Kinetics and Mechanism of the Oxidation of Amino Acids by Peroxomonosulphate. Part 1.

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The kinetics of oxidation of α-amino acids by peroxomonosulphate (PMS) were studied. The observed rate is first order in [oxidant] and [amino acid] and inverse first order in hydrogen ion concentration. Perusal of the kinetic results show that aldehyde, the oxidation product, enhances the rate of oxidation of all amino acids, except valine. This exceptional behaviour can be attributed to steric factors due to the presence of a methyl group in the β-position. Mechanisms of oxidations of amino acids are discussed in terms of the kinetic results.

The kinetics of oxidation of amino acids have gained in importance because of their biological significance. Oxidation of amino acids by the more common oxidants such as MnO_4^-, IO_4^-, Mn^{III}, $S_2O_8^{2-}$ *etc.* has been carried out, probably with an aim of investigating model systems for the enzymic oxidation of amino acids.[1-4] In all these reactions, one of the oxidation products of the amino acid is the corresponding aldehyde. It is a well known fact that aldehydes tend to condense with amino acids to give Schiff's bases. The importance of amino acid–aldehyde condensations in biosystems is well established.[5-7] The biologically active vitamin pyridoxal phosphate as the cofactor for enzymes catalyses a number of reactions of amino acids such as the transfer of the amino group to an oxo acid, a reaction central to biosynthesis and catabolism of most amino acids.[5-7] In earlier reports, the authors could not observe the effect of the product aldehyde because the reactions were carried out at high acid concentration wherein the existence of a Schiff's base is doubtful. Therefore to find out the effect of aldehyde on the oxidation of amino acids, the kinetics of oxidation of amino acids were studied and we report here the results of oxidation of amino acids (S) by peroxomonosulphate (PMS). This system was chosen because, unlike other oxidants, oxidation by PMS can be carried out conveniently at low acid concentration.

Peroxomonosulphate can be considered as a monosubstituted hydrogen peroxide in which one of the hydrogens is replaced by the SO_3 group. Peroxomonosulphate is a better oxidant than peroxodisulphate ($S_2O_8^{2-}$) in the reaction with halides.[8,9] In this report the oxidation kinetics of glycine, DL-alanine, DL-butyrine, DL-valine, L-leucine, and DL-norleucine are discussed.

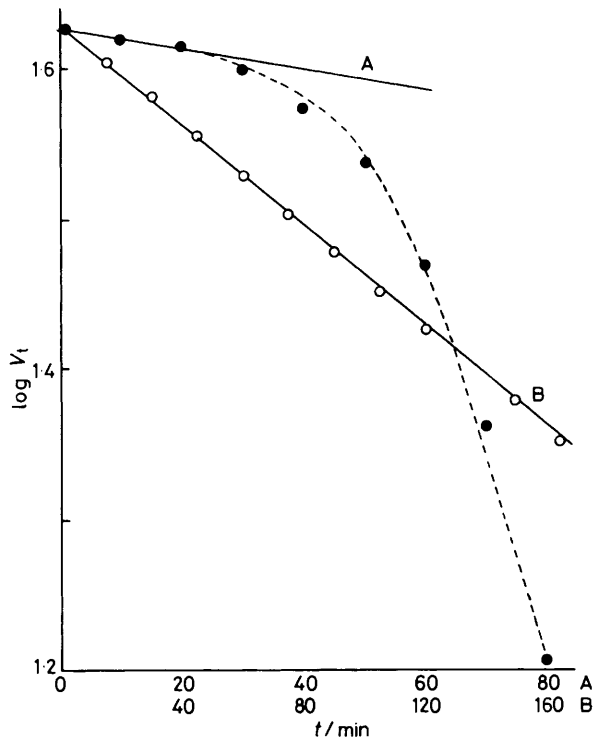


Figure 1. Plots of log V_t *versus* time: A, [Glycine] 0.15M, [PMS] 8.40×10^{-3}M, pH 4.0, μ 0.25, 35 °C; B, [Valine] 0.1M, [PMS] 8.44×10^{-3}M, pH 5.2, μ 0.25, 31 °C

