The Review of Physical Chemistry of Japan Vol. 32 No. 1 & 2 (1962)

PRESSURE INACTIVATION OF ENZYME*

Some Kinetic Aspects of Pressure Inactivation of Chymotrypsin

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(*Received January 10, 1963*)

The inactivation of chymotrypsin by pressure has been investigated kinetically including the effect of pH, concentration of enzyme, magnitude of pressure, repeated compression.

Inactivation process of chymotrypsin by high pressure is of the first order kinetics. The temperature coefficient of the rate of inactivation is positive, and thermodynamic functions in the activation process of inactivation are as follows; $\Delta H^{*}>0$, $\Delta F^{*}>0$, $\Delta S^{*}<0$ and $\Delta V^{*}<0$.

From the experimental results, it is found that the process of the inactivation of chymotrypsin by high pressure is almost similar to that of trypsin.

Introduction

A series of our works was made on the denaturation of proteins and inactivation of enzyme, and the pressure denaturation of albumin[1~3,6], hemoglobin[4~6] and pressure inactivation of trypsin[7] were already reported.

Though the inactivation of chymotrypsin by pressure had been already investigated by Curl and Jansen[8], kinetic treatment, however, has not been performed yet.

From the standpoint of kinetic behavior, it seems to be interesting to investigate whether the process of inactivation is of the same type as those observed for trypsin.

This paper is concerned with confirming the qualitative behaviors in the previous paper and obtaining more detail information about kinetical properties.

Experimental

Commercial crystalline chymotrypsin (N. B. C.) was used, the concentration of the test sample being about 4×10^{-2} mg/ml in 1/15 M phosphate buffer or distilled water. In the latter case, pH

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* Presented at the 15th Annual Meeting of the Chemical Society of Japan, Kyoto, April, 1962

1) K. Suzuki, *Memoirs Res. Inst. Sci. and Eng., Ritsumeikan Univ.*, **2**, 19 (1957)
2) K. Suzuki, K. Kitamura, S. Kagawa and K. Tamura, *ibid.*, **3**, 1 (1958)
3) K. Suzuki, *This Journal*, **28**, 24 (1958)
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5) K. Suzuki, K. Kitamura, *ibid*, **29**, 86 (1960)
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7) K. Miyagawa, K. Suzuki, *ibid.*,
8) L. Curl, E. Jansen, *J. Biol. Chem.* **184**, 45 (1950)

was adjusted by 0.1 N HCl or 0.1 N NaOH. The pressure apparatus, its procedures and the method of activity measurment were the same as those of trypsin[7].

Results and Discussion

Enzyme solution (pH 7.6) was compressed at pressure of 6000 and 8000 kg/cm² for 5 minutes, after pressure being released, the enzyme was kept at 5°C and activity was measured after definite time. As shown in Fig. 1 the reversibility of pressure inactivated chymotrypsin was not found within 3 hours. Therefore, the inactivation seems to be an irreversible reaction.

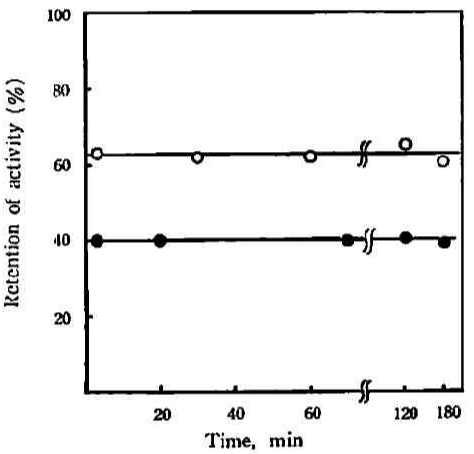

Fig. 1 Irreversibility of pressure inactivated chymotrypsin
(—○—) 600kg/cm²
(—●—) 8000kg/cm²
Duration of time: 5 minutes
pH: 7.6
Temperature: 25°C

The concentration of enzyme affected the inactivation by pressure. Five different concentrations of the enzyme were compressed for 5 minutes at 6000 kg/cm², at pH 7.6 and at 25°C. The results are presented in Fig. 2; the inactivation was increased with the increase of concentration.

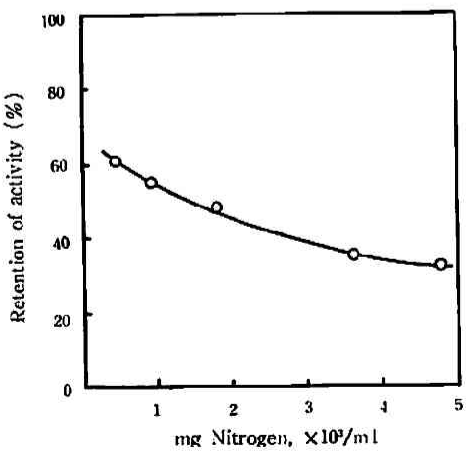

Fig. 2 Effect of concentration of chymotrypsin
Nitrogen is measured by micro kjeldahal method
Magnitude of pressure: 6000kg/cm²
Duration of time: 5 minutes
pH: 7.6

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The results of the pH dependence on the process of inactivation are shown in Fig. 3. It shows that the lower the pH, the higher the retention of activity.

