효소의 회전반경과 유전율 특성 분석을 통한 유기용매내 구조-활성 예측

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Prediction of Enzyme Structure-Activity Relationships by Measuring Radius of Gyration and Dielectric Values between Protein and Water-Miscible Organic Solvents

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Introduction

The study on the enzyme structure-activity correlation is of special interest, because it gives us important information for designing an organic solvent reaction media in order to avoid enzyme structure denaturation[1]. Furthermore, it may offer important rules to control the enzyme activity and selectivity by organic solvents, since the change of the structure will alter the intrinsic properties of the enzyme, which will govern stability, catalytic activity and some special functions such as regio- and stereo-selectivity, etc.

Our research topic, therefore, is to find out the interplay of the structure changes by organic solvents which can limit the biological activity. In our preliminary experiments(H. Joo and Y. J. Yoo, *Theories and Applications of Chem. Eng.*, 1995, **1**, 475), we introduced a promising parameter, grdius of gyratrion, to visualize the real changes of enzyme conformation in organic solvent media. The peroxidase activity for all the solvent tested were inversely proportional to the increments of radius of gyration, and the relation linearly depended on the solvent. Nevertheless, another activity loss with solvents of low dielectric was observed in that experiment, which was not the result from large-size expansion of protein structure. The non-structural deactivation was reflected as decrease of slopes in Figure 1. Initially, we did not speculate the fundamental understanding of the deactivation Currently, however, full understanding of such phenomenon is vital and inevitable because the loss of enzyme activity was too remarkable to ignore and some ambiguities about forces affecting the peroxidase deactivation were still remains unsolved.

Material and Methods

Horseradish peroxidase(HRP) was chosen as a model protein(EC 1.11.1.7, RZ 2.0, 150-200

units/mg solid; Sigma, St. Louis, MO). The remaining activities of the HRP in various solvent system was measured after the 20 minutes incubation. The activities were measured using the UV-detectable pyrogallol dye(λ_{max} at 420 nm).

Radius of gyration, the parameter in relating the conformational features of polymer to the physical properties of polymer and its solvent, was chosen in the present study to predict the overall expansion of the enzyme. Ubbelohde type capillary viscometer(Scott Gerate, Germany) was used to measure the radius of gyration in a given solvent system.

The real dielectric value of protein by using dielectric analyzer(DEA, TA Instruments) was measured. In DEA operation, well dried peroxidase powder stored in desiccator(2 weeks) was placed between two gold-plated ceramic sensors(thickness; 0.232 mm) and a sinusoidal voltage was applied with a frequency range(0.01 Hz to 10^5 Hz).

Results and Discussion

By taking the assumption that the dielectricity of the HRP surface is uniform and following the model of Tanford and Kirkwood[2], the value of the enzyme surface dielectricity in aqueous solution was easily estimated(ε=80). The surface dielectric value is generally accepted as reasonable, because enzymes have charged groups where water bound on the surface of the protein. It is not clear, however, how to determine the interior dielectric values of the protein by using the continuum models. The dielectric for a protein is somewhat variable with some factors, number of charged molecules, radius of the protein, hydrophobicities of the amino acids, etc. In order to verify and qunatify the forces affecting the protein denaturation, we must eliminate solution properties to the enzyme and know the net(intrinsic) dielectric values of the bulk protein, not in the solution state. In the present study, the real dielectric value of protein by using dielectric analyzer(DEA, TA Instruments) was measured. The result allows us to probe the molecular mobility(or relaxations) of peroxidase. High frequency domain reflecting the large-scale bulk motion of the protein is linked to high dielectric property and the low frequency reflecting the residual motion is linked to very low dielectric property of molecules. The two dielectric constants of the protein bulk and water(is referenced in the study as ε_{H2O}=80) were estimated by extraporating the permittivity(e') to the static(zero) frequency. The measured permanent dielectric constant for the portein bulk is about ε_{BULK}=0.84. The value is nearly same as the interior protein, since the protein consists of 96 % wt.frac. hydrophobic innerpart.

According to the Lifshitz theory[3] dealing interactions in a medium(Y-axis; the water is referenced in the Figure 2), the Hamaker constant at zero frequency between water(ε=80) and peroxidase bulk($\sim$ interior protein;ε=0.84), leads to a quite difference of van der Waals forces. The Hamaker constant indicating electrostatic or columbic interactions between peroxidase bulk($\sim$ interior) proteins in a water medium is about 0.297 x 10^{-20} J at 298.15 K. On the

contrary, the Hamaker constant for the interactions with dioxane is about 0.623 $\times 10^{-21}$ J at 298.15K. Van der Waals interaction energy for the dioxane-protein interaction is 5-times lower than in water. Note that van der Waals force is much reduced in low dielectric medium than in water, and the force was indicated as a slope in Figure 1. For the cases, the entropic force such as hydrophobic interaction not the dispersion force dominates the interaction between non-polar molecules.

