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ESR Simulation Analysis of pH-induced Conformational Change in PrP^c as Labeled by the Site-Directed Spin Labeling (SDSL) Method

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[Introduction] The prion (PrP) diseases such as BSE and CJD have been reported to attribute to the abnormality of protein conformation [1]. The conformational change of PrP may be induced by the pH-induced, binding of Cu-ion and the salt bridge. ESR spectroscopy with the site-directed spin labeling (SDSL) method is a powerful method for obtaining conformational information [1]. However, without the theoretical calculations, the analysis of the ESR spectra is ultimately difficult for the system of protein due to its complexity [2]. We employed the simulation method in order to obtain the best fitting to the experimental spectra by the theoretical calculations. The ESR simulation enables us to estimate the time-dependent parameters of molecular motion, i.e., the correlation time and the order parameter.

[Method] The theoretical scheme of simulation in the present study is based on the motional narrowing. The ESR spectrum of PrP showed a mixed spectrum as composed of different components with a fast molecular motion (Mb) and with a slow motion (Im). For this reason, we calculated the Mb and the Im components separately, then, they were superimposed with a suitable ratio that yields a best fitting spectrum to the experimental spectrum.

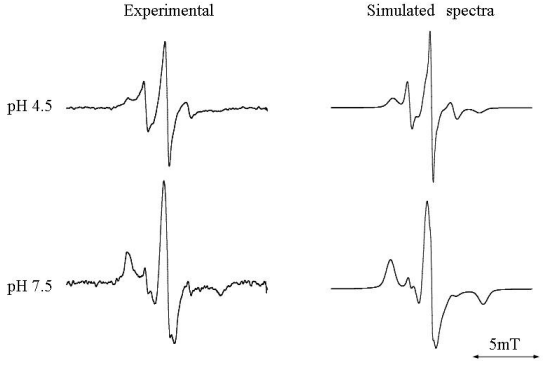

Fig. 1 Simulation (right-column) of experimental ESR spectra (left-column) of Prion at two representative pHs.

[Results and discussion] We carried out the simulation fitting to the experimental spectrum by adding the Mb and the Im components (Fig. 1). The Mb component exhibits the intense molecular motion of nitroxide moiety that yields an ESR spectrum of isotropic motion. The Im component indicates a spectrum that is not in the complete rigid limit, but in the restricted motion (Table 1). Motional mode of prion protein varies with pH. Namely, amplitude of molecular motion (β_0) changes at a transition point of pH6.0. This suggests that order parameter of motion also changes by pH. However, the correlation time (τ), the speed of a molecular motion is invariant during the pH change (Table 1).

[Reference] **[1]** O. Inanami et al Biochem. Biophys. Res. Commun, **335**, 785-792 (2005). **[2]** Y. Shimoyama et al. Biochemica et Biophysica Acta, **508**, 213-235 (1978).

Table 1 Parameters used for simulations of the immobile component in the ESR spectra of spin labeled PrP.

pH	W_L [mT]	ϕ_0 [deg]	β_0 [deg]	τ_t [ns]	τ_{at} [ns]	τ_{eff} [ns]	Im/Mb
4.5	0.60	90	35	1.0	0.5	0.33	5
5.0	0.60	90	30	1.0	0.5	0.33	5
5.5	0.60	90	28	1.0	0.5	0.33	5
6.0	0.60	90	28	1.0	0.5	0.33	5
6.5	0.60	90	25	1.0	0.5	0.33	30
7.0	0.60	90	25	1.0	0.5	0.33	30
7.5	0.60	90	25	1.0	0.5	0.33	100