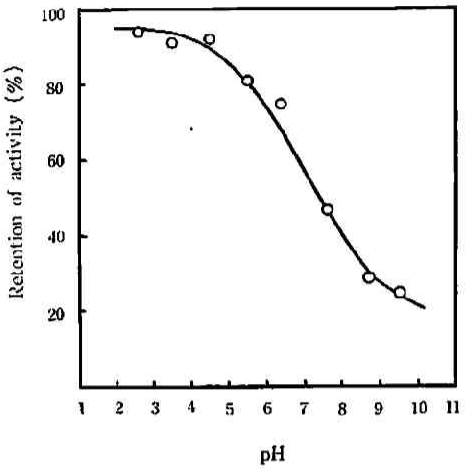

Fig. 3 The effect of the pH
pH was adjusted by 0.1*N* HCl and 0.1*N* NaOH
Magnitude of pressure: 6000kg/cm²
Duration of time: 5 minutes
Temperature: 25°C

The effect of magnitude of pressure and temperature are shown in Fig. 4; above a certain pressure, the increase in pressure results in a rapid increase of the inactivation, but above 6500 kg/cm², futher increase in pressure causes little or no change (we call it critical pressure), and it is found that the temperature coefficient is positive, that is, the increase in temperature lessens the retention of activity under the same pressure. It is to be noted that this constant value above 6500 kg/cm² depends on the temperature.

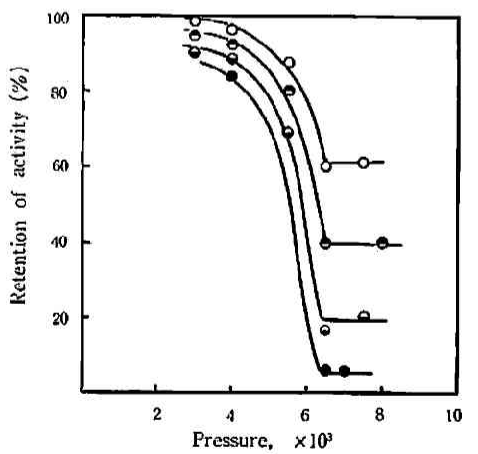

Fig. 4 The effect of magnitude of pressure and temperature
(—○—) 15°C
(—◓—) 25°C
(—◒—) 35°C
(—●—) 45°C
Duration of time: 5 minutes
pH: 7.6

Below the critical pressure, the repeated compression on the enzyme causes the same inactivation as that caused by a single compression of totally the same duration. On the other hand, above the critical pressure, the repeated compression causes much more inactivation than that caused by a single compression of the totally same duration, and those results are shown in Fig. 5.

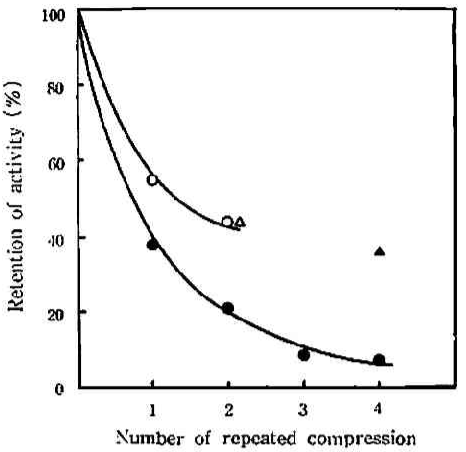

Fig. 5 The effect of repeated compression of pressure
(—○—) 6000kg/cm² for 5 minutes
(△) 6000kg/cm² for 10 minutes
(—●—) 8000kg/cm² for 5 minutes
(▲) 8000kg/cm² for 20 minutes
Temperature : 25°C
pH : 7.6

The experiments for the time course of inactivation were carried out at 5500 and 6000 kg/cm², and at 25°C and 35°C (pH 7.6) respectively, and the semilogarithmic plot of the time course of inactivation are shown in Fig. 6, where C_0 is the activity at zero time, C is that at time t and Ce is the final value of C at the given condition (cf. Fig. 4). The linear relationships are found and so the inactivation process is of first order.

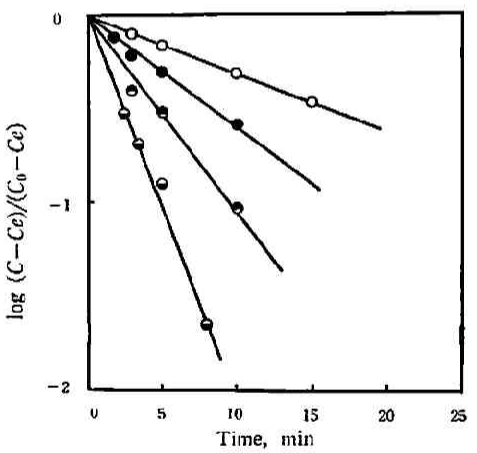

Fig. 6 Time course of inactivation
(—○—) 5500kg/cm² at 25°C
(—◓—) 6000kg/cm² at 25°C
(—●—) 5500kg/cm² at 35°C
(—◒—) 6000kg/cm² at 35°C
pH : 7.6
C_0 : initial activity of enzyme
C : activity at time t
Ce : final activity of C

