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Influence Of Substituents On The Mechanism Of Oxidation Of Amino Acids

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Abstract : The kinetics of oxidation of amino acids (AA) by peroxomonosulphate in aqueous alkaline medium at 35°C is studied. Based on the experimental results, a reaction scheme is proposed in which the electrophilic attack of HSO_5^- occurs at the amino nitrogen. The break down of the intermediate is influenced by the nature of the substitutents at the amino carbon atom. The formation of imine/imino acid is supported by the absorption spectra of the intermediate.

Kinetics of oxidation of α-amino acids (AA) by peroxomonosulphate (PMS) in buffered medium was reported earlier.[1,2] In the lower pH region, amino acids exist as zwitterions and peroxomonosulphate as monoprotonated species ie. HSO_5^-. Although there are few data available on the oxidation by peroxomonosulphate, evidence shows that HSO_5^- is more reactive than SO_5^{2-} towards some anions.[3] Therefore it would be interesting to study the reaction between amino acid anion and PMS. In this report, we present the results on the oxidation of twelve structurally different amino acids in aqueous alkaline medium at 35°C.

RESULTS AND DISCUSSION

The kinetics of oxidation of PMS are followed by monitoring unreacted peroxomonosulphate under psudo-first order condition ie [amino acid] $\gg$ [PMS]. All the kinetic studies are carried out at constant hydroxide concentration $[OH^-]_f$ where $[OH^-]_f = [OH^-]_T$ - [amino acid] - [PMS].

The rate of disappearance of PMS follows first order reaction, as shown by plot of log $[PMS]_t$ VS time (Fig.1), which is linear at even $>75\%$ conversion of $[PMS]_o$. The values of the pseudo-first order rate constants (k_{obs}) calculated from these plots are independent of $[PMS]_o$. This clearly shows that the rate is first

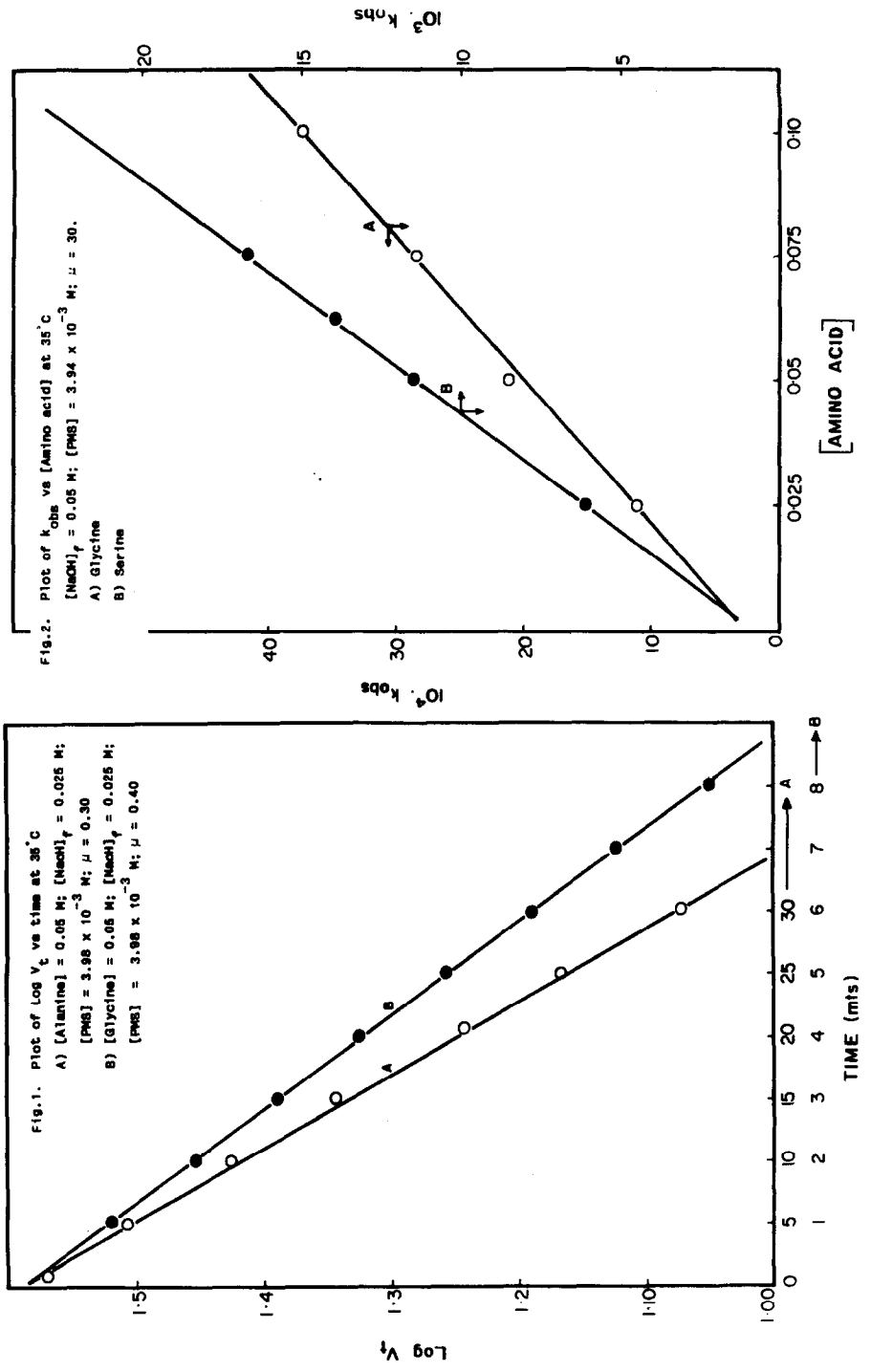
Fig.1. Plot of Log V_t vs time at 35°C
A) [Alanine] = 0.05 M; $[NaOH]_f$ = 0.025 M;
[PMS] = 3.98×10^{-3} M; μ = 0.30
B) [Glycine] = 0.05 M; $[NaOH]_f$ = 0.025 M;
[PMS] = 3.98×10^{-3} M; μ = 0.40
Log V_t
TIME (mts)
Fig.2. Plot of k_{obs} vs [Amino acid] at 35°C
$[NaOH]_f$ = 0.05 M; [PMS] = 3.94×10^{-3} M; μ = 0.30.
A) Glycine
B) Serine
$10^4 \cdot k_{obs}$
$10^3 \cdot k_{obs}$
[AMINO ACID]

