



## Oxidation of Dipeptide Glycylglycine By PMS In Aqueous Medium And Comparison With Monomer Glycine: A Kinetic And Mechanistic Study

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### ABSTRACT

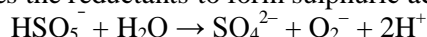
The kinetics of oxidation reactions of dipeptide Glycylglycine (GG) by Peroxomonosulphate (PMS) in aqueous medium, under the condition  $(PMS) \ll (GG)$  at different temperatures (313 – 323 K), to produce an aldehyde, and ammonia were studied. Perusal of the kinetic results showed that the first order dependence in  $[PMS]$  and fractional order dependence in  $[GG]$ . The effect of ionic strength and Acetonitrile (ACN) on rate was studied and thermodynamic parameters were also calculated. Michealis Menten type mechanism was proposed.

**Keywords:** The kinetics of oxidation reactions of dipeptide Glycylglycine, the effect of ionic strength, thermodynamic parameters.

### INTRODUCTION

A peroxide compound contains an oxygen–oxygen single bond or the peroxide anion  $([O-O]^{2-})$ [1]. Peroxides can be roughly classified into organic and inorganic compounds. Whereas the inorganic peroxides have ionic, salt-like character, the organic peroxides are dominated by the covalent bonds. Peroxy compounds like PMS[2-3], Peroxodisulphate (PDS)[4-5], Peroxomonophosphate (PMP)[6], Peroxodiphosphate (PDP)[7] and organic peroxides[8-12] are economically and environmentally preferred for oxidation of organic compounds than conventional metal ions oxidants[2]. These reagents react with wide range of functional groups affecting an array of molecular transformations.

PMP and PMS can be considered as substituted hydrogen peroxide in which one of the hydrogen is replaced by an oxy anion group of phosphorous or sulphur. Even though PMP and PMS are more powerful oxidizing agents than the corresponding peroxodiacids ( $H_4P_2O_8$  or  $H_2S_2O_8$ ), sometimes they exhibit reducing properties similar to  $H_2O_2$ . PMS has been used as an oxidant in the study of kinetics of oxidation of organic compounds like carbonyl compound, alcohols and benzylic compounds [4-10]. PMS, which is structurally similar to peroxodisulphate (PDS), and has been shown to be a better oxidant than PDS in its reaction with halides[4,5]. PMS oxidizes the reductants to form sulphuric acid and the reaction involves



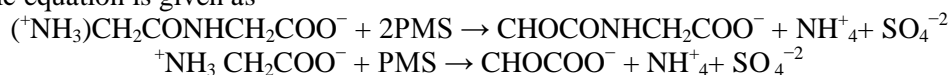
GG is a typical dipeptide which is the first member of dipeptide series. The oxidation of GG has been reported by a very few oxidants[13- 16]. GG undergo oxidation by two different routes based on the nature of the oxidant used and the reaction medium employed[17,18]. The first route is through C-C bond cleavage and second one is through N-H bond cleavage.

## MATERIALS AND METHODS

GG (E.Merck, analytical grade) was purified by column chromatography and used in experiment. PMS obtained from Aldrich-West Hermann with highest purity and analytical grade used as received in stock solution. All other chemicals were of analytical grade.

**Kinetics and Measurements:** The kinetic studies were made under Pseudo-first order conditions with GG concentrations always greater than the concentration of PMS. The progress of the reaction was monitored by estimating the unreacted concentration of PMS by iodometrically using freshly prepared starch as an indicator.

**Stoichiometry and product analysis:** Under the conditions  $[PMS] \gg [GG]$ , the reaction was allowed to go to completion and the unreacted PMS was estimated iodometrically. It was found that one equivalent of GG required two equivalents of PMS and one equivalent of Gly needed one equivalent of PMS. The stoichiometric equation is given as



Aldehyde formation was detected by chemical analysis 2,4-DNP reagent and TLC by comparing with standard, while ammonia was identified by Nessler's reagent.

