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Kinetics & Mechanism of Oxidation of Glutamine & Serine by Peroxomonosulphate in the Absence & Presence of Acetaldehyde & Propionaldehyde

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The kinetics of oxidation of three structurally different amino acids, viz. serine, glutamine and alanine by peroxomonosulphate (PMS) in the absence and presence of aldehydes have been investigated in HOAc-NaOAc buffer medium. Analysis of kinetic results in the absence of aldehyde shows that SO_5^{2-} is the reactive species. The kinetic parameters such as rate constant, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ show a correlation with pK_2 of the amino acid. The results have been analysed on the basis of a mechanism wherein the schiff base is proposed as the reactive intermediate.

In our earlier report[1] on the kinetics and mechanism of oxidation of amino acids (AA) by peroxomonosulphate (PMS)[1], it was observed that the first order plots showed curvature after 5-10% conversion of PMS which we believed was due to the effect of the product formed during the course of reaction. This was confirmed by studying the reactions in the presence of formaldehyde[2]. In order to have some insight in to the mechanism in the presence of aldehyde, presently we have investigated the kinetics of PMS oxidation of three structurally different amino acids namely alanine, serine and glutamine in the presence of acetaldehyde and propionaldehyde. Serine and glutamine have been chosen, in particular, to arrive at any correlation of the rate of oxidation with the structure of amino acids.

Materials and Methods

Potassium peroxomonosulphate (Du Pont) was found to be > 96% pure and its solution was always prepared afresh before use. The solution was standardised by cerimetry using ferroin as an indicator. Amino acids except glutamine were from Loba-Chemie Indo Austranal Co. Glutamine was from Koch-Light, England. Formaldehyde (S. Merck, 30%) was used as such and it was assayed by hypoiodite method[3]. Acetaldehyde and propionaldehyde were purified and assayed by standard procedures.

All the kinetic runs were followed by iodometry at constant $[H^+]$ using HOAc-NaOAc buffer. A high concentration of the buffer ($0.2M$) was maintained in the reaction mixture since the product HSO_4^- is a stronger acid than the oxidant HSO_5^-. No self-decomposition of PMS was observed under our experimental conditions.

Stoichiometry

Reaction mixtures containing large excess of [PMS] over [AA] or [AA+ aldehyde] were allowed to stand for several hours (24 to 48 hr) at room temperature and constant $[H^+]$ (pH 4.8) and the unconsumed PMS was determined iodometrically. The stoichiometries of the reaction in the absence and presence of aldehyde were:

$$2\text{PMS} + 1\text{AA} \rightarrow \text{products};$$

and

$$3\text{PMS} + 1\text{AA} + 1\text{aldehyde} \rightarrow \text{products}.$$

One of the oxidation products of serine with PMS both in the absence and presence of aldehyde was glycollic acid. An acid product was obtained in the case of glutamine also. However, in this case the product underwent further oxidation on long standing as in the case of glycollic acid (serine-PMS). In the presence of aldehyde the products of oxidation (CH_3COOH etc.) of aldehydes were also detected by spot tests.

Results

(i) Oxidation of serine and glutamine in the absence of aldehyde

All the experiments were carried out under pseudo-first order conditions: [AA] ≫ [PMS]. Plots of log V_t (V_t = volume of thiosulphate consumed) versus time were linear up to 60% conversion (Fig. 1A). At fixed [AA] and $[H^+]$ the values of pseudo-first order rate constant, (k_{obs}) were independent of the initial (PMS], indicating that rate was proportional to [PMS].

The values of k_{obs} increased linearly with [AA] and the plots of k_{obs} versus [AA] passed through the origin (Fig. 2A). The values of k_{obs} decreased with increase in $[H^+]$ and the plots of k_{obs} versus $1/[H^+]$ were linear and passed through the origin (Fig. 2B).

