

산화환원 효소의 최적 pH 예측

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The Optimum pH of Oxidoreductases: A Comparison Between Experimental and Calculated pH Optimum

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요 약. 본 논문에서는 산화 환원 효소의 최적 pH를 예측해 보았다. Boltzman 분배를 이용하여 어떤 아미노산의 side chain이 물에서 발견될 수 있는 상대적 확률이나 물에 대한 상대적 친화력을 구하였으며 pK_a 를 이용해 protonated 아미노산의 양과 deprotonated 아미노산의 양을 계산하였다. 효소의 최적 pH는 아미노산의 side chain이 물에서 발견될 수 있는 상대적 확률과 protonated 또는 deprotonated된 아미노산 양의 곱인 유효protonated된 양과 유효deprotonated된 양이 같아지는 pH로 예측하였다. 문헌 값과 비교해 보았을 때, 예측치는 상당히 일치하는 경향을 보였으며 이 결과로 효소 자체의 전도도가 생물학적 기능에 있어 매우 중요한 역할을 하는 것을 알 수 있었다.

주제어: 효소, 최적 pH, 산화환원효소, 전자 전달

ABSTRACT. For various oxidoreductases, the optimum pHs of the enzymes can be calculated using the rule based on proton transfer. Relative probability of a certain amino acid side chain to be in the water, or the relative affinity to the water was calculated using Boltzman distribution. Also, the protonated and deprotonated portions of a certain amino acid side chain were calculated using pK_a of that and the effective protonated and deprotonated portions were the product of relative probability and the protonated and deprotonated portions. Where the total effective protonated portion was equal to the effective deprotonated portion of amino acid side chains, it was expected that oxidoreductases have maximum activities. The optimum pHs calculated by our rule were compared with the experimental results.

Keywords: Enzyme, Optimum pH, Oxidoreductase, Proton transfer

INTRODUCTION

In enzymology, various parameters such as temperature, pressure, solvent, and pH affect the enzyme characteristics.¹⁻³ In extreme pH, enzymes lose their activities due to several reasons.^{4,5} Especially, enzymes have the optimum pH for reaction in various solvents including water,⁶ thus buffer solution has been used in enzymatic reactions to maintain the optimum pH of the reaction system. Furthermore, several reactions are accelerated or

inhibited by controlling pH of the reaction system. Optimum pH of enzyme was changed by modification of some residues or immobilization of enzyme.⁷ However, the optimum pH in enzymatic reaction has been determined experimentally until now, not theoretically, since the principle of optimum pH in enzymatic reaction has not been understood.

Ionic states of enzymes affect the overall dipole moments of the enzymes. This might determine the intrinsic conductivities of enzymes. Many researchers have investigated the ionic conductivities of

enzymes using dielectric measurements.⁸⁻¹¹ It was reported that α -dispersion is associated with charge carriers that hop between the localized sites^{8, 10} and the conductivity is suddenly increased at the long-range percolation of the charges along the pathways connecting the localized sites. For lysozyme, some researchers showed a relationship between the α -dispersion and the conductivity was dominated by mobile protons.^{12, 13} Hawkes and Pethig¹¹ emphasized the α -dispersion on lysozyme is caused by proton transfer between the ionizable side group with pH. Using dielectric-gravimetric technique, Careri *et al.*^{14, 15} indicated that the flow of proton is channeled through the enzymes active site. They analyzed their results using the percolation theory of the protonic conduction along the hydrogen-bonded water molecules.

It was reported that as the contents of the water are increased, the capacitance and the activity of enzyme are enhanced and enzyme has identical breakthrough point between the conductivity and the activity of the enzyme.¹⁵ Rosenberg¹⁶ showed that electron or hole contributes the conductivity of enzyme in almost water-free environment. However, as the content of water is increased, proton ion significantly contributes the conductivity of enzyme.^{16, 17} In general, the enzymatic reaction is performed in water. Therefore, the motion of proton ion in various pH of reaction system may affect the intrinsic conductivity of enzyme. Especially, the oxido-reduction by proton ion is important because it is involved in the reaction directly.

So, by calculating ion states of the enzymes, the motion of proton ion can be considered indirectly. In order to predict of the optimum pHs of several oxidoreductases the effective protonated and deprotonated portions were calculated using Boltzman distribution and pK_R .

MATERIALS AND METHODS

Enzymes

The optimum pHs of various oxidoreductases, such as alcohol dehydrogenase (E.C. 1.1.1.1), glucose oxidase (E.C. 1.1.3.4), dihydrofolate dehydro-

genase (E.C. 1.5.1.3), trypanothione reductase (E.C. 1.6.4.8), catalase (E.C. 1.11.1.6), horseradish peroxidase (E.C. 1.11.1.7), peroxidase (E.C. 1.11.1.7), and glutathione peroxidase (E.C. 1.11.1.9) were tested.