## RESULTS AND DISCUSSION

The kinetics of oxidation of GG by PMS was investigated at different  $[GG]$ , conducted under the conditions  $[PMS] \ll [GG]$ , the reaction was allowed to go for completion. The progress of the reaction was monitored by estimating the unreacted  $[PMS]$  at different intervals of time. The plots of  $\log(a/a-x)$  (where 'a' and (a-x) corresponds to the concentration of PMS at zero time and at time 't') Vs time were found to be linear passing through the origin fig-1 indicating first order dependence of rate in  $[PMS]$ . From the slopes of such plots pseudo-first order rate constants ( $k'$ ) evaluated were independent of  $[PMS]$ , conforming the first order dependence in  $[PMS]$ . The rate increases with increasing in  $[GG]$ .

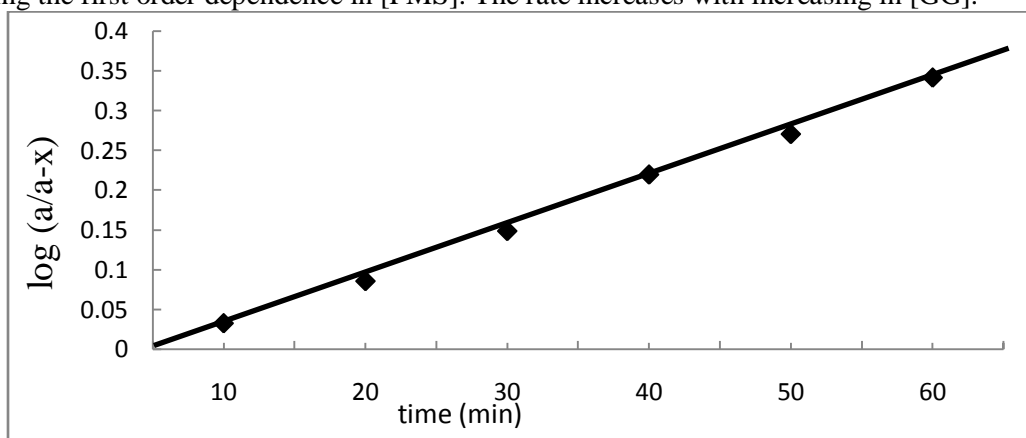


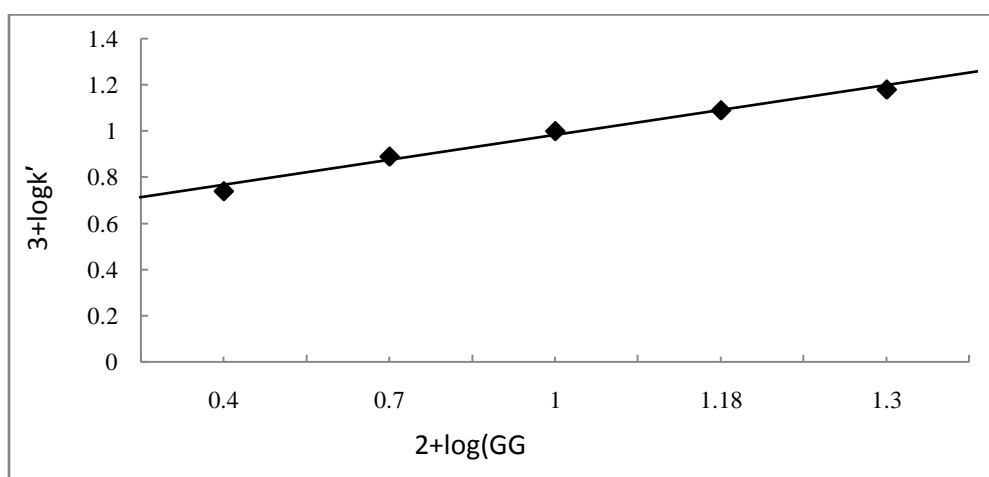
Fig.1. Oxidation of GG by PMS  $[PMS] = 5 \times 10^{-3} \text{ mol. dm}^{-3}$ ; Temp = 313 K