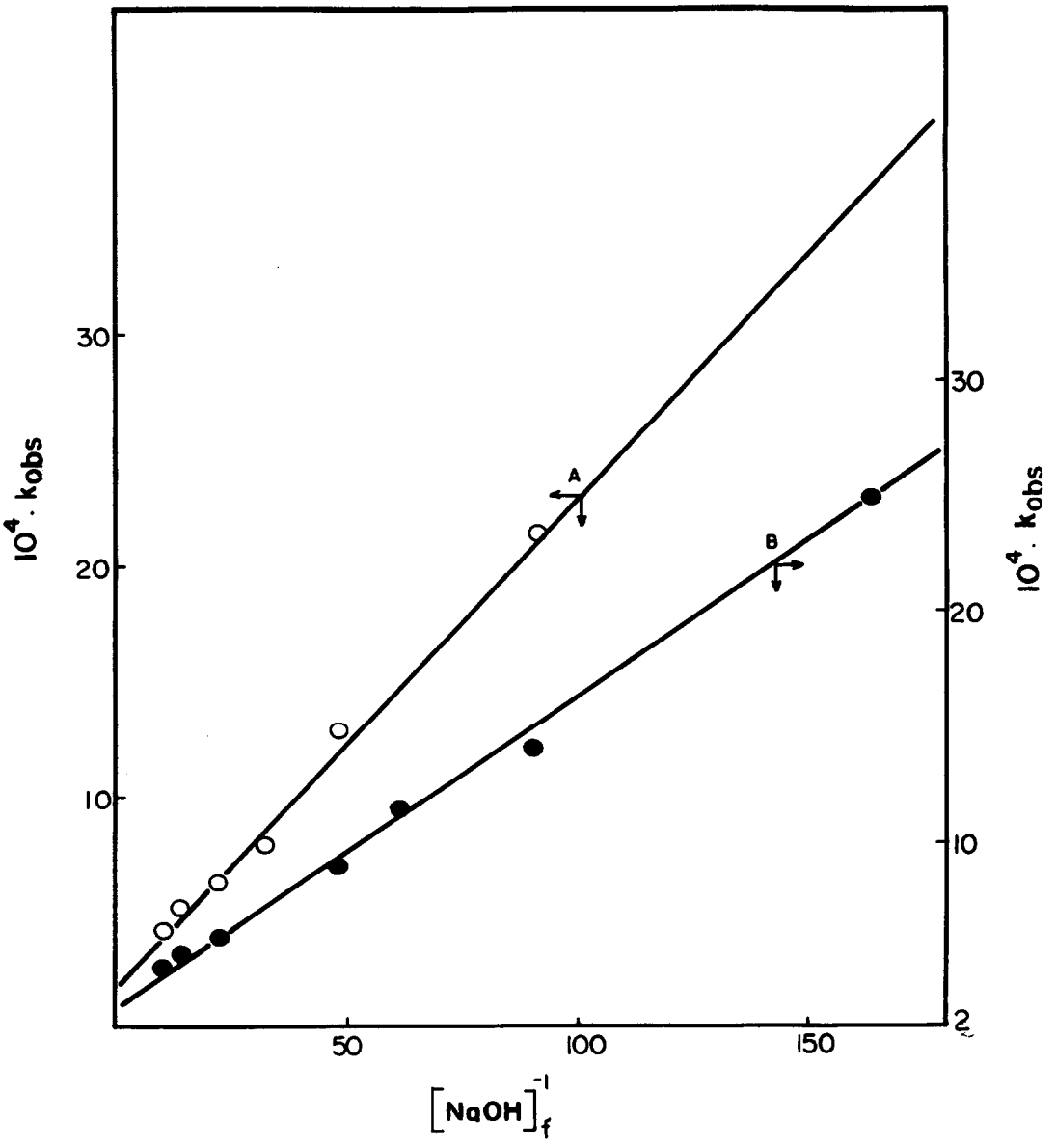

Fig.3. Plot of k_{obs} vs $[NaOH]_f^{-1}$ at 35°C
A) [Valine] = 0.05 M; [PMS] = 3.96×10^{-3} M; μ = 0.30
B) [Phenylalanine] = 0..05 M; [PMS] = 3.88×10^{-3} M;
μ = 0.40

order with respect to [PMS].

At constant $[OH^-]_f$ the pseudo-first order rate constants are found to increase with increase in [AA]. The plots k_{obs} VS [AA] are straight lines with positive intercept in all the amino acids (Fig.2). The slope of such plots shows an inverse relationship with $[OH^-]_f$.

At constant [AA], k_{obs} values decrease with increase in $[OH^-]_f$. Furthermore the plots, of k_{obs} VS $[OH^-]_f^{-1}$, are straight lines with a positive intercept (Fig.3). The rate of oxidation of N-methyl and N- phenyl glycine is studied at very high $[OH^-]_f$ (usually >0.1 M). Serine and threonine react very fast at 0.05 M $[OH^-]_f$. Hence the influence of OH^- ion on k_{obs} could not be studied in these amino acids.

The effect of ionic strength (μ) on k_{obs} is also studied. A noticeable but insignificant increase in k_{obs} with increase in μ is observed. The effect of dielectric constant is studied by increasing the percentage (weight) of acetonitrile in acetonitrile-water solvent. The k_{obs} values increase with increase in percentage weight of acetonitrile. The rate is not at all affected by the presence of nitrogen atmosphere. Sulphate ion has no effect on the rate.

The stoichiometry of the reaction can be representated as

$$PMS + AA \longrightarrow \text{Products.}$$

The oxidative product of AA is identified as the corresponding aldehyde. The percentage yield of aldehydes are; glycine > 90%, alanine > 95% & valine > 90%.

The nature of the reactant species, at the experimental conditions or involved in the reaction, is essential to have an idea about the reaction mechanism. For amino acids the following equilibria exist in acidic/alkaline solutions.

$$R\,CH(\overset{+}{N}H_3)\,COOH \rightleftharpoons R\,CH(\overset{+}{N}H_3)COO^- \rightleftharpoons RCH(NH_2)COO^-$$

The pK_1 and pK_2 values for most of the amino acids[4] are 2.1 ± 0.3 and 9.6 ± 0.7. Under the experimental conditions, namely at $[OH^-]_T$ > [AA], all the amino acids would be in the form of amino acid anion ($R.CH(NH_2)COO^-$) and the conversion may be quantitative. Therefore by AA we mean only amino acid anion.