Gibbs free energies of solvation (from vapor phase to water phase) and pK_R 's of amino acid side chains

Gibbs free energies of solvation and pK_R 's of amino acid side chains were reported.^{18, 19} Those were used in calculation of the effective protonated and deprotonated portions of amino acid side chains. According to the hydrophathy index,¹⁸ the Gibbs free energy of solvation of arginine was assumed to be -4.5 kcal mole⁻¹. For calculating Gibbs free energies of solvation of the amino acids, normalized Gibbs free energies of the transfer of the amino acids from the vapor phase to water were used. That is, the solid phases of the amino acids were assumed to be equivalent to the vapor phase of the amino acids.

Effective protonated and deprotonated portions of amino acid side chain

From Gibbs free energy of solvation of an amino acid side chain, the relative probability for the amino acid side chain to be in water was calculated by Boltzman distribution. From pK_R 's of an amino acid side chain, the protonated and deprotonated portions of the amino acid side chain were calculated. Only amino acid side chains having the ability of being protonated and deprotonated, such as cysteine, threonine, serine, tyrosine, histidine, glutamic acid, aspartic acid, lysine, and arginine were considered because other amino acid side chains are not significantly protonated or deprotonated.

Effective protonated and deprotonated portions of an amino acid side chain are defined as the product between the relative probability for the amino acid side chain to be in water, that is related to the hydrophathy index of the amino acid side chain, and the protonated and deprotonated portions of the amino acid side chain in a certain pH.

Prediction of the optimum pH of oxidoreductases

With the change in pH of reaction system, the total effective protonated and deprotonated portions of enzyme were calculated by summing up the effective protonated and deprotonated portions of

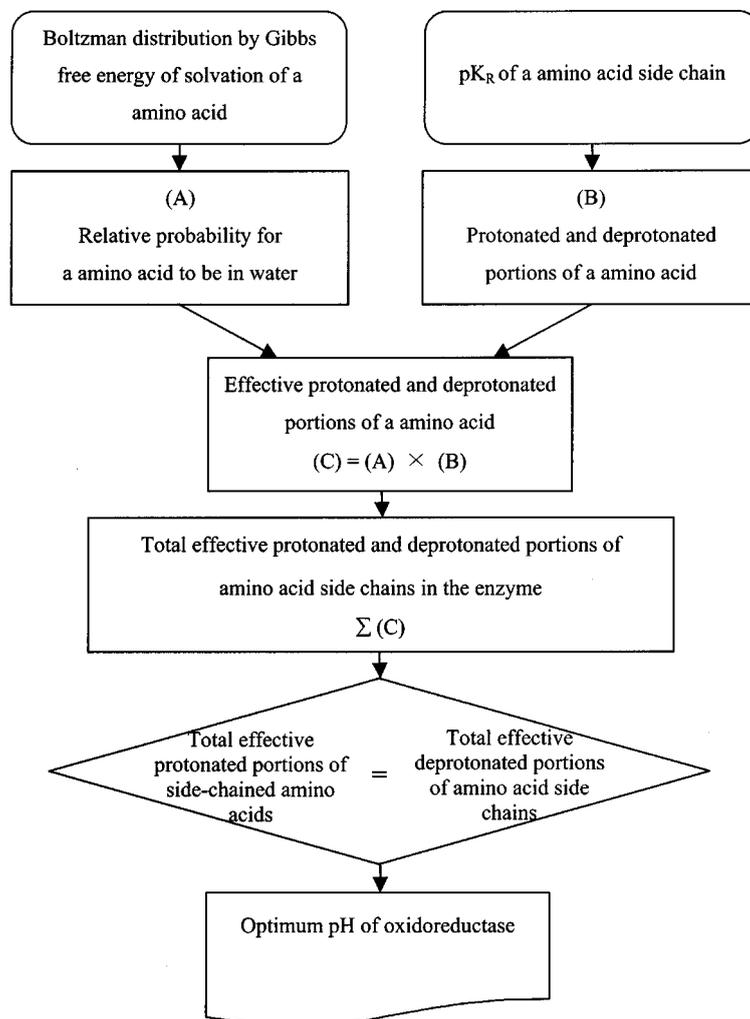


Fig. 1. Predicting method of optimum pH of oxidoreductase.

each amino acid side chain. Fig. 1 showed the schematic procedure of predicting optimum pH of oxidoreductases.