Results and Discussion

All the experiments were carried out under pseudo-first-order conditions. Plots of the logarithm of the volume of thiosulphate consumed (log V_t) *versus* time were found to be linear up to 70% conversion of PMS with valine as substrate. All other amino acids showed an interesting feature, namely plots of log V_t *versus* time were linear only in the initial stages of the reaction. After 2—5% conversion of PMS, the plots showed curvature towards the x-axis. Typical plots are shown in Figure 1. This shows an increase in k_{obs} as the reaction proceeds. This may be because either the product may also be oxidised or the product catalyses the reaction. Therefore k_{obs} values were calculated from the initial part of the reactions.† At constant amino acid and hydrogen ion concentrations the values of k_{obs} were found to be independent of the initial concentration of PMS. This shows that rate is directly proportional to [PMS].

The values of k_{obs} at different initial concentrations of amino acids, and at a fixed concentration of PMS and H^+, were found to depend linearly on amino acid concentration. Plots of k_{obs} *versus* [amino acid] were found to be linear and to pass through the origin (Figure 2). This shows that at constant $[H^+]$ the rate equation can be written as $-d[PMS]/dt = k_2$ [PMS] [amino acid] $= k_{obs}$ [PMS] where $k_{obs} = k_2$[amino acid]. From the plots of k_{obs} *versus* [amino acid], the second-order rate constants k_2 were evaluated and are given in Table 1.

The values of k_{obs} were found to decrease with increasing $[H^+]$, and the plots of k_{obs} *versus* $[H^+]^{-1}$ were found to be linear with a positive slope and a non-zero intercept. This shows an inverse first-order dependence on $[H^+]$. Consequently the plots of k_{obs} $[H^+]$ *versus* $[H^+]$ were linear with an intercept on the ordinate (Figure 3). This clearly proves that the reaction

† k_{obs} for all amino acids except valine is the value calculated from plots of log V_t *versus* time for conversions of PMS not more than 5%.

Table 1. Rate constants and thermodynamic parameters for the oxidation of amino acids by PMS

	Glycine	Alanine	Butyrine	Valine	Leucine	Norleucine
$10^5 k_2$/l mol^{-1} s^{-1}	9.9[a]	10.8[b]	15.0[b]	10.1[b]	6.9[b]	16.2[b]
$10^5 k_a$/l mol^{-1} s^{-1}	2.40[c]	2.4[d]	3.4[d]	0.6[d]	4.2[d]	5.3[d]
$10^{-1} k_b$/l mol^{-1} s^{-1}	2.0[c]	2.1[d]	2.8[d]	1.0[d]	0.7[d]	2.8[d]
$\Delta H^{\neq}$/kJ mol^{-1}[e]	88.7	57.7	95.0	115.5	65.7	
$\Delta S^{\neq}$/J K^{-1} mol^{-1}[e]	−20.2	−118.0	6.7	65.5	−10.2	

[a] pH 4.0; temperature 35.0 C; μ 0.25. [b] pH 4.0; temperature 31.0 C; μ 0.25. [c] μ 0.25; temperature 35.0 C. [d] μ 0.25; temperature 31.0 C. [e] μ 0.25; pH 4.0.

