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Measurement of electric field gradient at ^{117}In on the Cu-site in mavicyanin by perturbed angular correlation of γ–rays

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Abstract The structure around the metal site of mavicyanin, a protein molecule with a copper site, was investigated in solution by using time-differential perturbed angular correlation of ^{117}In. The electric field gradient (EFG) of the metal site was deduced from the measurement. It demonstrated that the site in a mutant-type mavicyanin, Thr15Ala-Mav, gives an EFG different from that in the wild-type mavicyanin does. The pH dependence of the EFG was also observed for both proteins.

Keywords, Perturbed angular correlation of gamma-rays · In-117 · mavicyanin · cupredoxin · pH dependence

1 Introduction

Mavicyanin isolated from zucchini peelings is a member of the cupredoxin family or blue copper protein, but its physiological role in plants is still unknown [1-3]. The protein was found to have a copper ion coordinated with two His, one Cys residues and a Gln residue with an amide-oxygen atom as shown in Figure1 [3]. It was chosen as a sample in the present study because it has a relatively low molecular weight and the biosynthetic technique for it was well established by one of the authors (K. Kataoka) [3].

In order to investigate the electric field gradient (EFG) at a site occupied by a nuclear probe, perturbed angular correlation of γ-rays (PAC) has an advantage in application for a relevant atom at a site of interest replaced by a gamma-ray emitter, irrespective of the physical state of a sample [4]. Thus, it is especially a powerful method to measure the EFG at a metal site of a biological molecule in an aqueous solution representative of physiological conditions [5].

In this study, the structure around the metal site of mavicyanin was investigated by using time-differential PAC of ^{117}In. Its parent nucleus ^{117}Cd with a half-life $t_{1/2} = 2.49$ h deexcites by β^-decay mode to populate the 749 keV excited state of ^{117}In, decaying to the 315 keV excited state through the 660 keV intermediate state having a spin $I = \frac{3}{2}$, $t_{1/2} = 53.6$ ns, and an electric quadrupole moment $Q = (-)0.59(1)$ b [6]. The aim of the study is the assessment of this technique for the study of biological molecule in living matter. For this purpose, we utilized the PAC method for the first time for wild-type mavicyanin and mutant-type mavicyanin (Thr15Ala-Mav), which have redox potentials of 213 mV and 283 mV, respectively, although their UV-vis spectra are indistinguishable [7].The difference in redox potential is assumed to reflect the difference of the hydrogen-bonding network in the reduced Cu site.

2 Experiments

Site-directed mutagenesis and preparations of wild-type and mutant-type mavicyanins were performed as described previously [3]. Recombinant mavicyanin expressed as inclusion bodies in transformed *Escherichia coli* cells was isolated and solubilized by urea. Then, the proteins were refolded through five stepwise dialysis. Finally, copper-free proteins of mavicyanin were prepared with dialysis procedures against 0.1 M KCN.

The parent nuclei ^{117}Cd was obtained from enriched ^{116}CdO (96.53%) irradiated at Kyoto University Research Reactor Institute. The Cd ions from the oxide were introduced in mavicyanin, from which Cu ions were extracted beforehand. It is reasonable to assume that ^{117}Cd enters the Cu site from the well known fact that ^{111m}Cd occupies the Cu site of other small blue copper proteins (see [8]). Finally, sucrose was added at a concentration of 50 weight % in the solution to slow down rotational motion, which would otherwise damp an oscillatory pattern of the PAC time spectrum. The sample solution thereby obtained was subjected to a PAC measurement at a temperature of some degrees below room temperature while being cooled on a Peltier device.

The PAC measurement system used in this study is the same that was mentioned in [9]. The directional anisotropy $A_{22}G_{22}(t)$ derived from the measurement, denoted as $R(t)$ in Figure 2, is expressed as follows for an ensemble of randomly oriented molecules in liquid but in inactive molecular motion.

$$A_{22}G_{22}(t) = A_{22}\,[1+4\cos\omega_0 t]/5 \quad \text{with} \quad \omega_0 = 6\omega_Q(1+\eta^2/3)^{1/2}. \tag{1}$$

The value of A_{22} for ^{117}In ($\leftarrow$ ^{117}Cd), which depends only on the nuclear transitions, is −0.36 [4]. The perturbation factor $G_{22}(t)$ is a function of the electric quadrupole frequency ω_Q and the asymmetry parameter η of EFG. The ω_Q is defined as $\hbar\omega_Q = -eQV_{zz}/[4I(2I-1)]$, where V_{zz} is the largest component of EFG in the principal axes system.