The optimum pH of the enzyme was supposed to the pH where the total effective protonated portions of the amino acids in the enzyme is the same as the total effective deprotonated portions of the amino acids in the enzyme. For example, the calculation of the effective protonated and deprotonated portions of tyrosine is as follow.

$$M_D = \frac{pK_R \times M_T}{10^{pH} \cdot pK_R}$$

$$M_p = M_T - M_D$$

$$P_{ED} = M_D \times e^{\Delta G_s / RT}$$

$$P_{EP} = M_p \times e^{-\Delta G_s / RT}$$

M_D : moles of deprotonated tyrosine

M_T : moles of tyrosine in enzyme

M_p : moles of protonated tyrosine

P_{ED} : effective deprotonated tyrosine portion

P_{EP} : effective protonated tyrosine portion

ΔG_s : ΔG of solvation of tyrosine

R: gas constant

T: temperature (K)

This prediction rule has some assumptions: 1) proton transfer paths have constant distances. 2) in narrow pH change, the conformational change is not critical. 3) changes of pK_R 's of amino acid side chains were not significant or cancelled in the overall protein. 4) the affinity between the enzyme and the substrate is not changed with the variation of pH.

RESULTS AND DISCUSSIONS

The optimum pHs of oxidoreductases from many references and our calculation results are listed in Table 1. Our predicted optimum pHs accorded with the experimental results, except for dihydrofolate dehydrogenase and trypanothione reductase. These results suggest that the optimum pHs of oxidoreductases could be related to the intrinsic conductivity of enzyme. In the FTIR studies of dehydration-induced conformational transitions, poly(L-lysine) has a sheet structure rather than helix or inordered structure in a lower degree of solvation of the water to compensate for the loss of hydrogen bonding to water on the dehydration.^{20,21} In the FTIR studies of Subtili-

sin Carlsberg, lysozyme, chymotrypsinogen, serum albumin, bovine pancreatic trypsin inhibitor, RNase A, and porcine insulin, sheet conformation appears more frequently in the water solution than in the lyophilized powder and the suspension in organic solvents.²²⁻²⁴ Therefore, it could be recognized that the intrinsic conductivity of protein should be supposed to be more contributed by proton ion in a helix-rich enzyme than sheet-rich enzyme. For this reason, the optimum pHs of the oxidoreductases that have more helix conformation than sheet conformation were tested in this study.

In our rule, the amounts of the total effective protonated amino acid side chains and the total effective deprotonated amino acid side chains were considered while distances between the effective protonated portions of amino acid side chains and effective deprotonated portions of amino acid side chains were not considered. The affinity of a certain amino acid side chain to be in the water is relatively different among various amino acid side chains. Using Gibbs free energies of solvation of amino acid side chains, those affinities were consid-

Table 1. Optimum pH of oxidoreductases

Enzyme	Predicted		Experimental		
	Source	Optimum pH	Source	Optimum pH	Ref.
Alcohol dehydrogenase (1.1.1.1)	Human liver	9.4	Human liver	8.5	25
				(oxidation of ethanol)	26
Glucose oxidase (1.1.3.4)	<i>Aspergillus niger</i>	5.85	<i>Aspergillus niger</i>	7.2 (reduction of acetaldehyde)	27 28
				5.5	
Catalase (1.11.1.6)	Bovine liver	6.3	Bovine liver	7.0	29
Horseradish peroxidase (1.11.1.7)	Horseradish	10.7	Horseradish	7.0	30
				11-12	31
Peroxidase (1.11.1.7)	<i>Arthomyces ramosus</i>	6.2	<i>Arthomyces ramosus</i>	(primary alcohol)	
				6.0	32
Glutathione peroxidase (1.11.1.9)	Bovine erythrocyte	9.95	Bovine erythrocyte	8.8	33
Dihydrofolate dehydrogenase* (1.5.1.3)	<i>E. coli</i>	5.15	<i>E. coli</i>	Two optima 4.5 and 7.0	34
Trypanothione reductase* (1.6.4.8)	<i>Crithidia fasciculata</i>	6.15	<i>Crithidia fasciculata</i>	7.5-8.0	35

*enzyme having relatively more sheet conformation.

ered in the form of probabilities. In this case, the maximum intrinsic conductivity of oxidoreductase was obtained when the total effective protonated portions of amino acid side chains were equal to that of the total effective deprotonated portions of amino acid side chains. This average sense cause the discrepancies between the optimum pI's of oxidoreductases from our prediction rule and references. Also some assumptions of calculation rule, mentioned in *Materials and Methods*, might be the causes of the discrepancies.

Since our prediction rule was mainly based on the proton transfer, oxidoreductases having more sheet moiety cannot be applied, and the rule can be applied for oxidoreductase, not any other enzymes. However, the results obtained in this study can be further used in understanding of enzymes behavior, especially for different pI buffer solutions.