Peroxomonosulphuric acid ($H\text{-}O\text{-}O\text{-}SO_3H$) has two ionizable protons, one is the sulphuric acid proton and the other is the hydrogen peroxide proton[5]. The pK_a value of the sulphuric acid proton lies in the high acidity region and that of hydrogen peroxide proton is 9.4. This suggest that the commercially available salt such as $KHSO_5$ will quantitatively exist only as SO_5^{2-} when $[OH^-]$ > [PMS]. Therefore it is reasonable to assume that

$$[OH^-]_f = [OH^-]_T - [AA] - [PMS].$$

The absorption spectra of the reaction intermediates may throw some light on

the reaction mechanism. The intermediate in alanine oxidation shows an absorption spectrum with a maximum at ~228.0 nm and the absorption increases with time (Fig.4). Similar spectral behaviour is observed in other amino acids (valine ~225.0 nm β-alanine ~223.0 nm). But in glycine a broad spectrum, probably a mixture of two, with maximum at ~226.0 nm and ~260.0 nm is observed (Fig.5). Imines, as the oxidative intermediate of α-amino acids by NBS at pH ~4.0, with absorption ~240 nm were reported.[6] Therefore the absorption around ~230 nm in our experiments may also be ascribed to the intermediate imine.

One of the interesting observation is that almost all the amino acids show an inverse dependence on $[OH^-]_f$. Hydroxide ion catalysed reaction also occurs in addition to the inverse dependence in some amino acids. This hydroxide ion catalysed reaction is well pronounced in glycine, β-alanine and γ-aminobutyric acid and to a smaller extend in alanine and phenylalanine. Strong alkali reacts with α-hydrogen atom of the α-amino acids.[7] Experimental results from this laboratory show hydroxide ion interacts with the hydrogen atom of the amino carbon in glycine and to a smaller extend in alanine[8]. Such interaction can also be assumed in this study also.

Thompson et al[3] observed that HSO_5^- is more reactive than SO_5^{2-} towards N_3^- and HN_3. This is explained by the electrostatic effect and weakening of the peroxide bond by proton. The same effect may also operate here and the inverse hydroxide ion dependence can be explained by the assumption that HSO_5^- may be the reactive form of PMS due to the equilibrium.

$$SO_5^{2-} + H_2O \underset{}{\overset{K_1}{\rightleftharpoons}} HSO_5^- + OH^-$$

and the value of the hydrolysis constant K_1 is 2.5×10^{-5} at 25°C.

Based on the experimental results, we can propose a reaction scheme as follows.

$$SO_5^{2-} + H_2O \overset{K_1}{\rightleftharpoons} HSO_5^- + OH^-$$

$$\text{Amino acid} + OH^- \overset{K_2}{\rightleftharpoons} \text{complex}$$

$$\text{Amino acid} + HSO_5^- \xrightarrow{k_1} \text{products}$$

$$\text{Complex} + HSO_5^- \xrightarrow{k_2} \text{products}$$

$$SO_5^{2-} \xrightarrow[\text{hydrolysis}]{k_3} \text{products.}$$

The observed rate constant for the disappearance of PMS is given as

$$k_{obs} = \left\{ \frac{k_1K_1 + k_2K_1K_2[OH^-]}{[OH^-]} \right\} [AA] + k_3$$

The first step in the reaction scheme is the electrophilic attack of HSO_5^- at the nitrogen atom of the amino group (Fig.6). This intermediate may decompose through a non concerted reaction pathway. Oxygen-Oxygen bond cleavage may precede carbon-nitrogen

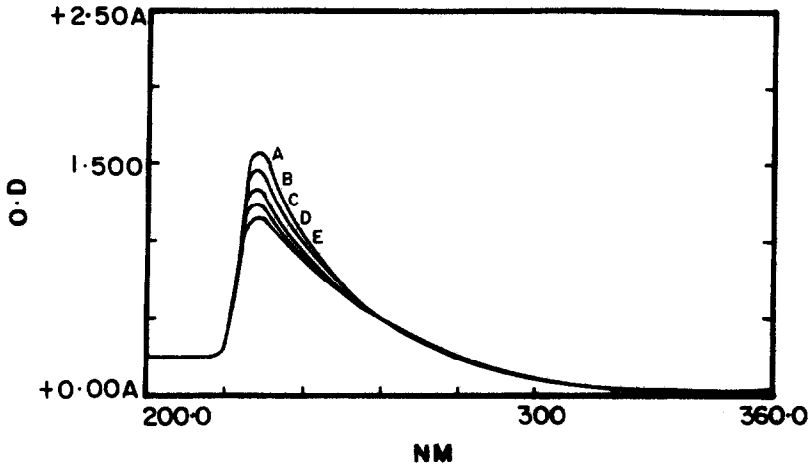

Fig.4. Absorbtion spectrum of alanine anion PMS mixture at various time.
[Alanine] = 0.05 M; [NaOH] = 0.05 M;
[PMS] = 4.00 x 10^{-3}M
A) 1.5 min; B) 5 min; C) 10 min;
D) 15 min; E) 20 min.