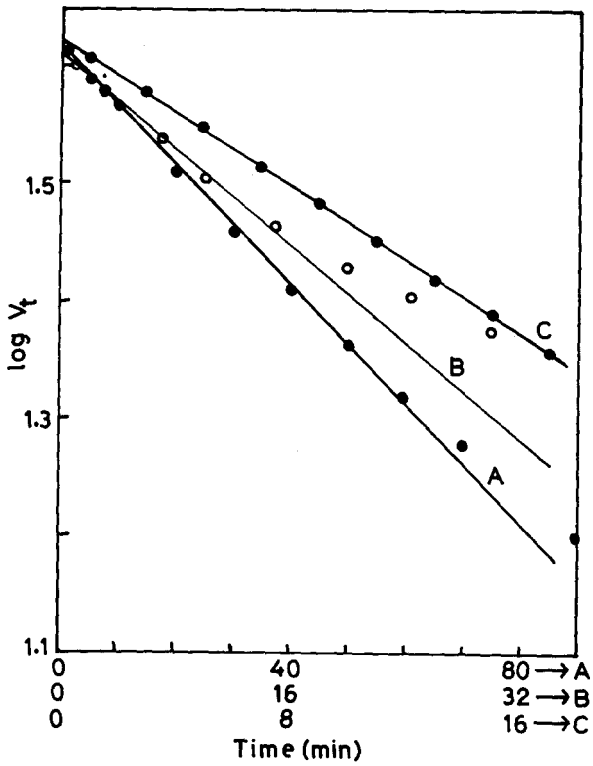


Fig. 1—(A) Plot of log V_t versus time at 31°C. ([glutamine] = 0.05 mol dm^{-3}, [PMS] = 4.19×10^{-3} mol dm^{-3}, pH = 5.2, μ = 0.25 mol dm^{-3}, (B) [alanine] = 0.05 mol dm^{-3}, [PMS] = 4.18×10^{-3} mol dm^{-3}, pH = 4.0; μ = 0.25 mol dm^{-3}, [acetaldehyde] = 2.8×10^{-3} mol dm^{-3}, (C) [serine] = 0.05 mol dm^{-3}, [PMS] = 4.2×10^{-3} mol dm^{-3}, pH = 4.0, μ = 0.25 mol dm^{-3}, [formaldehyde] = 3.03×10^{-3} mol dm^{-3})

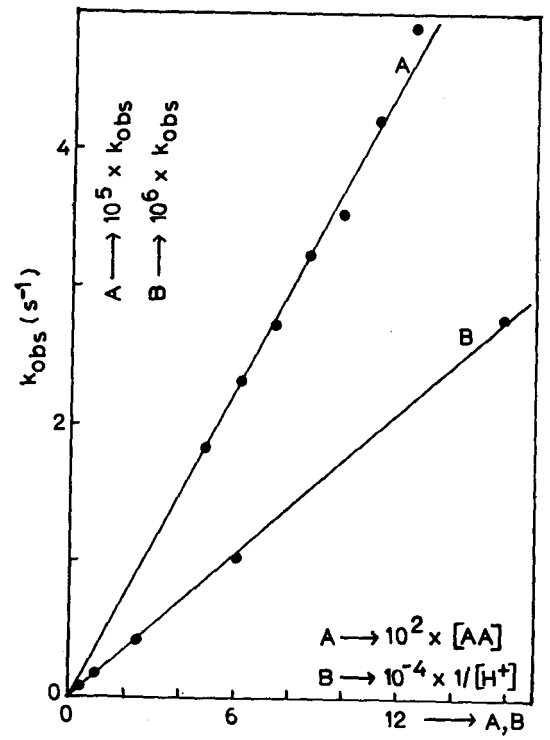


Fig. 2—(A) Plot of k_{obs} versus [amino acid] (AA) at 31°C ([AA] = [serine], [PMS] = 3.93×10^{-3} mol dm^{-3}, pH = 4.0, μ = 0.25 mol dm^{-3}, (B) Plot of k_{obs} versus $1/[H^+]$ at 31°C [Serine] = 0.05 mol dm^{-3}, [PMS] = 3.93×10^{-3} mol dm^{-3}, μ = 0.25 mol dm^{-3})

The change in ionic strength of the medium from 0.25 to 0.55 by adding potassium sulphate showed almost negligible increase in k_{obs}. The reactions were studied at four different temperatures (30-45°C) and from the temperature dependence of k_{obs} the activation parameters were calculated.

