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Curve- Fitting Program for Reaction Progress Curves

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Abstract

Saturation growth model was evaluated to predict the formation of product as a function of time. Good agreement was observed with homogenous and heterogenous reactions. Prediction of product yield can be made reasonably using this model. In addition, accuracy of measured values can be roughly evaluated by this model. User-friendly computer program in BASIC was written to evaluate the constants Pmax and K as well as averages of relative errors.

Introduction

Prediction of product formation upon extended hydrolysis time is necessary to predetermine the production scale and related cost. Reaction kinetic studies on enzymatic hydrolysis of protein were mostly focused on initial velocity of reaction. In some cases, product yieid counts more than reaction time depending upon the purpose of reaction and price of final products.

In this study, empirical model was employed to predict the progress of reaction. Homogenous reacton was evaluated with saturation model. Data were taken from published papers. For heterogenous reaction, myofibrillar protein from Alaska pollack was used. In both cases, the fitness of data to the model was determined by the average of relative error.

Materials and Methods

Materials

Alaska pollack fillets were used because of commercial availablity. Those fillets were rapidly frozen using liquid nitrogen and stored at −15℃. Myofibrillar protein was prepared by the method of Katoh et al.[1] Alcalase was obtaind through Novo and used for hydrolysis of protein without further purification.

Hydrolysis of myofibrillar protein

Five ml of 0.1 M sodium phosphate buffer (pH 7.5) was added to 0.5 mg of myofibrillar protein. After incubating the mixture in 45℃ water bath for 5 min, 0.1 ml of alcalase was added to start reaction. Degree of hydrolysis was determined by the method of Hagihara, et al.[2]

Theoretical

Nonlinear model, saturation-growth rate equation was evaluated to predict the degree of enzymatic hydrolysis of protein upon varying reaction time.[3] Since depletion of substrate and inactivation of enzymes contribute to reduction of reaction rate, this empirical model may be able to predict the progress of reaction. Formation of product can be predicted as a function of time as equation (1).

$$P(t) = Pmax * t/(K+t) \quad (1)$$

Constants, Pmax and K, are the function of concentration of enzyme and substrate. At a given ratio

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of enzyme to substrate, these constants can be determined by equation (1). After obtaining Pmax and K, the fitness of data was evaluated by the average relative errors.

Results and Discussion

Homogenous reaction

Data published by Chung et al.[4] agreed well with saturation model (Fig. 1). Averages of relative errors were lower than 6.65% (Table 1). If reaction at 35℃ was not considered, the average of relative error would be lower than 1.6%, Thus, data from 35℃ reaction might be somewhat inaccurate from the viewpoint of enzymatic reaction. It became rather obvious when comparison of data was made with predicted values designated by solid line (Fig .1).

Results implied that saturation model could be successfully applied to the homogenous reaction for the prediction of product yield as a function time at given conditions of reaction.

Heterogenous reaction

In the case of heterogenous reactions, saturation model showed reasonably good agreement with the measured values (Fig. 2). With two different sets of enzyme dilutions, measured values fitted well to the model throughout the reaction time except for 55min or over. Averages of relative errors were somewhat larger than those of homogenous reaction. However, considering the difficulty of controlling heterogenous reaction, those extents of errors could be quite acceptable (Table 2).

Therefore, saturation model also showed the possible application to the heterogenous reactions.

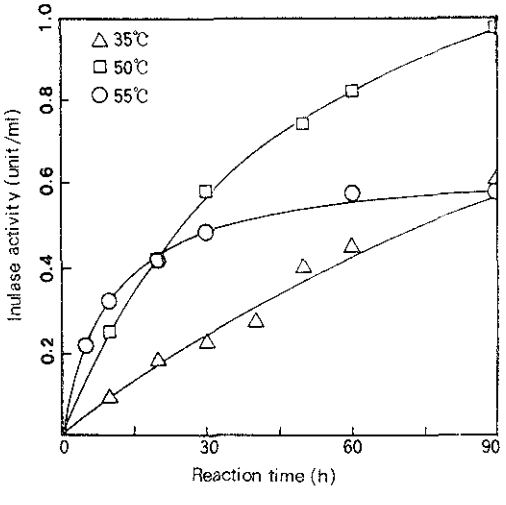

Fig. 1. Data fitting for homogenous reactions with saturation model.
(data source from Chung et al., 1981.)

