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## **Theoretical Analysis of Chemical Reaction in Frozen State (Part 2) -Introduction of a More Accurate Freezing Point Depression -**

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Acceleration of chemical reactions in the frozen state has become of interest in recent years because of its unique characteristics. To give a theoretical interpretation for the acceleration mechanism of such reactions, a new equation is derived. The equation is essentially the product of Arrhenius formula of temperature effect and the term of liquid concentration varied with freezing ratio. In the previous paper (Murata et al., 1992), for calculating the freezing ratio the Heiss formula was used. In this case, a more accurate freezing point depression is introduced and the error of the optimum temperature of reaction between calculated result and experimental result is reduced from 1.8°C to 0.2°C.

For calculation, the kinetic data obtained for the acid-catalyzed deformylation of N-acetyl-N'-formyl-L-kynureninamide (Yamasaki et al., 1976b) are used.

The results clearly indicate that the calculated first-order rate constants at various temperatures are in good agreement with the experimental data.

The acceleration of the deformylation of N'-formylkynurenine in the frozen acidic solutions can be interpreted as being due to the concentration of the reactants in the liquid region in ice crystals.

### INTRODUCTION

It has recently been shown that acceleration of chemical reactions in frozen state and has become of interest of its unique characteristics (Aso, 1990, Grant, 1961, Kiofsky, 1966, Prusoff, 1963 and Yamasaki, 1976b). These reactions are closely related with food industries, then if a theoretical interpretation for the acceleration mechanism of such reactions are given, it may be obtained some basic information for the deterioration of foods during partial freezing storage or freeze drying process.

From this point of view, the acceleration of the deformylation of N-acetyl-N'-formyl-L-kynureninamide (NFK) in the frozen acidic solutions is studied. NFK is a medium product of tryptophan metabolism, which is obtained from tryptophan by using tryptophan-2,3-Oxygen or which is obtained as oxidation product of tryptophan by ozone. Furthermore, formic acid and kynurenine (Kyn) are obtained from the acid-catalyzed deformylation of NFK by acid or formamylase. Yamasaki et al. (1976) have found that the reaction of acid-catalyzed hydrolysis of the formamide linkage of NFK was accelerated in frozen dilute hydrochloric acid solution. By applying the above chemical modification method to the oxidized lysozyme, they have obtained a lysozyme derivative, 1-NFK-lysozyme, in which tryptophan 62 was substituted by N'-formylkynurenine and this finding shows that above chemical modification may be useful for

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analysis of relationship between lysozyme structure and that function (Yamasaki et al., 1976ab, 1980, 1988a, 1990, 1991).

The reaction of the deformylation of NFK in the frozen state had very unique characteristics and the kinetic analysis was carried out in detail by using the model chemical compound (N-acetyl-N'-formyl-L-kynurenineamide). But, the theoretical interpretation for this acceleration of chemical reaction had never been given. In this study, to give a theoretical interpretation for the mechanism of this reaction, a new equation was derived. The equation was based on Arrhenius formula and the theory of "the concentration effect" which was founded on solid-liquid phase equilibrium. It was clear that the reaction of deformylation of NFK in dilute hydrochloric acid solution could be explained theoretically by using this equation.

## THEORY

### Rate equation

The reaction of the deformylation of N'-formylkynurenine in the frozen and liquid of acidic solutions follows first-order kinetics (Yamasaki, 1976b), and the rate of reaction was correlated by the eq. (1).

$$-\frac{d[A]}{d\theta} = k[A] \quad \dots\dots(1)$$

where as :

[A] : the concentration of reactant

$\theta$  : reaction time

k : first-order rate constant

The first-order rate constant which was given as following Arrhenius formula (2)

$$k = k_0 \exp\left(-\frac{E}{R \cdot T}\right) \quad \dots\dots(2)$$

where as :

E : activation energy in kJ · Mol<sup>-1</sup>

R : ideal gas constant (=8.314 kJ · K<sup>-1</sup> · Mol<sup>-1</sup>)

T : the absorbed temperature in K

### The introduction of new simulation equation of freezing point depression

The rate constants of reaction in the liquid solution should be calculated in accuracy by Arrhenius formula eq. (2), of course, as stand above. But the reactants were concentrated in liquid region in ice crystal when the solutions were frozen, so it was needed for giving a theoretical interpretation to take eq. (1) into account of "the effect of concentration", i.e. the left side term [A] ([A]<sub>L</sub>) in eq. (1) was initial concentration of solution but the other side [A] ([A]<sub>R</sub>) was the concentrated fraction caused on freezing the solution. Hence [A]<sub>R</sub> should be calculated from freezing fraction.