(ii) Oxidation of alanine, serine and glutamine in the presence of aldehyde

In these experiments pseudo-first order conditions were maintained only with respect to [AA]. The [aldehyde] was approximately equal to [PMS] in the oxidation reactions of amino acids in the presence of formaldehyde. But [acetaldehyde] and [propionaldehyde] were slightly less than [PMS]. In the presence of formaldehyde the plots of log V_t versus time were linear (Fig. 1C) even up to 70% conversion of PMS. In the presence of acetaldehyde and propionaldehyde the first order plots were curved (Fig. 1B) and the percentage of [PMS] conversion, corresponding to the point at which the curvature appeared, increased proportionally with increase in [acetaldehyde] and [propionaldehyde]. When the [aldehyde] in the case of acetaldehyde and propionaldehyde was greater than [PMS], the first order plots were linear upto 50% conversion. At fixed [AA], [H^+] and [aldehyde] the values of k_{obs} were independent of the initial [PMS], indicating first order dependence in [PMS].

The values of k_{obs} increased with increase in [AA]. Plots of k_{obs} versus [AA] were linear with a definite positive intercept (Fig. 3). At fixed [H^+] and [AA], the value of k_{obs} increased with increase in [aldehyde] and the plots of k_{obs} versus [aldehyde] were linear passing through the origin.

The values of k_{obs} decreased with increase in [H^+] and the plots of k_{obs} versus $1/[H^+]$ were linear with a positive intercept, indicating clearly that the reactions proceeded through two independent paths: one was $[H^+]^{-1}$ dependent and the other was [H^+] independent.

The change in ionic strength from 0.25 to 0.55 had negligible effect on k_{obs}. The reactions were studied at four different temperatures in the range 30-45°C and the activation parameters were calculated.

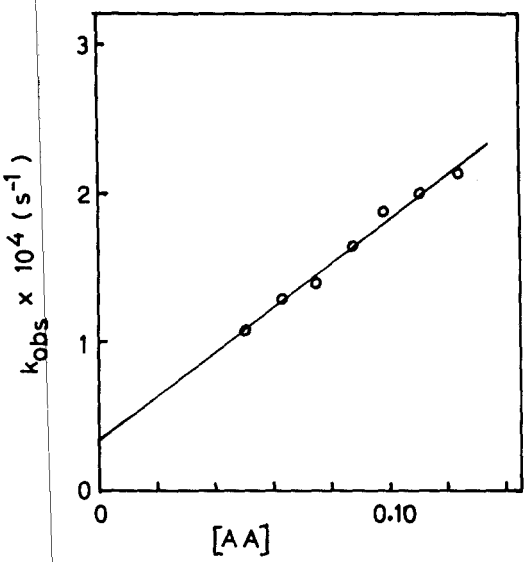


Fig. 3—Plot of k_{obs} versus [amino acid] (AA) at 31°C ([AA]=[glutamine], [PMS]=4.2×10^{-3} mol dm^{-3}, pH=4.0, μ = 0.25 mol dm^{-3}, [propionaldehyde]=7.9×10^{-4} mol dm^{-3})

Discussion

Peroxomonosulphuric acid ($HO-OSO_3-H$) has two ionizable protons, viz. sulphuric acid proton and hydrogen peroxide proton. The pK_a value[4] of the sulphuric acid proton lies in a high acidity region and that of hydrogen peroxide proton is 9.4.

For amino acids, the following equilibria (1) exist in acidic/alkaline medium.

$$R'\text{-}\underset{\overset{+}{N}H_3}{\underset{|}{CH}}\text{-}COOH \overset{K_i}{\rightleftharpoons} R'\text{-}\underset{\overset{+}{N}H_3}{\underset{|}{CH}}\text{-}COO^- \overset{K_{ii}}{\rightleftharpoons} R'\text{-}\underset{NH_2}{\underset{|}{CH}}\text{-}COO^- \quad \ldots (1)$$

The values of pK_1 and pK_2 for the amino acids[5] studied are 2.2 and 9.1-9.7 respectively and the values are given in Table 1. Under our experimental conditions namely at pH = 4.0 all the amino acids would be in the form of zwitter ions. Therefore the amino acid in its zwitterionic form may be the reactive species.

