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Oxidation of Dipeptide Glycylglycine by Chloramine-T 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 Chloramine-T(CAT) in aqueous medium to produce aldehyde, ammonia and carbon dioxide, under the condition [CAT] << [GG] at different temperatures (308-318 K) have been studied. The kinetics revealed that the fractional order dependence in [GG] and first order dependence in [CAT]. Michealis Menten type mechanism was proposed. Thermodynamic parameters have been evaluated. The rates of oxidation reactions were compared to that of the monomer glycine (Gly).

Keywords: Glycylglycine (GG), Glycine (Gly), Chloramine-T (CAT), Chloramine-B (CAB) and Acetonitrile (ACN).

INTRODUCTION

Proteins are essential constituents of all organisms. Most tasks performed by living cells require proteins. The variety of functions that perform is astonishing, and in which, oxidation of peptides and proteins is one major task. Oxidation of peptides and proteins involves various biochemical events ranging from normal metabolism to ageing and disease process[1]. Glycylglycine (GG) is a typical dipeptide which is the first member of dipeptide series. The oxidation of Glycylglycine has been reported by a very few oxidants[2,3]. GG undergo oxidation by two different routes based on the nature of the oxidant used and the reaction medium employed[4, 5]. The first route is through C-C bond cleavage resulting in decarboxylation and the second one is through N-H bond cleavage.

Chloramine-T (CAT) is N-haloarenesulfonamide derivative. The prominent members of these N-halo compounds, Chloramine-T (CAT), Bromamine-T (BAT), Chloramine-B (CAB) and Bromamine-B (BAB) have been employed as oxidants[6] in organic transformation and also for the determination of various organic, inorganic and pharmaceutical compounds. Among the N-haloarenesulfonamides, Chloramine-T (CAT) is gaining importance as strong oxidant and sometimes it act as vigorous oxidant[7]. It undergoes reduction to form 4-methyl benzenesulfonamide ($TsNH_2$) and the half cell reaction involves

$$TsNHCl + H^+ + 2\bar{e} \rightleftharpoons TsNH_2 + Cl^-$$

The redox potential for the couple Chloramine-T / $TsNH_2$ in aqueous medium was found to be >1.00 V at room temperature, indicating that Chloramine-T is considerably strong oxidant.

MATERIALS AND METHODS

GG (E.Merck, analytical grade) was purified by column chromatography and used in experiment. CAT and p-toluene sulphonamide obtained from Aldrich (U.S.A) with highest purity and analytical grade used in stock solution[8] of the oxidant preparation. All other chemicals were of analytical grade.

Kinetics and Measurements: The kinetic studies were made under pseudo-first order conditions with (CAT) << (GG) in aqueous medium. The progress of the reaction was monitored by estimating the unreacted (CAT) at different intervals of time. CAT content was estimated by iodometrically using a 1% solution of freshly prepared starch as an indicator. The concentration of CAT was calculated using the following stoichiometric equation.

$$p\text{-}MePhSO_2NClNa + 2I^- + 2H^+ \longrightarrow p\text{-}MePhSO_2NH_2 + I_2 + NaCl$$

Stoichiometry and product analysis: Under the conditions (CAT) >> (substrate) the reaction was allowed to go for completion. The unreacted (CAT) was estimated and based on the results, one mole of glycylglycine needed two moles of CAT and one mole of glycine needed one mole of CAT to get oxidised and the stoichiometric equation is given as

$$NH_2CH_2CONHCH_2COOH + 2\ p\text{-}MePhSO_2NClNa + 3H_2O \longrightarrow 2HCHO + 2\ p\text{-}MePhSO_2NH_2 + 2CO_2 + 2NH_3 + 2Na^+ + 2Cl^-$$

$$NH_2CH_2COOH + p\text{-}MePhSO_2NClNa + H_2O \longrightarrow HCHO + p\text{-}MePhSO_2NH_2 + CO_2 + NH_3 + Na^+ + Cl^-$$

Formaldehyde was detected by Chromo tropic acid[9] test, while ammonia was identified by Nessler's reagent[3], and carbon dioxide was detected by gas evolution apparatus. Formation of $TsNH_2$ identified by TLC, comparing with standard solution of pure $TsNH_2$.