The freezing fraction was shown by the thermodynamic expression of phase equilibrium for non-ideal solution (unpublished paper by Murata). In this study the phase equilibrium on the assumption that the solid reactants insolve together but these

liquids dissolve together, were used as the following eq. (3).

$$\Delta T = - \frac{R \cdot T \cdot T_0}{\Delta H} \cdot \ln \left[ \frac{1 - X_s}{1 - X_s + \alpha X_s + \beta X_s^2} \right] \dots\dots\dots (3)$$

where as :

- ΔH** : latent heat of melting in kJ/Mol
- $X_s$  : mass fraction of solution in decimal
- 0 : denotes the freezing point of pure water

Parameters ( $\alpha$  and  $\beta$ ) were calculated from typical data for hydrochloric acid solution (Weast, 1985) by using the Deming method, i.e.  $\alpha$  was 0.809 and  $\beta$  was 7.164.

Then the freezing fraction ( $\psi$ ) was represented by the following eq. (4).

$$\psi = 1 - \frac{X_f}{X_s} \dots\dots\dots(4)$$

Rearranging eq. (4), the unfrozen fraction ( $1 - \psi$ ) became

$$1 - \psi = \frac{X_f}{X_s} \dots\dots\dots(5)$$

$$X_s = \frac{- (1 - \delta + \alpha \cdot \delta) + \{ (1 - \delta + \alpha \cdot \delta)^2 - 4 \beta \cdot \delta (\delta - 1) \}^{0.5}}{2 \beta \cdot \delta} \dots\dots\dots(6)$$

where as :

$$\delta = \exp \{ - (\Delta H \cdot \Delta T) / (R \cdot T \cdot T_0) \} \dots\dots\dots(7)$$

Then the concentration of liquid region in ice crystals was  $A_0(X_s/X_f)$

where as :

$A_0$  : initial concentration of solution.

Eq. (1) was rearranged to the following :

$$- \frac{d[A]}{d\theta} = A_0 \cdot (X_s/X_f) \cdot \exp \left\{ \frac{- E}{R \cdot (273.15 + t)} \right\} \dots\dots\dots(8)$$

After all, the simplest expression of chemical reaction which had a maximum rate point in frozen state was shown by above eq. (8). This equation was essentially based on the Arrhenius formula and new simulation equation of freezing point depression. Eq. (8) were derived by taking into account of the concentration effect caused on freezing the solution.

The reaction in liquid state, however, follows eq. (9).

$$- \frac{d[A]}{d\theta} = A_0 \cdot k_0 \cdot t \cdot \exp \left\{ \frac{- E}{R \cdot (273.15 + t)} \right\} \dots\dots\dots(9)$$

## RESULTS AND DISCUSSION

## Determination of parameters

For analyzing the acceleration of chemical reactions in frozen state, the data of the spectral absorption change at 360nm observed during the acid-catalyzed deformylation reaction of the model kynurenine derivative in the frozen and liquid of 0.025M dilute hydrochloric acid solution was used (Yamasaki, 1976b). This data in Table 1 and figure 1 indicates the plot of  $\log k$  against  $1/T$  in liquid and in frozen solutions at constant initial concentrations. The first - order rate constant in liquid solution decreases when the temperature degree decreasing. The broken line in Fig. 1 was calculated resulting from eq. (10), it should be confirmed that the reaction of the deformylation of NFK in liquid acidic solution Arrhenius was depending on the type of temperature. At this time, activation energy ( $E$ ) and parameter ( $k_0$ ) were introduced by the least squares method, i.e.  $E = 61700\text{kJ} \cdot \text{Mol}^{-1}$ ,  $k_0 = 19.1\text{min}^{-1}$  (standard deviation was  $0.24 \times 10^{-3}\text{min}^{-1}$ ). Further, this-depending to realized in super-cooled liquid solution at  $-8^\circ\text{C}$ , its deformation reaction was very slow and  $k$  was  $0.15 \times 10^{-3}\text{min}^{-1}$ . It was supposed that the reaction in liquid region in frozen solution as follow the Arrhenius formula.