It is well known that amino acids react with aldehyde to give schiff bases. The catalytic effect of pyridoxal phosphate in the amino acid metabolism by enzymes is attributed to the schiff base formed between the amino acid and pyridoxal phosphate[6-8]. We can therefore assume that the following equilibrium (2) exists under the present experimental conditions.

$$R'-\underset{\overset{+}{N}H_3}{\underset{|}{CH}}-COO^- + RCHO \rightleftharpoons R'-\underset{\overset{+}{N}H=CHR}{\underset{|}{CH}}-COO^- \quad \ldots (2)$$

Mechanism of oxidation of serine and glutamine by PMS in the absence of aldehydes

From a knowledge of the possible reactive species that exist under the present experimental conditions oxidation of serine and glutamine in the absence of aldehydes may proceed as shown in Eqs (3) and (4).

$$HOOSO_3^- \overset{K}{\rightleftharpoons} {}^-OOSO_3^- + H^+ \quad \ldots (3)$$

$$\text{Amino acid} + {}^-OOSO_3^- \overset{k_1}{\rightarrow} \text{products} \quad \ldots (4)$$

Rate law corresponding to Eqs (3) and (4) is given by Eq. (5).

$$\frac{-d[\text{PMS}]}{dt} = k_1[\text{Amino acid}][SO^{2-}_5] = Kk_1\frac{[\text{Amino acid}][HSO_5^-]}{[H^+]}$$

$$k_{obs} = Kk_1\frac{[\text{Amino acid}]}{[H^+]} \quad \ldots (5)$$

The rate equation (5) explains all the experimental facts. Using the literature[4] value of $K = 3.98\times10^{-10}$ the rate constants for the oxidation of SO_5^{2-} (k_1) have been obtained and the values are given in Table 1.

It is interesting to note that though serine is similar to threonine, it reacts only with SO_5^{2-} whereas threonine has been shown to react[9] with both SO_5^{2-} and HSO_5^-. Moreover the reaction of threonine with PMS was studied at pH 3.6, 4.0 and 4.4. In order to

Table 1—Kinetic Parameters for oxidation of Amino Acids at 31 °C in the Absence of Aldehydes[a]

Amino acid[b]	k_1		$\Delta H^{\ddagger}_{k_1}$ (K cal mol^{-1})	$\Delta S^{\ddagger}_{k_1}$ (cal deg^{-1} mol^{-1})	pK_2
Alanine	24.5	(21.0)	—	—	9.87
Butryne	32.0	(28.0)	—	—	9.83
Valine	10.6	(10.0)	22.6	62.6	9.74
Leucine	7.3	(7.0)	—	—	9.74
Isoleucine	18.4	(17.6)	16.4	46.9	9.76
nor-Leucine	28.0	(28.0)	—	—	9.83
Phenylalanine	47.2	(47.2)	18.0	50.5	9.18
Serine	87.0		17.6	51.4	9.21
Glutamine	60.0		13.4	35.5	9.13
Threonine	88.7	(76.2)	8.6	20.6	9.10

[a] Values given in parentheses are reported values taken from ref (2) and (9).

[b] Since in the oxidation of alanine, butryne and nor-leucine the first-order plots are linear only upto 10% conversion, a large error may be introduced in the calculation of k_{obs}. Therefore the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values are not calculated. We have calculated the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values only for the systems where the first order plot is linear atleast upto 50% conversion.

ascertain the above fact the kinetics were reinvestigated at *p*H 3.6 to 5.2 and from the plot of k_{obs} versus $1/[H^+]$ it was found that only SO_5^{2-} alone reacts with threonine since this plot also passed through origin. This led us to reexamine our earlier report[1] because in the earlier report we have studied the kinetics only upto 5-10% conversion of [PMS] and therefore there may arise some error in the calculation of k_{obs}. The recalculated results are given in Table 1. The values reported in Table 1 are the average of k_1 obtained as a result of $[H^+]$ and [AA] variations. Comparison of k_1 (Table 1) with the earlier reported values for the oxidation by SO_5^{2-} shows only a small difference.

