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Prediction of optimum pH of hydrolases

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Abstract

Hydrolase is a group of the most widely used enzymes in industrial biological processes. Generally, their activities are easily changed with pH. With this characteristics, research for the optimal pH of hydrolases is required to obtain the optimization of process conditions. We selected xylanase, lysozyme, glucoamylase and barnase as model enzymes. To predict optimum pH of hydrolases, the calculation program based on Tanford-Kirkwood(TK) model[(1)] was used. Results show that charge difference of catalytic residues is an important parameter deciding optimum pH and when charge difference of catalytic residues is maximum, optimum pH of the hydrolase establishes.

Introduction

Industrial exploitation of enzymes is making the development of cleaner, environmentally friendly processes possible. In industrial processes, however, some of its characteristics are not suitable in real process conditions, such as higher or lower pH, higher temperature, high concentration of salts and nonaqueous solvent, etc. Especially, the optimum pH prediction and modification for enzymes are necessary in several process like starch process and cheese manufacture etc.

Up to now, few approaches to predict optimum pH of enzymes have been tried. Only in the case of some hydrolases, following facts have been reported.

1. pH dependence of an enzyme is determined by the ionization of the catalytic groups, which is affected by various interactions involved in their microenvironments[(2)].
2. A local network of hydrogen bonds involving the active site residues and surrounding residues may have an influence on the rate and optimal pH of substrate cleavage[(3)].
3. The pKa values of titratable groups in proteins are determined by the strength of the electrostatic field, and both the local hydrogen bonding network

and the solvent accessibility of the titratable group influence the electrostatic field dramatically[4].

These facts show that it is possible to predict optimum pH using electrostatic forces based on hydrogen bonding network and solvent accessibility. This study gives information about optimum pH prediction of some hydrolases based on electrostatic forces. Using solvent accessibility of titratable groups and pKa value using hydrogen bonding forces, catalytic residues' charges of target enzymes were calculated. Predicted optimum pH was the pH when charge difference between catalytic residues is maximum. Based on these results, it would be possible to consider the new strategy to change optimum pH of enzyme.

Material and Methods

Fig. 1 shows the flow chart for predicting optimum pH of target hydrolases

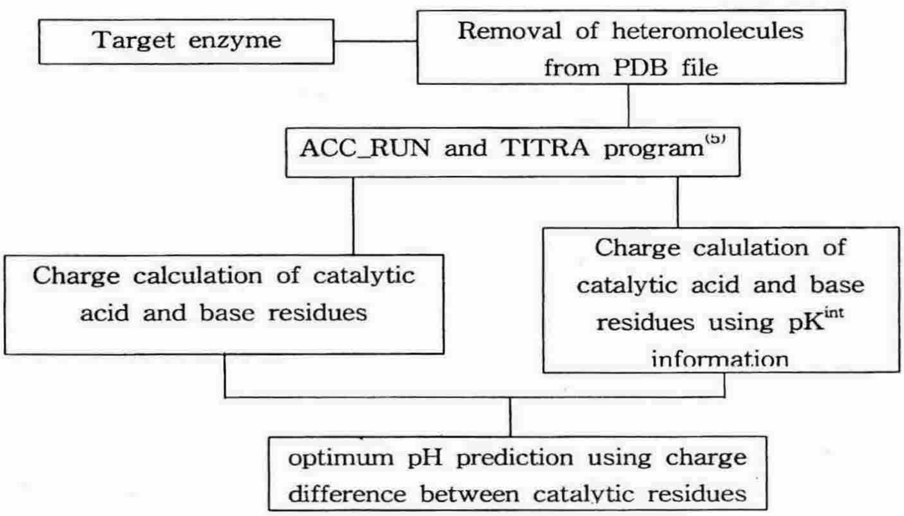

Fig 1. Flow chart for predicting optimum pH of target hydrolases

Results and Discussion

Target enzymes are xylanase, glucoamylase, lysozyme and barnase. Fig. 2~5 show that optimum pH of each hydrolases is the pH when charge difference between catalytic residues with pH is maximum.