REFERENCES

1. Washabaugh, M. W.; Collins, K. D. *J. Biol. Chem.* **1986**, *261*, 2477.
2. Mitraki, A.; Betton, J. M.; Desmadril, M.; Yon, J. M. *Eur. J. Biochem.* **1987**, *163*, 29.
3. Mulkerrin, M. G.; Wetzel, R. *Biochemistry* **1989**, *28*, 6556.
4. Thannhauser, T. W.; Scheraga, H. A. *Biochemistry* **1985**, *24*, 7681.
5. Hickel, A.; Grauper, M.; Lehner, D.; Hermetter, A.; Glatter, O.; Griengl, H. *Enzyme Microb. Technol.* **1997**, *21*, 361.
6. Palmer, T. *Understanding Enzymes 4th ed.*; Prentice Hall-Ellis Horwood: New York, NY, U.S.A., 1995.
7. Weetall, H. H.; Pitcher, W. H. *Science* **1986**, *232*, 1396.
8. Takashima, S.; Schwan, H. P. *J. Phys. Chem.* **1965**, *69*, 4176.
9. Eden, J. P.; Gascoyne, R. C.; Pethig, R. *J. Chem. Soc. Faraday Trans. 1* **1980**, *76*, 426.
10. Bone, S.; Eden, J.; Pethig, R. *Int J Quantum Chem: Quant Biol Symp.* **1981**, *8*, 307.
11. Hawkes, J. J.; Pethig, R. *Biochim. Biophys. Acta* **1988**, *952*, 27.
12. Morgan, H.; Pethig, R. *J. Chem. Soc. Faraday Trans. 1* **1986**, *82*, 143.
13. Lawton, B. A.; Lu, Z. H.; Wei, Y.; Pethig, R. *J. Mol. Liquids* **1989**, *42*, 83.
14. Careri, G.; Geraci, M.; Giansanti, A.; Rupley, J. A. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 5342.
15. Careri, G.; Giansanti, A.; Rupley, J. A. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 6810.
16. Rosenberg, B. *Nature* **1962**, *193*, 364.
17. Maricic, S.; Pifat, G.; Pravidic, V. *Biochim. et Biophys. Acta* **1964**, *79*, 293.
18. Kyte, J.; Doolittle, R. F. *J. Mol. Bio.* **1982**, *157*, 105.
19. Lehninger, A. L.; Nelson, D. L.; Cox, M. M. *Principles of biochemistry* 2nd ed., New York: Worth Publishers, U.S.A., 1993; pp.111-133.
20. Carpenter, J. F.; Crowe, J. H. *Biochemistry* **1989**, *28*, 3916.
21. Prestrelski, S. J.; Tedeschi, N.; Arakawa, T.; Carpenter, J. F. *Biophys. J.* **1993**, *65*, 661.
22. Mishra, P.; Griebenow, K.; Klibanov, A. M. *Biotechnol. Bioeng.* **1996**, *52*, 609.
23. Griebenow, K.; Klibanov, A. M. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 10969.
24. Griebenow, K.; Klibanov, A. M. *Biotechnol. Bioeng.* **1997**, *53*, 351.
25. Yin, S. J.; Bosron, W. F.; Magnes, L. J.; Li, T. K. *Biochemistry*, **1984**, *23*, 5847.
26. Schneider-Bemlqhkwr, H.; Formicka-Kozłowska, G.; Brieler, R.; Wartburg, J. P.; Zeppezauer, M. *Eur. J. Biochem.* **1988**, *173*, 275.
27. Bright, H.; Appleby, M. *J. Biol. Chem.* **1969**, *244*, 3625.
28. Weibel, M.; Bright, H. *J. Biol. Chem.* **1971**, *246*, 2734.
29. Maehly, A.; Chance, B. *Methods of Biochemical Analysis I*; Glick, D., Ed.; Interscience Publishers: New York, U.S.A., 1987; pp. 357-424.
30. Maehly, A. *Methods in Enzymology Vol II*; Colowick, S.; Kaplan, N., Eds.; Academic Press: New York, U.S.A., 1955; pp. 764-775.
31. Meunier, B. *Biochimie* **1987**, *69*, 3.
32. Shinmei, Y.; Amachi, T.; Asami, S.; Shimizu, S.; Yamada, H. *Agric. Biol. Chem.* **1986**, *50*, 247.
33. Wendel, A. *Methods in enzymology Vol. 77*; Jakoby, W. B. Ed.; Academic press, New York, U.S.A., 1981; pp. 325-333.
34. Baccanari, D.; Phillips, A.; Smith, S.; Sinski, D.; Burchall, J. *Biochemistry* **1975**, *14*, 5267.
35. Shames, S. L.; Fairlamb, A. H.; Cerami, A.; Walsh, C. T. *Biochemistry*, **1986**, *25*, 3519.