The plot of  $\log k'$  versus  $\log [GG]$  is linear ( $r=0.99$ ), with a slope of  $n=0.5$  indicating a fractional order dependence of rate in  $[GG]$  Fig-2. No altering of order in GG further increasing  $[GG]$ , the reaction obeying the Michealis-Menten kinetics (Table-1). The rate of oxidation of GG was decreased marginally with the

increase in ionic strength (addition of  $\text{NaClO}_4$ ). The reaction rate was found to increase with increase in concentration of ACN content varied by the addition of 0-20% (v/v) ACN in the reaction medium. Blank experiment showed that ACN was not oxidised by PMS under experimental condition.

**Table-1:** Order in [GG] in the oxidation of [GG] by PMS in aqueous medium.  $[\text{PMS}] = 5 \times 10^{-3} \text{ mol. dm}^{-3}$ ; Temp = 313 K

$10^2 \times [\text{GG}]$ ( $\text{mol dm}^{-3}$ )	$k \times 10^3 \text{ min}^{-1}$	$2+\log[\text{GG}]$	$3+\log k'$
2.5	5.5	0.40	0.74
5.0	7.7	0.70	0.89
10.0	10.1	1.00	1.00
15.0	12.3	1.18	1.09
20.0	15.1	1.30	1.18

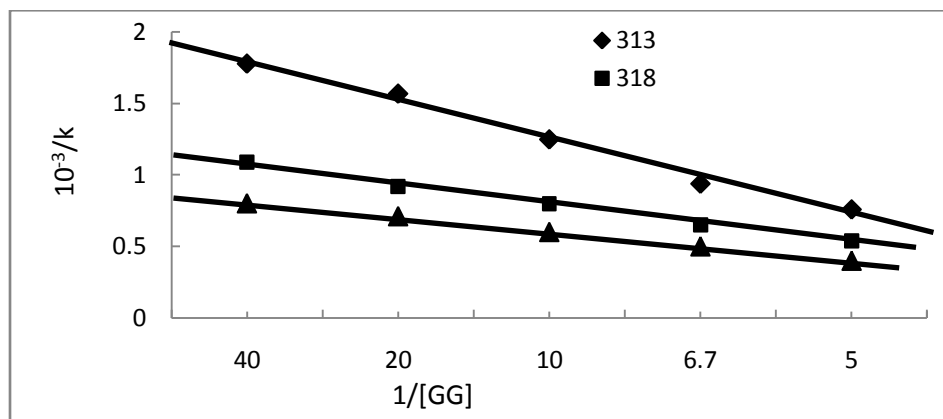


**Fig.2** Effect of [GG] on  $k'$  in PMS-GG reaction  $[\text{PMS}] = 5 \times 10^{-3} \text{ mol. dm}^{-3}$ ; Temp = 313 K

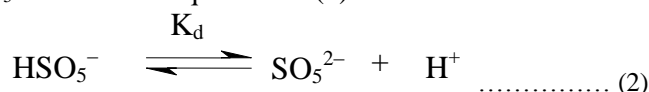
The kinetic study of the reaction is studied at different temperature from the range 313-323K. The substance concentration was changed at each temperature to Michealis-Menten kinetics. Activation parameters for the rate limiting step have been computed as follows:

**Table-2:** Activation parameters for the oxidation of GG by PMS.

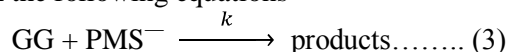
Substrate	$E_a$ ( $\text{kJ mol}^{-1}$ )	$\Delta H^\#$ ( $\text{kJ mol}^{-1}$ )	$\Delta G^\#$ ( $\text{kJ mol}^{-1}$ )	$\Delta S^\#$ ( $\text{J mol}^{-1} \text{ K}^{-1}$ )
GG	27.2	24.6	107.8	-266
Glycine	13.1	10.5	133.8	-394