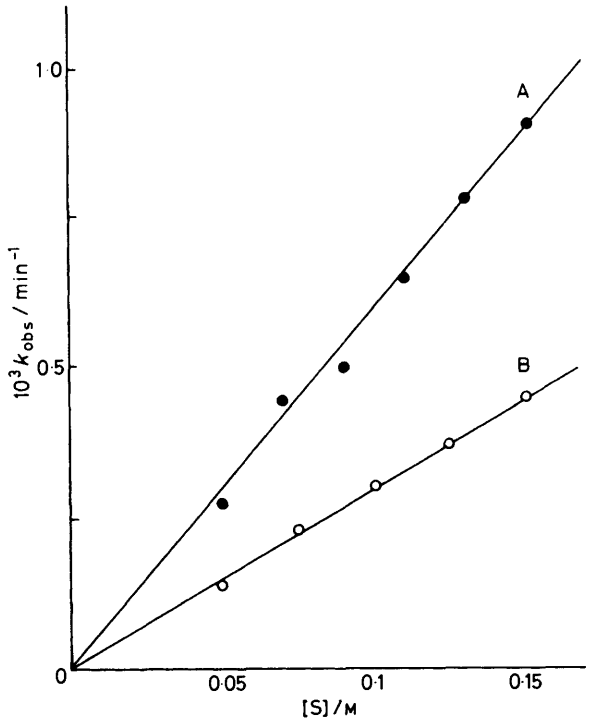


Figure 2. Plots of k_{obs} *versus* [amino acid]: A, glycine–PMS: [PMS] 4.3 × 10^{-3}M, pH 4.0, μ 0.25, 35 °C; B, valine–PMS: [PMS] 8.44 × 10^{-3}M, pH 4.0, μ 0.25, 31 °C

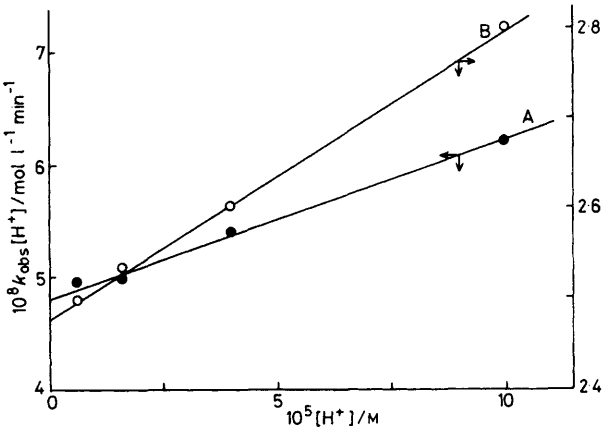
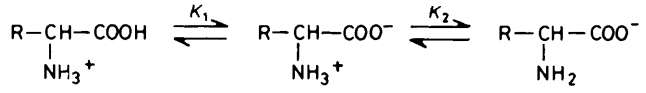


Figure 3. Plots of k_{obs} [H$^+$] *versus* [H$^+$]: A, [Glycine] 0.10M, [PMS] 4.3 × 10^{-3}M, μ 0.25, 35 °C; B, [Valine] 0.10M, [PMS] 8.44 × 10^{-3}M, μ 0.25, 31 °C

proceeds through two independent paths; one is H$^+$ ion dependent and the other is H$^+$ ion independent.



Scheme 1.

Table 2. Structures and dissociation constants* of α-amino acids RCH(NH$_2$)COOH

R	pK_1	pK_2
H	2.35	9.78
CH_3	2.35	9.87
CH_3CH_2	2.10	9.80
$CH(CH_3)_2$	2.29	9.72
CH_3–C(CH_3)H–CH_2	2.33	9.74
$CH_3CH_2CH_2CH_2$	2.34	9.83

* Lange's Handbook of Chemistry, ed. J. A. Dean, McGraw-Hill, New York, 1973, 11th edn., pp. 5—15.

A change in ionic strength, over the range 0.25—1.00, showed no pronounced effect on k_{obs}. The reactions were studied at three different temperatures (30—45 °C) and, from the temperature dependence of k_{obs}, the activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were calculated (Table 1).

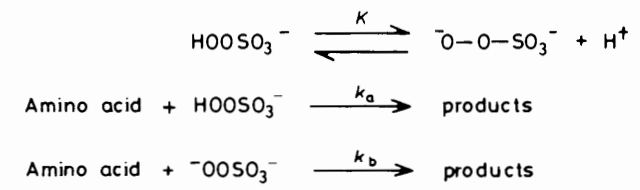
For amino acids the equilibria in Scheme 1 exist in acid–alkali solutions. The values of pK_1 and pK_2 for the amino acids along with their structures are in Table 2. Under our experimental conditions namely at pH 4.0 all the amino acids are in the form of zwitterions. Therefore the amino acid in its zwitterionic form may be the reactive species.