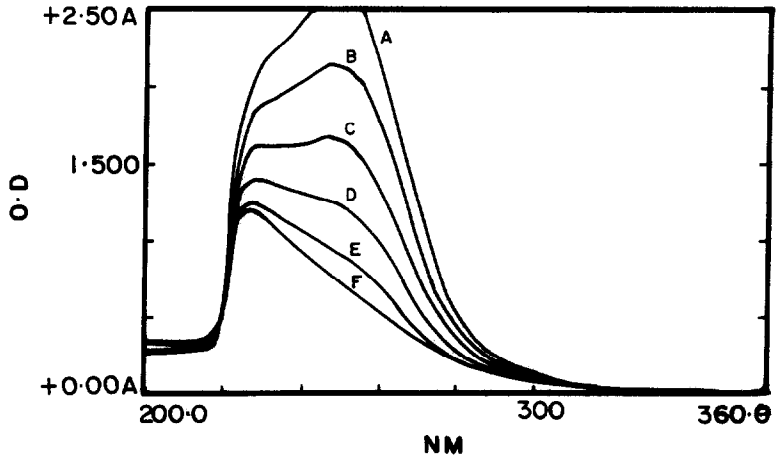

Fig.5. Absorbtion spectrum of glycine anion PMS mixture at various time.
[Glycine] = 0.05 M; [NaOH] = 0.05 M;
[PMS] = 3.90 x 10^{-3}M
A) 1.5 min; B) 3 min; C) 5 min;

bond formation. The carbon-nitrogen bond formation may be accelarated by the polarisation of the C-H bond through the interaction with OH^-. The inductive effect of the alkyl substituent at the amino carbon may inhibit the OH^- catalysed reaction. This may explain that hydroxide ion catalysed reaction occurs readily in glycine, β-alanine and γ-aminobutyric acid. Moreover the similarity of the observed kinetics in these three amino acids also support the fact that HSO_5^- attack at the amino nitrogen. The influence of electro static effect may be the reason for the electrophilic attack of HSO_5^- at the amino nitrogen instead of an electron rich carboxylato group.

The high reactivity of N-methyl and N-phenyl glycine compared to glycine can be explained by the electron donating effect of substituent at the amino nitrogen which may favour the oxygen-oxygen bond cleavage. Based on the inductive effect of the substitutent one would expect that N-methyl glycine should be more reactive than N-phenyl glycine. This is borne out by the observation that the rate is measurable only with 0.2 M $[OH^-]_f$ in N-methyl glycine and 0.1 M in N-phenyl glycine. The high reactivity of serine and threonine may be attributed to the α-effect as in the reactions with N-halo oxidants[8].

The values of $k_1 K_1$, $k_2 K_1 K_2$ and k_3 calculated from different plots, such as (i) k_{obs} VS [AA] at different $[OH^-]_f$ and (ii) k_{obs} VS $[OH]_f^{-1}$, are agreeable within the limits of experimental error. The values are given in Table 1. This is supported by the independant study of thermal decomposition of PMS which is independant of $[OH^-]$ with a rate constant 0.11×10^{-4} (s^{-1}). Analysis of the results in Table 1 shows that in α-amino acid the breakdown of the intermediate to imine (non-catalysed reaction) is not influenced by the nature of the alkyl substituent at the α-carbon atom.

The mechanistic scheme for the oxidation of amino acids by PMS is shown in Fig.6 with glycine as an example. Imino acid may also be formed in addition to imine in glycine and alanine. Only imino acids are formed through hydroxide ion catalysed as well as the normal reaction pathway in β-alanine and γ-amino butryic acids. α-Amino acids with alkyl substituent at α-carbon give imine as the only reactive intermediate. These observations may explain the presence of two absorption peaks in glycine while only a single maximum in other amino acids. The absorption peak due to the imino acid is not observed in alanine. This may be due to the fact that formation of imino acid is slower (compared to glycine) which may be merged with the imine spectrum. The intermediates imines and imino acids may be hydrolysed to corresponding aldehydes which are estimated in the product analysis.