Peroxomonosulphuric acid (HO<sub>2</sub>OSO<sub>3</sub>-H) has two ionizable protons, one is the sulphuric acid proton and the other is the hydrogen peroxide proton. The pK<sub>a</sub> value of the hydrogen peroxide proton[19] is 9.4. The oxidant peroxomonosulfate is a potential two equivalent oxidant and in most of the reaction usually the terminal peroxide oxygen atom is transferred to the reductants. In aqueous solution, PMS exists as a mixture of HSO<sub>5</sub><sup>-</sup> and SO<sub>5</sub><sup>2-</sup> due to the equilibrium (2).



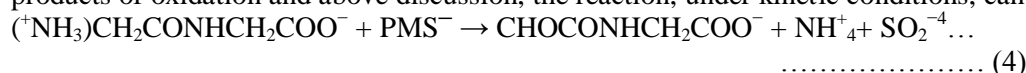
The dissociation constant  $K_d$  is given in the literature as  $4.0 \times 10^{-10}$  M at 25°C<sup>20</sup>. Under the experimental conditions,  $K_d / [\text{H}^+]$  value will be  $< 10^{-3}$  or even lesser and hence, all PMS will exist in the form of HSO<sub>5</sub><sup>-</sup>. Therefore, HSO<sub>5</sub><sup>-</sup> may be the active form of the oxidant under the experimental conditions. The kinetic scheme can be represented as in the following equations



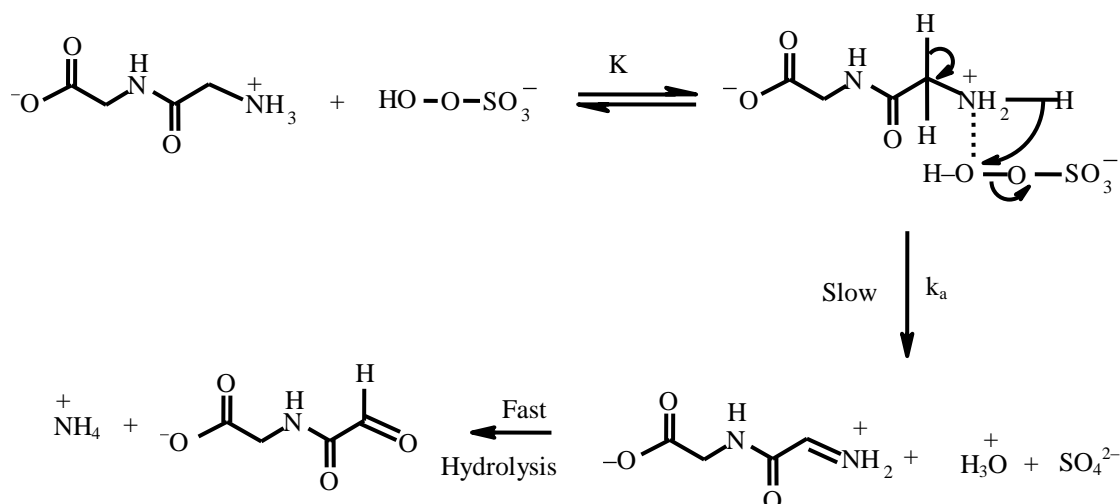
Equation (3) represents the interaction of the HSO<sub>5</sub><sup>-</sup> with GG. Similar mechanisms were proposed in the oxidation of simple α-amino acids by PMS[8, 9].

In general, the reactions of peroxides are liable to acid catalysis. The higher reactivity of SO<sub>5</sub><sup>2-</sup> than that of HSO<sub>5</sub><sup>-</sup> may be considered to be in favor of nucleophilic attack by the peroxide[10-12,21,22]. Based on this PMS can exist in two anionic forms, viz. PMS<sup>-</sup> and PMS<sup>2-</sup> depending upon the pH in aqueous solution. In the present kinetic study, under experimental pH condition PMS exist as PMS<sup>-</sup>. Therefore, PMS<sup>-</sup> can be assumed to reactive species in the oxidation of GG in aqueous medium. GG exists as Zwitterion, cation, anion and neutral molecule depending on the pH of the medium[23-28]. Under the experimental conditions (pH= 3.42-5.89) zwitterionic form of GG is supposed to be the reactive species. Addition of olefinic monomer such as acrylamide or acrylonitrile did not induce polymerization confirming the absence of free radicals in the reaction mixture.