Peroxomonosulphuric acid (HO–OSO_3–H) has two ionisable protons; one is the sulphuric acid proton and the other is the hydrogen peroxide proton. The pK_a value of the sulphuric acid proton lies in the high acidity region and that of the hydrogen peroxide proton [10] is 9.4. Since the rate was found to increase as the pH was increased, and decrease as [H$^+$] was increased; and also from the nature of the k_{obs} [H$^+$] *versus* [H$^+$] plot leaving an intercept on the ordinate (Figure 3), the mechanism for oxidation involving acid-independent and inverse acid-dependent paths may be as in Scheme 2. Rate equations (1) and (2) for the disappearance of PMS are based on Scheme 2. k_a'

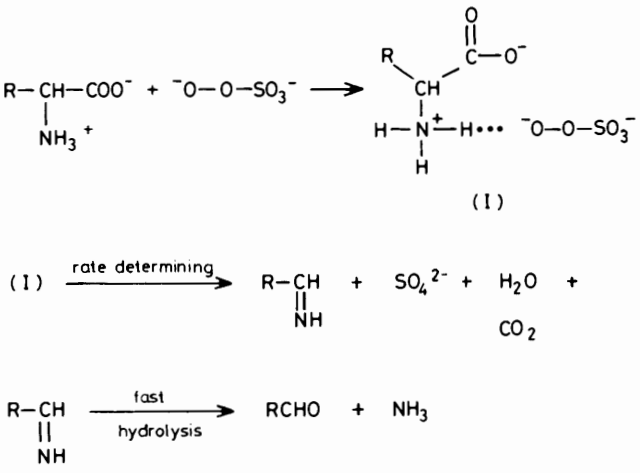
$$-\mathrm{d[PMS]}/\mathrm{d}t = k_a\,[\text{amino acid}]\,[HSO_5^-] + k_bK\,[\text{amino acid}]\,[HSO_5^-]/[H^+] \quad (1)$$

$$-\mathrm{d[PMS]}/\mathrm{d}t\,[\mathrm{PMS}] = k_{obs} = k_a[\text{amino acid}] + k_bK\,[\text{amino acid}]/[H^+] = k_a' + (k_b'K/[H^+]) \quad (2)$$

and k_b' denote the pseudo-first-order rate constants. This mechanistic scheme explains the acid dependence of k_{obs}. Plots of k_{obs} [H$^+$] *versus* [H$^+$] were drawn and from the slope of these plots the values of k_a were obtained. From the intercepts the values of k_b were obtained using the literature value[10] K = 3.98 × 10^{-10}. The values are given in Table 1. It is seen that k_b



Scheme 2.



Scheme 3.

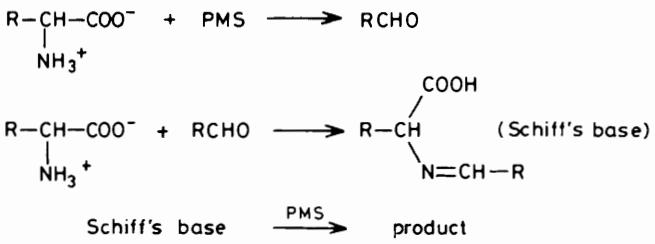
is approximately six orders of magnitude higher than k_a. This is in accord with earlier observations.[11]

In general, the reactions of peroxides are liable to acid catalysis. The present investigation is interesting in that an inverse acid dependence is observed. The higher reactivity of SO_5^{2-} than that of HSO_5^- may be considered to be in favour of nucleophilic attack by the peroxide.[11] The foregoing kinetic results may suggest a nucleophilic substitution mechanism on the NH_3^+ group in the amino acid by PMS as shown in Scheme 3.