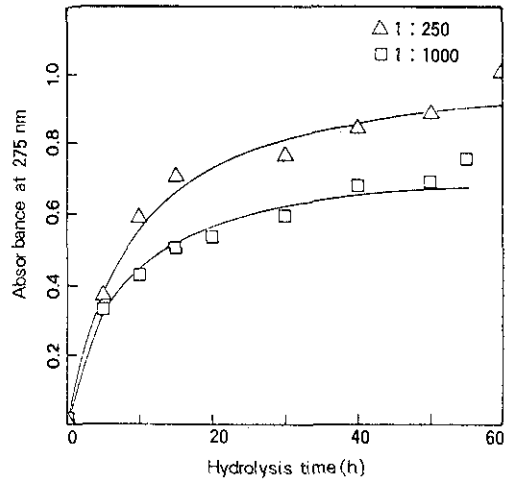

Fig. 2. Data fitting for hydrolysis of myofibrillar protein with saturation model.

Table 1. Pmax, K, and % error estimated by the saturation model program

Temperature(℃)	Pmax	K	% Error
35	1.66	174	6.65
50	1.52	50.9	1.34
55	0.64	9.6	1.52

Another interesting outcome was that saturation model can serve as a criterion for the accuracy of experimental data in terms of reaction progress. As shown in Fig. 1 and 2, the reliability of measured values could be roughly evaluated by looking into the direction of deviation from the predicted values.

Program written in BASIC

To estimate constants Pmax and K, user-friendly computer program was written in BASIC (Fig. 3). This program can be utilized with personal computer of 64K memory or over. Using double-reciprocal plot, equation (1) becomes equation (2). Then linear regression can be used to determine Pmax and K.

$$1/P(t) = (K/Pmax)(1/t) + 1/Pmax \quad (2)$$

This program consists of three subprograms : input of data, linear regression, and error analysis. Output will be displayed on screen. Equation (1) with computed Pmax and K will be shown ; in addition, averages of relative errors will be expressed as % error.

Table 2. Pmax, K, and % error estimated by the saturation model program

Dilution ratio	Pmax	K	% Error
1 : 250	1.05	8.89	3.71
1 : 1000	0.762	6.96	4.69

```
5 CLS
10 REM MAIN PROGRAM
20 GOSUB 100
30 GOSUB 300
40 GOSUB 600
50 END
60 REM
100 REM SUBROUTINE DATA INPUT
110 REM
120 INPUT "Number of data points" ; N
130 DIN X(N), Y(N)
140 REM
150 FOR I=ℓ TO N
160     PRINT "X(" ; ili ; "), Y(" ; ili ; ")=" ;
170     INPUT X(I), Y(I)
180 NEXT I
190 RETURN
300 REM SUBROUTINE STAURATION MODEL
310 REM .
313 SX=0
315 SY=0
316 XY=0
317 X2=0
320 FOR I= 1 TO N
330 REM
332     X(I) = I/X(I)
335     Y(I) = I/Y(I)
340       SX=SX+X(I)
350       SY=SY+Y(I)
360 X2=X2+X(I)*X(I)
370 XY=XY+X(I)*Y(I)
380 NEXT I
390 XM=SX/N
400 YM=SY/N
410 REM
420 SLOPE=(N*XY-SX*SY)/(N*X2-SX*SX)
430 YINY=YM-SLOPE*XM
440 REM
450 K1=1/YINT
460 K2=SLOPE*K1
470 REM
480 PRINT "P=" ; K1 ; "*t/(" ; K2 ; "+t)" ;
490 RETURN
500 REM
600 REM SUBROUTINE ERROR ESTIMATE
610 REM
615 EA=0
620 FOR I=1 TO N
622       X(I)=1/X(I)
624       Y(I)=1/Y(I)
630     EY(I)=K1*X(I)/(K2+X(I))
640     EA=EA+ABS((Y(I)-EY(I))/Y(I))
650 NEXT I
660 EAC=EA/N*100
670 REM
680 PRINT "Error estioate = " ; EAC ; "%" ;
690 RETURN
```

Fig. 3. User-friendly computer program for estimation of Pmax and K using saturation model.

References

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Curve-Fitting Program을 이용한 반응진행곡선의 예측에 관한연구

홍정화 · 최진호 · 변대석

부산수산대학교 식품영양학과

요 약

포화성장모델을 이용하여 반응시간에 따른 생성물의 양을 예측하였다. 수용성 기질－수용성 효소간의 반응뿐만 아니라 비수용성 기질－수용성 효소간의 반응 결과에도 이 모델은 뛰어난 예측성을 나타내었다. 그외에도 측정치의 정확도도 이 모델로 대략 평가할 수 있었다.

사용자가 편리하도록 컴퓨터 프로그램을 BASIC 언어를 사용하여 제시하였고 이 프로그램을 이용하여 Pmax와 K 상수 값을 결정하고 상대오차의 평균값도 계산하였다.