Perusal of the results in Table 1 shows that there exists an approximate correlation between k_1 and pK_2 of the amino acid as expected since the reaction is a nucleophilic attack of SO_5^{2-} on $R'-CH(\overset{+}{N}H_3)COO^-$ at the $-\overset{+}{N}H_3$ group. From Table 1, we can infer that alanine, butryne etc. which are linear amino acids are more reactive than the branched chain amino acids such as leucine, isoleucine, etc. Similarly if we consider the reactivities of phenylalanine, glutamine and threonine, the reactivity order is threonine > glutamine > phenylalanine, as expected on the basis of pK_2 values. Based on this, the k_1 values of serine would be approximately equal to that of phenylalanine. But the observed value is equal to that of threonine. The above three amino acids, in comparison to serine have a different substituent at the β-position. The effect of the different bulkier groups at β-position may be complex and therefore it is difficult to arrive at any correlation in the case of serine.

From the effect of temperature on k_{obs} the activation parameters have been calculated (Table 1).

$$\Delta H^{\ddagger}_{obs} = \Delta H^{\ddagger}_{K} + \Delta H^{\ddagger}_{k_1}$$

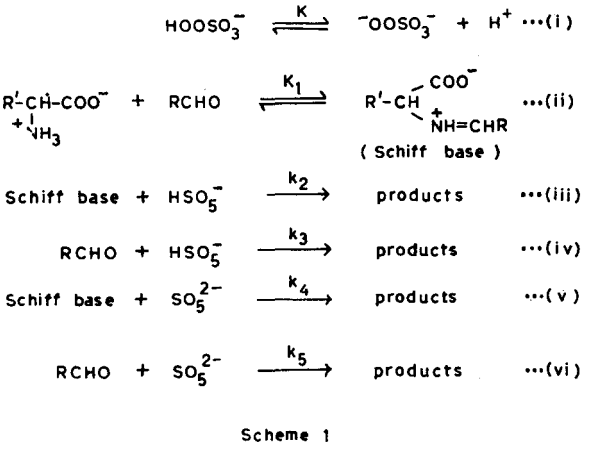
$$\Delta S^{\ddagger}_{obs} = \Delta S^{\ddagger}_{K} + \Delta S^{\ddagger}_{k_1}$$

The values of $\Delta S^{\ddagger}_{k_1}$ for all the systems are surprisingly positive and this shows that more than one intermediate/transition state is involved in the reaction pathway.

From the results we can generalize the following facts: (i) The linear α-amino acids (i.e. with no β-substituent) show a curved first order plot after 5-10% conversion of PMS. This is due to the fact that the product aldehyde reacts with the amino acids to give a schiff base which reacts faster than the amino acid. (ii) α-Amino acids having a β-substituent, with the exception of threonine, do not show such a behaviour. This may be due to the fact that the product aldehyde, is not able to form a schiff base due to steric effet. Therefore the first order plots are linear even up to 50% conversion. In the case of threonine a curvature towards X-axis (k_{obs} increases) is noticed in the first order plot after 5-10% conversion and this may be due to further oxidation of product, aldehyde. (iii) All the amino acids are oxidised by SO_5^{2-} and the rate constants (k_1), $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ can approximately be correlated with pK_2. In the case of β-branched α-amino acids the observed rate constant is smaller than the unsubstituted one.

Mechanism of oxidation of alanine, glutamine and serine in the presence of aldehydes

The mechanism of oxidation of amino acids in the presence of HCHO has been discussed earlier in detail[2,9]. The kinetics of oxidation of amino acids in the presence of both acetaldehyde and propionaldehyde are similar to those of formaldehyde. Hence, the mechanism identical to that proposed in the presence of formaldehyde is valid in the case of acetaldehyde and propionaldehyde (see Scheme 1).



Scheme 1

Based on Scheme 1, the rate law at constant *p*H can be expressed by Eq. (6)

$$\frac{-d[PMS]}{dt} = \{k_a[AA][RCHO] + k_b[RCHO]\}[PMS]$$

$$k_{obs} = k_a[AA][RCHO] + k_b[RCHO] \quad \ldots (6)$$

where k_a and k_b represent the rate constants for oxidation of schiff base and aldehydes at constant *p*H. Equation (6) explains the effect of [AA] and [RCHO] variation on the rate. The values of k_b can be calculated from the plots of k_{obs} versus [AA] (Fig. 2) and these values can be compared with the values obtained from the direct oxidation of aldehydes[10]. These two values given in Table 2 agree well and this confirms the validity of the reaction mechanism shown in Scheme 1.