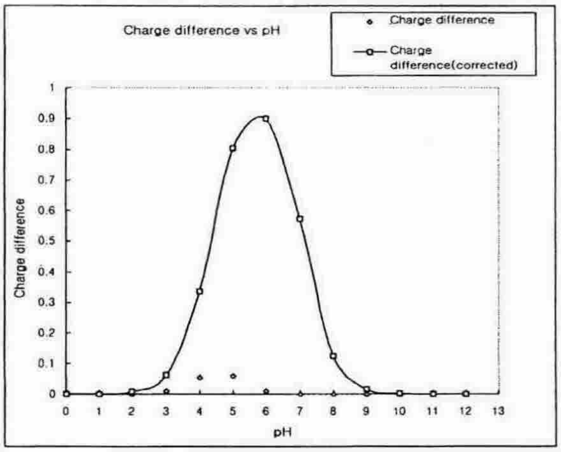

Fig 2. Charge difference of xylanase

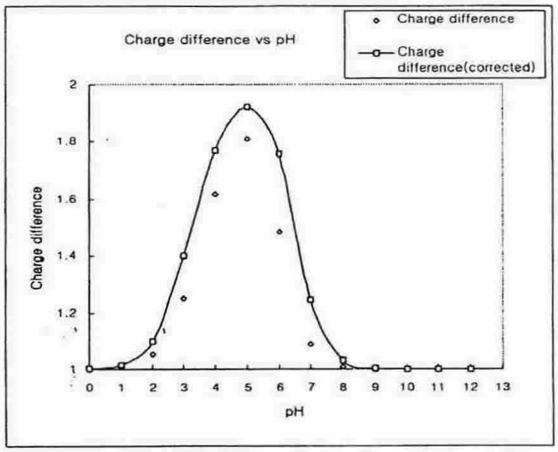

Fig 3. Charge difference of barnase

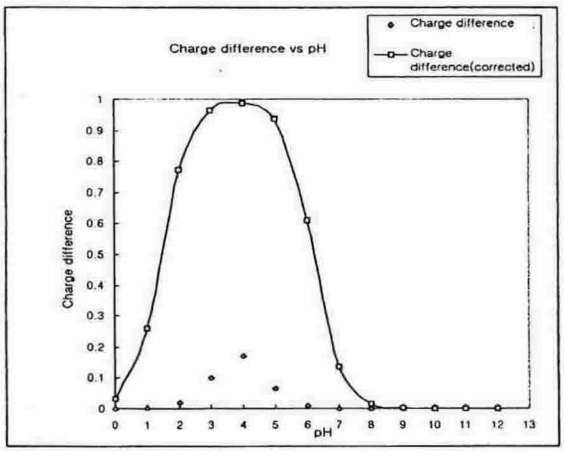

Fig 4. Charge difference of lysozyme

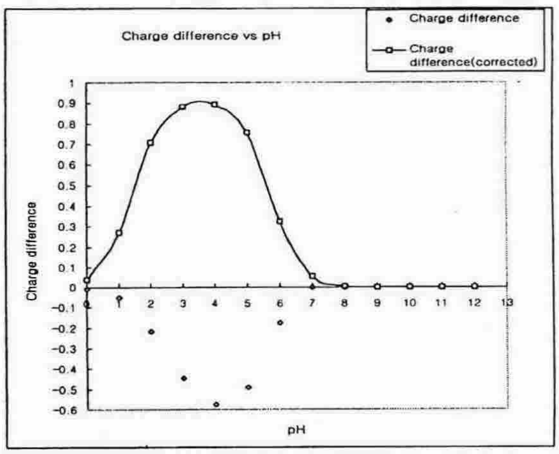

Fig 5. Charge difference of glucoamylase

When the charge difference between catalytic base and catalytic acid reaches to maximum, optimal reactive condition between two residues establishes.

In case of glucoamylase, no corrected data are reverse values for acid-base catalytic residues. But corrected data through pK^{int} information are good results. This study shows that the degree of charge profile s difference has relationship with the degree of pH-activity profile s opt. pH range broadness. Also hydrolases with many heteromolecules(water molecules, metal ion, carbohydrate etc) show more difference between predicted opt. pH· and experimental opt. pH.

Table 1. Comparison of experimental optimum pH and predicted optimum pH for target enzymes

Source		catalytic residue(acid)	catalytic residue(base)	Experimental opt. pH	Predicted opt. pH
Xylanase	*Bacillus circulans*	GLU172	GLU78	5.7	5.7
Barnase	*Bacillus amyloliquefaciens*	HIS102	GLU73	5.0	5.1
Lysozyme	Hen egg	GLU35	ASP52	4.6-5.0	4.1
Glucoamylase	*Aspergillus awamori*	GLU179	ASP400	4.2-4.5	4.0

요약

Hydrolase는 생물학적 공정에서 가장 널리 사용되는 효소군 중의 하나이다. 본 연구에서는 hydrolase들 중에 xylanase, lysozyme, glucoamylase, barnase가 대상 hydrolase로 선택하여 최적 pH를 예측하는 방법론을 제시하였다. Tanford-Kirkwood(TK) model에 기반을 두고 선택된 효소들의 catalytic residue들의 전하 변화가 optimum pH에 중요한 인자로서 그 catalytic residue들의 전하 차이가 최대일 때의 pH가 optimum pH로 나타났다. 이렇게 예측된 optimum pH 결과들은 실험적으로 결정된 optimum pH와 서로 잘 일치하고 있음을 보여준다.

Reference

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