**Table 1.** Observed first-order rate constant for acid-catalyzed hydrolysis of the formamide linkage of N-acetyl-N'-formyl-r-Kynurenineamide

| Frozen state                 |  | Liquid state                 |  |
|------------------------------|--|------------------------------|--|
| Temp<br>( $^\circ\text{C}$ ) | $k \times 10^3$<br>( $\text{min}^{-1}$ ) | Temp<br>( $^\circ\text{C}$ ) | $k \times 10^3$<br>( $\text{min}^{-1}$ ) |
| -26                          | 4.15                                     | - 8                          | 0.15**                                   |
| -17                          | 5.76                                     | + 7                          | 0.60                                     |
| -16                          | 6.45                                     | +15                          | 1.42                                     |
| -13                          | 7.37                                     | +20                          | 2.26                                     |
| -12                          | 7.60                                     | +27                          | 3.70                                     |
| - 8                          | 8.75                                     | +30                          | 4.95                                     |
| - 7.5                        | 9.35                                     | +36                          | 8.19                                     |
| - 5                          | 7.60                                     | +40                          | 10.20                                    |
| - 3                          | 6.67                                     |                              |  |
| - 2                          | 5.29                                     |                              |  |
| - 1                          | 4.60                                     |                              |  |

\*\*Super-cooled liquid solution.

The rate constant was calculated from the spectral change (at 360nm) measured during the deformylation of N-acetyl-N'-formyl-L-kynurenineamide in frozen and liquid of 0.025 M dilute hydrochloric acid solutions.

On the other hand, the reaction rate was accelerated in frozen solution and the maximum rate of reaction was found at  $-7.5^\circ\text{C}$  and approximated that of the reaction in liquid solution at  $40^\circ\text{C}$ .

Hence, to give a theoretical interpretation for the acceleration mechanism of such reaction, a new theoretical equation ((8)) was used.

## Calculated results

The curved broken line in Fig. 1 was calculated resulting from fitting to eq. (9) by the least squares method. And the curved solid line 1 was calculated from eq. (10) (refer appendix) and it had a maximum rate point at  $-9.3^{\circ}\text{C}$  (the standard deviation was  $1.145 \times 10^{-3} \text{min}^{-1}$ ). The curved solid line 2 was calculated from eq. (8) and its maximum rate point was  $-7.3^{\circ}\text{C}$ . The calculated optimum temperature using eq. (8) for hydrolysis of NFK in frozen solution was in better agreement with experimental data than calculated from eq. (10). The results clearly indicated that the calculated first-order rate constant (from eq. (8)) under various temperatures were quite agreed well with the experimental data, and its standard deviation was  $1.144 \times 10^{-3} \text{min}^{-1}$ . The standard deviations of calculated first-order rate constant using eq. (8) and eq. (10) were almost same (see Table 2). And the standard deviation of the difference between data and simulated values from eq. (8) (frozen state) was little bigger than that from eq. (9) (unfrozen state).

Table 2. Parameters and its standard deviations

|          | Parameters                                |   |
|----------|---|---|
| Eq. (8)  | $\alpha=0.809, \beta=7.164$               | $E=61700 \text{ (kJ}\cdot\text{Mol}^{-1}\text{)}$ |
| Eq. (10) | $t_f = -0.137 \text{ (}^{\circ}\text{C)}$ | $k_0 = 19.1 \text{ (min}^{-1}\text{)}$            |
|          | Maximum rate point                        | Standard deviation                                |
| Eq. (8)  | $-7.3 \text{ (}^{\circ}\text{C)}$         | $1.144 \times 10^{-3} \text{ (min}^{-1}\text{)}$  |
| Eq. (10) | $-9.3 \text{ (}^{\circ}\text{C)}$         | $1.144 \times 10^{-3} \text{ (min}^{-1}\text{)}$  |

\*The experimental maximum rate point is  $-7.5^{\circ}\text{C}$

The above results showed that the solution associated with its frozen state, continues to progress chemical reactions having the same constant activation energy as when it is under a unfrozen state. And the acceleration of chemical reaction in the frozen acidic solutions could be interpreted as being due to the concentration of the reactants in the liquid region in ice crystals.