From the result, we conclude that two types of peroxidase deactivation occur in water-water miscible organic solvent systems. One is the structural deformation related enzyme deactivation, another is deactivation by the loss of surface flexibility. In relatively polar solvents, large size expansion of the enzyme molecule is expected because the solvents can replace water from the innerpart of the protein. In this manner, small decrease of enzyme activity is observed. In non-polar(low polar) solvents, severe activity loss is arising owing to the lowering nature of the surface flexibility by non-polar solvents, whereas minor(or none) structure changes are observed. This is due to the fact that the polar amino acid side chains are located in the enzyme surface, not in the protein bulk, correspondingly the liberative movement of this group involves higher dielectricity(or dipolarity) than that of large-scale macromolecule motion to which the side amino acid are attached, as shown in DEA result(Figure 2). The similar molecular behaviors are also observed in the dielectric absorption spectrum of amorphous polymethyl(or polypropyl) methacrylate[3].

Conclusions

To summarize, the aim of this work was to find ways of visualizing and figure out the enzyme structure-activity relationships by introducing a parameter, radius of gyration, in the water-miscible solvent system. In our earlier experiment, the change of radius of gyration ranging 3.45 Å(in water) to a max 24.5 Å(in water-methanol binary mixture) for peroxidase was served as a good parameter for the prediction of the enzyme activity in the organic solvent. Dielectric properties(ε/μ) of the solvent and enzymes surface were also served as a good parametric values for the prediction. Finally, In the present study, we could find the meaning of the dielectric property(ε/μ) and forces affecting the protein stability.

References

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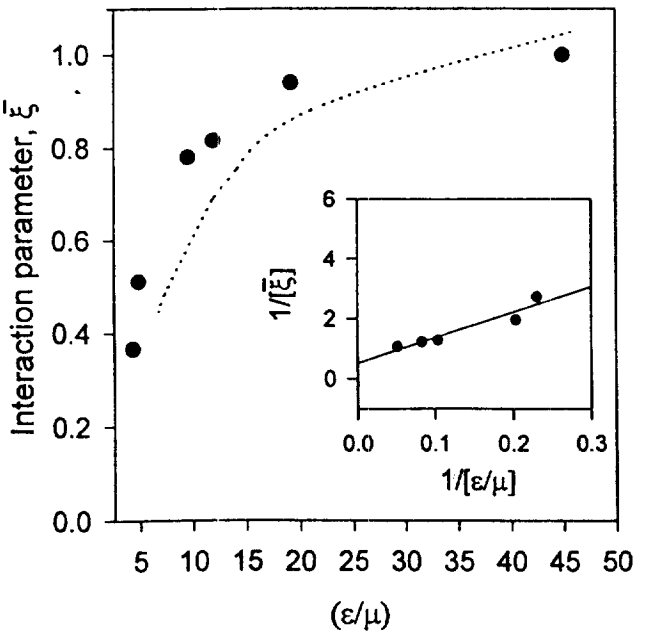

Fig.1 Effect of the peroxidase structure-activity interaction on the solvent dielectric property(ε/μ). Dotted line shows simulation result. *from (H. Joo, Y.J. Yoo,* Theories and Applications of Chem. Eng. *1, 475, 1995).*

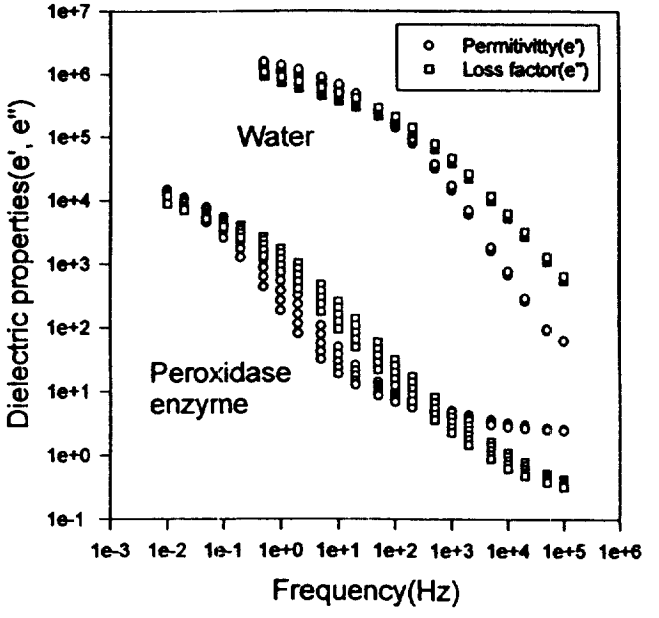

Fig. 2 Mreasurement of dielectric values for the bulk protein and water.