EXPERIMENTAL METHODS

Amino acids were from either Loba-Chemie (India) or Sigma (USA). Potassium peroxomonosulphate, the triple salt $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ under the trade name "Oxone" was from Dupont Chemical Co., USA. The purity of the sample was found to be 95%. Absence of free H_2O_2 was ensured by the test with permanganate. Other chemicals used were of analytical grade. The kinetics of the reaction was followed by measuring the

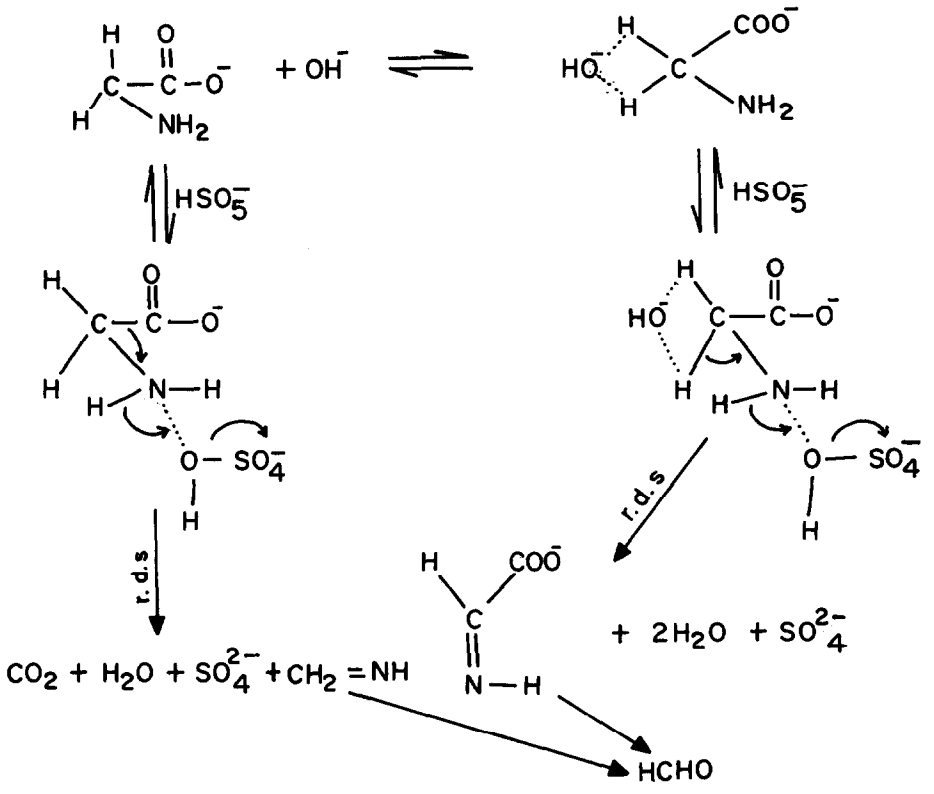

Fig.6. Reaction Scheme

TABLE 1: Kinetic Parameters* For The Oxidation Of Amino Acids By PMS At 35°C

Amino acids	$10^4 \times k_1K_1$ ($M^{-1}s^{-1}$)	$10^2 k_2K_1K_2$ ($M^{-1}s^{-1}$)	$10^4 k_3$ (s^{-1})
Glycine	7.00	2.10	2.83
β -Alanine	3.45	3.02	0.10
γ - Aminobutyric acid	2.39	3.28	0.10
Alanine	4.65	0.70	0.98
Phenylalanine	2.65	0.71	0.63
Valine	5.38	-	0.72
α - Aminoisobutyric acid	3.36	-	0.37
Aspartic acid	5.65	-	0.00

* The average values are given here

concentration of unreacted PMS by iodometry at different time intervals.

Stoichiometry: The stoichiometry of the reactions was determined by taking a known excess concentration of PMS over amino acids. After making corrections for self decomposition of PMS the observed stoichiometry can be written as

$$\text{PMS} + \text{Amino acid} \longrightarrow \text{Products}$$

Product Analysis : The product, aldehyde was estimated by 2,4-dinitro-phenyl hydrazione method, as given by Wells,[9] under kinetic conditions ie [AA] > [PMS]. The percentage yield of aldehye was calculated based on [PMS]. The product from β - alanine 3-oxopropionic acid ($CHO-CH_2-COOH$) could not be estimated since the compound enolises to give $CHOH=CHCOOH$ and free formyl acetic acid has not been isolated so far [10]. Similarly the product from γ -aminobutyric acid gives an oily hydrazone.[10] The presence of aniline in the oxidation of N-phenyl glycine is confirmed.

Absorption spectra of the intermediates were recorded in the UV region (360-200 nm) on a Schimatzu UV-160 spectrophotometer using 1 cm matched cells. Corrections for the absorption of amino acid anion if any were made.

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