The ratd constant k and the influence of pressure p on the rate are related by the following equations :

$$k=1/t\cdot\ln(C_0-Ce)/(C-Ce)$$

$$\partial\ln k/\partial p=-\Delta V^{\neq}/RT$$

where R is the gas constant, T the absolute temperature and $\Delta V^{\neq}$ the molar volume change of activation. Accordingly, the semilogarithmic plots of the rate constants against pressure afford the value of $\Delta V^{\neq}$ (cf. Fig. 7). As shown in Table 1, the values of ΔV by$^{\neq}$ high pressure are negative in the whole temperature range examined, and their absolute values decrease as the temperature increases.

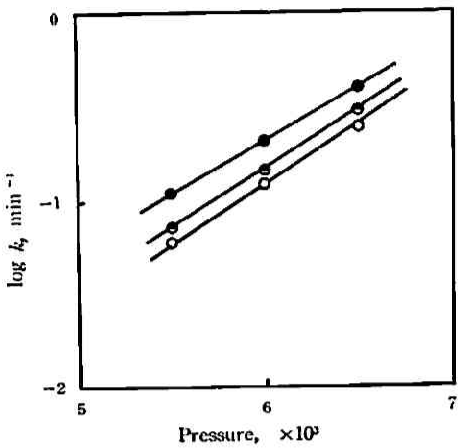

Fig. 7 Relation between semilogarithmic plots of the rate constant k and pressures
(—○—) 15°C
(—◒—) 25°C
(—●—) 35°C
pH : 7.6

Table 1 Molar volume change of activation, $\Delta V^{\ddagger}$cc/mole (pH 7.6)

Pressure, kg/cm²	Temperature, °C		
	15	25	35
5500–6500	−40	−39	−36

The studies on the temperature dependence of rate constant give the values of apparent activation energy E from the relation between the logarithm of rate constant and the reciprocal of the absolute temperature.

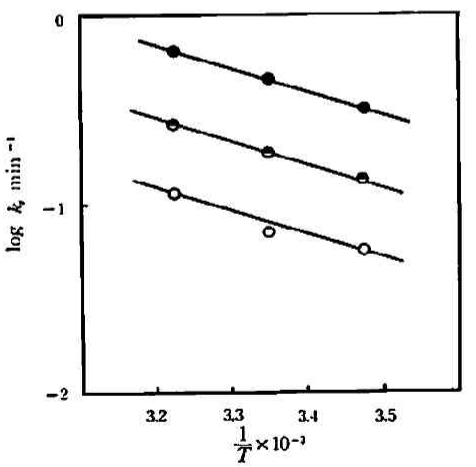

Fig. 8 Relation between the logarithm of rate constant k (min.$^{-1}$) and the reciprocal of the absolute temperature
(—○—) 5500 kg/cm²
(—◒—) 6000 kg/cm²
(—●—) 6500 kg/cm²
pH : 7.6

From the slopes of Fig. 8, we can calculate the apparent activation energies at 5500, 6000, and 6500 kg/cm² at pH 7.6. The obtained values are 4.6, 5.5 and 5.5 kcal/mole respectively.

From the following equations which are deduced from the equation of absolute reaction rates, the thermodynamic quantities in the activation process of inactivation, the free energy of activation, $\Delta F^{\ddagger}$, the enthalpy of activation, $\Delta H^{\ddagger}$, and the entropy of activation, $\Delta S^{\ddagger}$, are calculated.

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Table 2 Kinetics of inactivation of chymotrypsin by pressure (pH 7.6)

Temperature °C	Pressure kg/cm²	k sec^{-1}	ΔF^* kcal/mole	ΔH^* kcal/mole	ΔS^* cal/mol. deg.
15	5500	2.3×10^{-4}	24	4.0	−69
	6000	5.5×10^{-4}	24	4.9	−64
	6500	8.2×10^{-4}	24	4.9	−62
25	5500	1.2×10^{-3}	24	4.0	−69
	6000	3.1×10^{-3}	25	4.9	−67
	6500	9.3×10^{-3}	25	4.9	−65
35	5500	1.9×10^{-3}	25	4.0	−73
	6000	5.4×10^{-3}	26	4.9	−71
	6500	1.2×10^{-2}	26	4.9	−69

$$\Delta F^* = RT\ln(KT/hk)$$

$$\Delta H^* = E - RT$$

and $$\Delta S^* = (\Delta H^* - \Delta F^*)/T$$

where k is the rate constant of the first order, K the Boltzman constant, h the Planck constant, T the absolute temperature, E the apparent activation energy stated above, and R the gas constant. Some of the results obtained are summarized in Table 2.

From the results of our experiment, we can find that the qualitative behaviour and kinetics of the pressure inactivation of chymotrypsin are almost same as those of trypsin, and it is suggested that chymotrypsin is nearly similar to trypsin in its character.

This investigation was supported, in part, by a grant from the Ministry of Education, to which the author's thanks are due.

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