The first order dependence of rate on [PMS] and fractional order [GG] suggest that the reaction might occur via an adduct formation which dissociates in the slow rate determining step to give final products. A further support for the participation of ionic PMS species in the rate limiting step can be obtained from the rate enhancement with a decrease in the dielectric constant. On the basis of observed kinetic results, products of oxidation and above discussion, the reaction, under kinetic conditions, can be written as in



The mechanism of oxidation of GG by HSO<sub>5</sub><sup>-</sup> may be written as



The oxidation products, namely an aldehyde (2-formylformamido acetic acid) and ammonia, suggest that the reaction center is the terminal amino group. The terminal carboxyl group is far away from the reaction center. Therefore, we can assume that the nature of the terminal carboxyl group, that is, charged or uncharged form, will have a least effect on the rate. This means the zwitterionic and the cationic form of GG will have the same rate. Only for simplicity, we have used only the zwitterionic form of GG in Eqs. (3). The rate equation for the disappearance of PMS can be given as following.

$$\text{Rate} = \frac{-d[\text{PMS}]}{dt} = \frac{kK[\text{GG}][\text{PMS}]}{1+K[\text{GG}]} \quad \text{.....(5)}$$

$$\frac{\text{rate}}{[\text{PMS}]} = \frac{kK[\text{GG}]}{1+K[\text{GG}]} \quad \text{.....(6)}$$

Reciprocal of equation (2) yields

$$\frac{[\text{PMS}]}{\text{rate}} = \frac{1}{Kk[\text{GG}]} + \frac{1}{k} \quad \text{.....(7)}$$

A plot of  $1/k'$  Vs  $1/[\text{GG}]$  is linear ( $r=0.99$ ) with positive slope and intercept on Y-axis. This has been observed in the present investigation, supporting the proposed mechanism and Michealis –Menten[29] type kinetics. From the intercepts and slopes of  $[\text{PMS}]/\text{rate}$  Vs  $1/[\text{GG}]$  plots at various temperatures  $k$  values are calculated ( $1.63 \text{ min}^{-1}$  at  $313\text{K}$ ,  $2.89 \text{ min}^{-1}$  at  $318\text{K}$  and  $4.12 \text{ min}^{-1}$  at  $323\text{K}$ ) from which the activation parameters were calculated (Table-2).

Comparison of rates of GG- PMS reaction with glycine (GLY)- PMS reaction under identical condition, revealed that the rate of oxidation is faster in case of glycine. The difference of reaction rates may be due to the (i) weaker electrostatic effects may occurs in the case of GG than Glycine (ii) Glycylglycine ( $pK_1$  3.2 and  $pK_2$  8.2) is weaker both as an acid and a base when compared to glycine ( $pK_1$  2.4 and  $pK_2$  9.8). Thus the oxidation of dipeptide GG is expected to be slower than the monomer.

## APPLICATIONS

The present study will helps in the study of reactivity of organic and inorganic peroxides and peroxy derivatives with various biomolecules like proteins, nucleic acids (RNA & DNA), biopolymers like enzyme and carbohydrates etc. From which we can study the bonding interactions of peroxides with Biomolecules.

## CONCLUSIONS

The rate of oxidation of GG and Glycine with PMS both shows first order dependence with respect to [PMS] and fractional order dependence with respect to [Substrate]. The rates were compared. The oxidation of GG and Glycine in the identical reaction conditions, the rate is slower in the case of GG than Glycine. It may be due to weaker electrostatic interactions and functional group separation factors. Hence, the oxidation of GG is expected to be slower than Glycine.

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