The rate law consistent with Scheme 1 can also be expressed by Eq. (7)

$$\frac{-d[\mathrm{PMS}]}{dt} = (k_4K_1[\mathrm{AA}] + k_5)[\mathrm{RCHO}]_T \frac{K[\mathrm{HSO}_5^-]}{[\mathrm{H}^+]} + (k_2K_1[\mathrm{AA}] + k_3)[\mathrm{RCHO}]_T[\mathrm{HSO}_5^-] \quad \ldots (7)$$

The values obtained for $(k_4K_1[\mathrm{AA}] + k_5)$ and $(k_2K_1[\mathrm{AA}] + k_3)$ from the plots of k_{obs} versus $1/[\mathrm{H}^+]$ are lower than the values of k_5 and k_3 respectively, which are the values from the direct oxidation of the concerned aldehydes[10]. This clearly shows that the rate constants k_5 and k_3 in the presence of amino acids are always smaller than the values obtained by the direct oxidation of aldehydes. This is due to our experimental conditions, viz. [AA] > [PMS] and [RCHO] ≤ [PMS]. Because of this the oxidation of RCHO by PMS may not follow the pseudo-first order kinetics. Therefore, as an approximation k_5 and k_3 can be neglected in Eq. (7) and approximate values of k_4K_1 and k_2K_1 are given in Table 3.

It is well known that the aldehydes in aqueous solution exist as a mixture of free carbonyl form and hydrated form[11]. From the foregoing results we could not say which form of aldehyde reacts with the amino group to form a schiff base. Moreover the values of the equilibrium constant (K_1) for the schiff base formation may be different for different aldehydes. Because of this we could not correlate the rate constants obtained for the oxidation of serine and glutamine in the presence of formaldehyde, acetaldehyde and propionaldehyde.

If the complex between SO_5^{2-} (HSO_5^-) and $-N=CHR$ is assumed as the activated complex, then the formation and decomposition of this activated complex depend on the electron density at the $-N=CHR$ group. According to the +I effect of alkyl group in $-N=CHR$, the electron density at the $-N=CHR$ may be in the order: propionaldehyde > acetaldehyde > formaldehyde. The observed $\Delta H^{\dagger}$ values for oxidation of glutamine, serine and alanine in the presence of aldehyde show that the order is: propionaldehyde < acetaldehyde < formaldehyde. This clearly indicates that the electronic effect is more pronounced on the decomposition of the activated complex since the reverse order is expected if the complex formation is more pronounced due to increased electron density at $-N=CHR$. Moreoever, in our earlier work[1] the values of $\Delta H^{\ddagger}$ are almost same for various amino acids in the presence of formaldehyde, showing that $\Delta H^{\ddagger}$ values do not depend upon the alkyl part of the amino acid but depend only on the $-N=CHR$ group. This is also clearly borne out by the $\Delta H^{\ddagger}$ values obtained in the presence of acetaldehyde and propionaldehyde (Table 3).

Table 2—Rate Constants (k_b)* for Oxidation of Aldehydes at 31°C

	k_b (from Eq. 6)		
	HCHO	CH_3CHO	CH_3CH_2CHO
Alanine	—	0.08	0.08
Serine	0.06	0.07	0.07
Glutamine	0.09	0.06	0.05
Experimental*[a]	0.11	0.26	0.19

*At pH = 4.0; μ = 0.25; temp. = 31°C.
[a]Obtained from direct oxidation of aldehydes by PMS (see ref. 10).

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Table 3—Kinetic Parameters for Oxidation of Amino Acids at 31°C in the Presence of Aldehydes

	Serine			Glutamine			Alanine	
	HCHO	CH_3CHO	CH_3CH_2CHO	HCHO	CH_3CHO	CH_3CH_2CHO	CH_3CHO	CH_3CH_2CHO
$10^1\ k_2K_1$ ($dm^6\ mol^{-2}\ s^{-1}$)	19.8	13.6	9.0	14.8	9.8	7.2	17.8	12.0
$10^{-5}\ k_4K_1$ ($dm^6\ mol^{-2}\ s^{-1}$)	8.3	5.8	5.0	8.2	7.4	8.4	3.2	2.8
$\Delta H^{\ddagger}$ (k cal mol^{-1})	14.5	9.2	8.2	13.2	8.1	7.4	9.0	7.8

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