. [2] Thus, the coordination environment in ETR1 should differ from blue copper protein.

To suppress the oxidation, 10 mM thiol reductant (β-mercaptethanol) was added to the all buffers used in purification steps. The XANES spectra of ETR1 purified in the presence of the reductant exhibited a weak shoulder around 8984 eV, which is typical of Cu (I) (**A** in Figure 1) and different from the previous spectra for Cu (II). The sample treated with ethylene gas showed the same feature and no differences were observed. Because the 8984 eV peak is correlated with site geometry, binding of ethylene to Cu (I) ion should lead to the change of the peak feature.

The work needs to be continued to determine the structure change upon the ethylene binding.

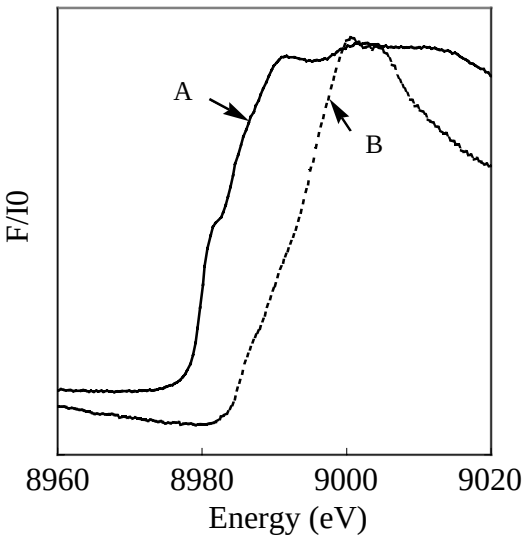

Figure 1 Cu K-edge XANES spectra for the ethylene-binding domain of ETR1 purified in the presence (A) and absence (B) of a thiol reductant.

References

[1] F. I. Rodríguez et al., Science. 283, 996 (1999).
[2] E. I. Solomon et al., Chem Rev. 96, 2563 (1996).

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