3 Results and discussion

A TDPAC spectrum obtained for mutant-type mavicyanin is shown as an example in Figure 2. The TDPAC spectra were fitted with

Equation 1 with the parameter ω_0 of one component, as drawn in Figure 2 with the solid line. The frequency was thus obtained and the $|V_{zz}|$ was derived by tentatively using the η value of 0.45 [8] for stellacyanin having a structure similar to mavicyanin. Although the true η value for mavicyanin can be different from 0.45, the resultant error of $|V_{zz}|$ is at most 12%. The $|V_{zz}|$ values thus obtained are 2.08×10^{22} Vm^{-2} at pH =7.5 and 1.90×10^{22} Vm^{-2} at pH = 8.0 for the wild type and 1.49×10^{22} Vm^{-2} at pH = 7.5 and 1.40×10^{22} Vm^{-2} at pH = 8.0 for the mutant type. The statistical uncertainties of EFG were estimated to be about 5% at most. The $|V_{zz}|$ values obtained in this work seem to be reasonable, compared with that for stellacyanin 1.71×10^{22} Vm^{-2} at pH = 7.2-7.9 [8] and show a meaningful difference between the two types of mavicyanin irrespective of the adopted value of η.

For the mutant type, there seems a large decrease of $|V_{zz}|$ at pH = 6.0, namely, a change of the perturbation between pH = 6.0 and 7.5 compared to the difference between pH = 7.5 and 8.0, while the change is not clear for the wild type probably due to poor statistics of the spectrum at pH = 6.0. The change between pH 6.0 and 7.5 might be related to a structural change around the metal site. However, more data in relation to the pH dependence as well as the aid of theoretical calculations are necessary for detailed discussions on the structure and relevant function of the molecule.

What should be noted is that the V_{zz} values for the mutant-type mavicyanin are smaller than the corresponding values for the wild type. This observation indicates that V_{zz} is sensitive to some structural change between the two types of mavicyanin, probably related to redox potential change found for them, although their UV-vis. spectra agree with each other and do not provide information on the structure change [10].

A PAC probe of ^{111}Cd produced by decay of ^{111}Ag or ^{111m}Cd has been often used to study copper proteins although it is still not clear how the cadmium ion could mimic a copper ion in a biomolecule. The present ^{117}In probe has the same problem and also an indium ion normally takes a charge state of +3 unlike a copper ion. However, we expect that the data with ^{117}In help us to obtain valuable information on the nature of the chemical bonding of a metal atom with its surroundings through, for example, an observation of a structural relaxation after decay accompanying the charge state and ionic size changes, if they are compared with those from the other probe ^{111}Cd.

The PAC technique was applied to the measurement of the electric field gradients in small biological molecules in solution where they could function in life. We consider that the PAC method is useful to observe a structural change of the metal site which plays an important role in physiology. We are planning to develop the technique with the aid of theoretical calculations to further investigate the structure of the molecules in life.

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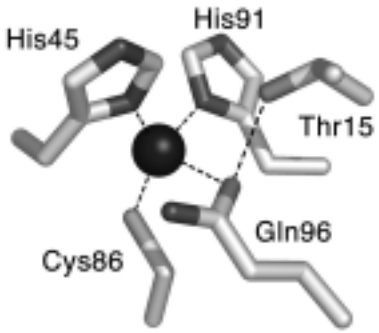

Figure 1. Structure of the copper-ion binding site in mavicyanin [3].

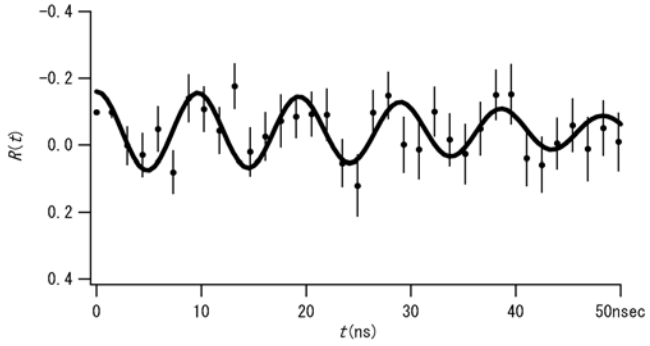

Figure 2. ^{117}In-TDPAC spectrum for a mutant-type mavicyanin, Thr15Ala-Mav, measured at pH 8.0.