It had been indicated that "the effect of concentration" was very important factor for deterioration of protein in frozen foods (Yamasaki, 1988b), so it was expected that this theory became a useful method for frozen food science.

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## APPENDIX

In the previous paper (Murata *et al.* 1992), the freezing ratio was calculated from the Heiss formula, and next equation was derived.

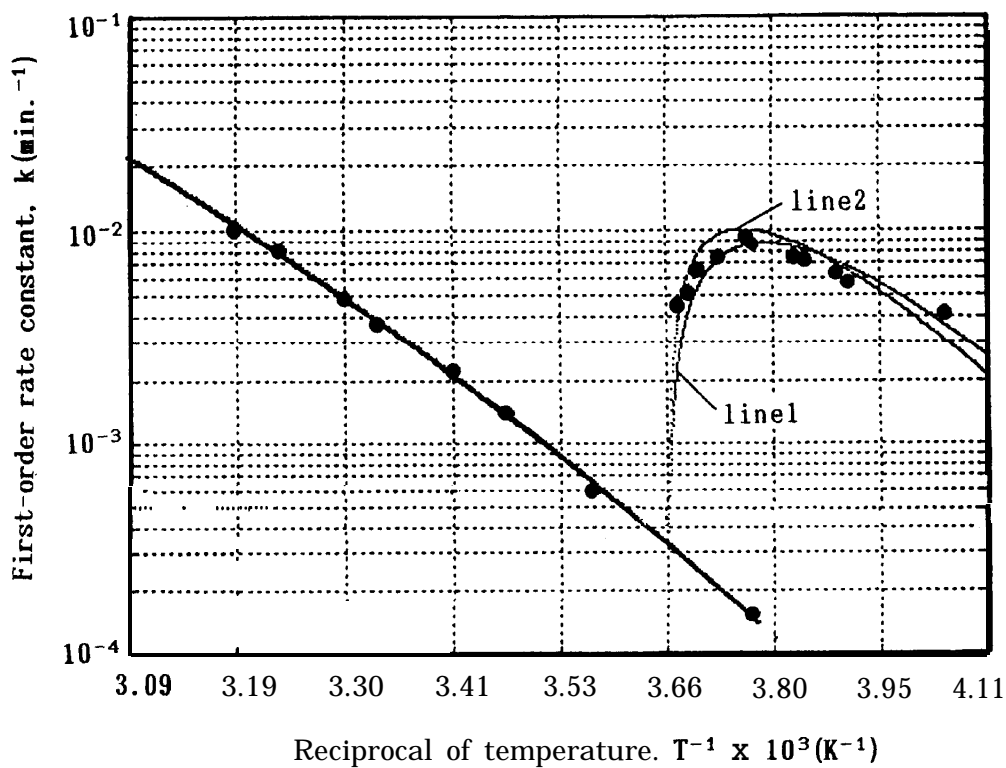
$$-\frac{a \cdot [A]}{d \theta} = A_0 \cdot k'_0 \cdot t \cdot \exp \left\{ \frac{-E}{R \cdot (273.15+t)} \right\} \dots\dots\dots (10)$$

where as :

$t$  : temperature in °C

and  $k'_0 = k_0/t_f$

Hence freezing point,  $t_f = -0.137^\circ\text{C}$  was determined from experimental data in frozen and super-cooled liquid solutions at  $-8^\circ\text{C}$ . This freezing point is lower than theoretical that of 0.025M dilute hydrochloric acid solution ( $-0.092^\circ\text{C}$ ), but by assuming  $t_f = -0.137^\circ\text{C}$ , the calculated rate constants of the present reaction at various temperature were in good agreement with experimental data.



**Fig. 1.** Temperature dependence of observed rate constant (curved lines are calculated from eq. (8) and eq. (10))