

## Spectral Simulation of ESR from Site-directed Spin-labeling Prion: Salt Bridge Formation of Protein

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**Introduction** The abnormal prion (PrP<sup>SC</sup>) is a transformation of normal prion (PrP<sup>C</sup>) by the various causes, such as, the accumulation of the copper ion in the cell, decrease of pH and the salt bridge formation. The ESR spectrum of the site-directed mutagenesis spin label (SDSL) prion exhibits two components, i.e., the mobile (Mb) and immobile (Im) spectra. ESR of Y161R1 demonstrates a very strong Im component. Y161R1 is the pH sensitive region. PrP<sup>C</sup> has special amino acid - amino acid interaction unit that is influenced by the pH near by Y161R1. The histidine residue at pH of 6.0 changes the electric potential and forms the salt bridge with other amino acids. It is known that D177 and Y127 form a salt bridge. Since, in PrP<sup>C</sup>, H176 and D177 are located near by Y161R1, one needs to examine the pH sensitivity on the salt bridge by ESR and SDSL methods. We will analyze motional parameters using theoretical calculations.

**Methods:** We prepared three samples that are the modified proteins. The preparative methods of the prion proteins were described elsewhere [1]. ESR measurements were also seen elsewhere [1]. The theoretical scheme of simulation in the present study is based on the motional narrowing [2]. The ESR line position and line width were determined by the spin Hamiltonian undergoing restricted motions and the correlation times, respectively. The Mb and Im components were calculated separately; then, they were added with a suitable ratio that yields the best fitting spectrum to the experimental one.

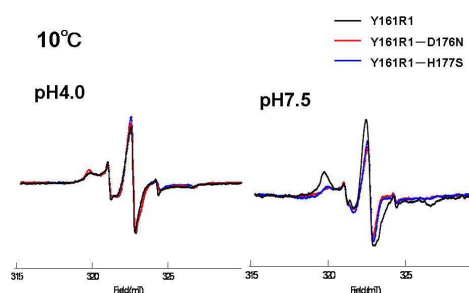


Fig.1 ESR spectra of Y161R1, Y161R1/D177N and Y161R1/H177S at pH 4.0 and 7.5 and 10

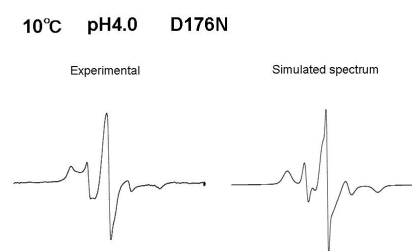


Fig.2 Experimental and simulation spectra of Y161R1/D177N in pH 4.0 at 10

**Results and Discussion:** Figure 1 shows stack plots of ESR spectra from the modified PrP<sup>C</sup> at the pH of 4.0 and 7.5. All the spectra in Fig. 1 indicate that Im contribution is always stronger than that of Mb, and reflecting that SDSL moieties in PrP<sup>C</sup> are somewhat undergoing the restricted motions. At pH 7.5, in particular, wild type of prion shows the strongest Im contribution to the spectrum. This means that breakage of the salt bridge decreases the Im contribution, simultaneously increases the fast motion. On the other hand, at the pH 4.0, ESR signals show essentially the same spectra. This indicates that salt bridges are in the thermal

equilibrium with pH 4.0 environment.

The simulation spectrum for the wild type at pH 7.5 yielded highest Im/Mb ratio of 40, indicating that the immobilization undergoes during the conservation of salt bridge in PrP<sup>C</sup>. However, upon bridge breakage Im/Mb ratio decreased down to 10, four times smaller than the wild type. Salt bridge conserves restricted motion; hence the higher order structure (3D or further). Moreover, the simulation shown in Fig.2 indicated that order parameter of the Im in Y161R1 at pH7.5 is not different from Y161R1, Y161R1/D177N, and Y161R1/H176S at pH4.0.

**References** [1] Y. Watanabe et al **B.B.R.C.**, submitted. [2] Y. Shimoyama et al. **B.B.A. Biomembranes** 508, 213-235 (1978).