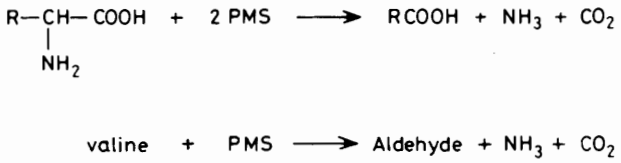
There is also abundant evidence that peroxoanions are strongly nucleophilic as pointed out by earlier workers.[12,13]

If we compare the values of k_a, we observe that they increase with increase in chain length, except for valine. This may be due to steric hindrance since in valine the methyl group is β to the COOH group. In the case of the k_b values we observe an anomalous effect, namely the value for leucine is less than for valine although the methyl group in leucine is β to the NH_3^+ group. This may be due to the stabilisation of the activated complex of the leucine–PMS system, probably by hydrogen bonding between the methyl group and SO_5^{2-}. However, the erratic variation of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ values from one amino acid to another prevents us from deriving any definite and supportive information. The increase in k_a or k_b with chain length can be attributed to the hyperconjugation effect proposed by Manikyamba and Sundaram[14] in the oxidation of amino acids by *N*-bromosaccharin.

Finally, one important observation not yet explained is the increase in k_{obs} after the conversion of a small amount of PMS. This can be very easily explained by the fact that the product aldehyde initially formed reacts with the amino acid to give



Scheme 4.



Scheme 5.

an aldimine (Schiff's base) which reacts more readily with PMS (Scheme 4).*

In fact the catalytic effect of pyridoxal phosphate (vitamin B_6) in amino acid metabolism by enzymes is attributed to the Schiff's base formed between the amino acid and pyridoxal phosphate.[16–18] The catalytic effect of aldehydes in the oxidation of amino acids by PMS is also corroborated by our investigation on the oxidation of amino acids by PMS in the presence of formaldehyde.[15] The exceptional behaviour of valine may be due to the fact that the resultant aldehyde $(CH_3)_2CHCHO$ can't form a Schiff's base with valine. This may be due to the steric factor because valine is found to form Schiff's bases with pyridoxal[19] and formaldehyde.[15] This fact is also confirmed by the final products detected; in all amino acids the final product is acid whereas valine gives only aldehyde.

Experimental

Potassium peroxomonosulphate was from the Dupont Chemical Co. under the trade name Oxone. It is a triple salt with the composition $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$. The purity of the salt was found to be 96%. The peroxomonosulphate solution was prepared daily just before starting the experiments and the concentration was estimated by cerimetry using ferroin as indicator. The absence of free hydrogen peroxide was established by tests with permanganate. Amino acids were from Loba-Chemie Indo Austranal Co. Other chemicals used were all of analytical grade.

Experiments were carried out in buffered media. High concentration of the buffer (0.1M) in the reaction mixture–final solution was maintained since the product HSO_4^- is a stronger acid. pH was measured in a Elico (India) pH meter model L1-10T. All the experiments were carried out at pH 4.0, unless otherwise stated. Under the present experimental conditions, no self-decomposition of PMS was observed. The kinetics of the reaction were followed iodometrically by estimating the amount of PMS which had disappeared at different time intervals. The rate constants were obtained using the integral method.

The stoicheiometry of the reactions was determined by taking a known excess concentration of peroxomonosulphate over amino acids and allowing the reaction to complete at room temperature. Different ratios of amino acids and PMS were taken and, after the reactions were over, the remaining oxidant was estimated by iodometry. The stoicheiometry of the reactions is represented in Scheme 5. The evolution of CO_2 and NH_3 was determined by tests with lime water and Nessler's

*Our experiments on the oxidation of amino acids by PMS in the presence of formaldehyde show that the Schiff's base formed between an amino acid and formaldehyde reacts 10^5 times faster than the amino acid itself (see ref. 15).

reagent, respectively. The formation of formic acid was detected by spot tests [20] in the case of glycine. In alanine, acetic acid was detected by spot tests [21] and in all other amino acids the formation of acid was confirmed. The oxidation of valine results in the formation of an aldehyde, confirmed by Schiff's reagent. In the case of glycine, the number of mol. equiv. of PMS consumed or required is greater than two and this may be due to further oxidation of product or formic acid. The oxidation of formic acid by PMS was also confirmed.[22]

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