RESULTS AND DISCUSSION

The kinetics of oxidation of GG by CAT was investigated at different (GG). Under the conditions of (GG) >> $[CAT]_0$, plots of log[a/a-x] (where 'a' and (a-x) corresponds to the concentration of Chloramine-T at zero time and at time 't') versus time were linear indicating a first order dependence of rate on $[CAT]_0$. Values of *k'*, calculated from these plots were independent of $(CAT)_0$, confirming the first order dependence on $(CAT)_0$ (fig 1). At constant $(CAT)_0$, the rate increases with increase in $(GG)_0$.

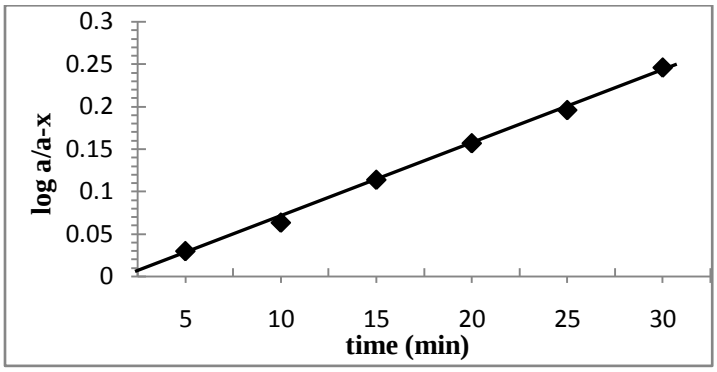


Fig. 1. Oxidation of glycylglycine by Chloramine-T.
[CAT]= 5 x 10^{-3} mol dm^{-3} **;** [GG] = 2.5 x 10^{-2} mol. dm^{-3}, T=308 K.

A plot of log *k′* versus log(GG) was linear (r=0.92,) with a slope (s=0.5) indicating a fractional order in (GG). Order in (GG) becomes zero as (GG) is increased further, obeying the Michealis-Menten kinetics (table-1). Increasing the [$HClO_4$], addition of $NaClO_4$ at fixed [H^+] and ionic strength did not affect the rate. The addition of p-CH_3-$PhSO_2NH_2$ also did not alter the rate of reaction. No significant change in rate was observed when the solvent composition of the medium was varied by the addition of 0-20% (v/v) Acetonitrile (ACN). Blank experiment shows that ACN was not oxidised by CAT under the experimental conditions.

Table-1: Order in [GG] in the oxidation of GG by CAT in aqueous medium; [CAT]= 5 x 10^{-3} mol dm^{-3};Temp = 308 K

10^2 x [GG] (mol dm^{-3})	k′x 10^3 min^{-1}	3+log[GG]	3+***logk′***
2.50	6.4	0.40	0.81
5.00	8.4	0.70	0.92
10.00	10.4	1.00	1.02
15.00	14.1	1.20	1.15
20.00	17.0	1.30	1.23

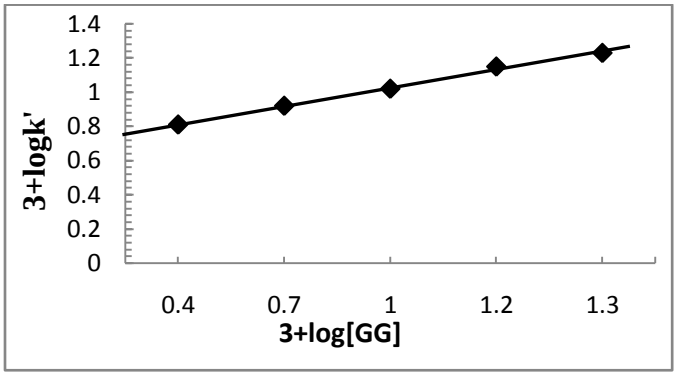


Fig 2. Effect of [GG] on k′ in CAT-GG reaction:
[CAT]= 5 x 10^{-3} mol dm^{-3}: Temp =308 K

The reaction was investigated at different temperatures in the range 308-318K. The substrate concentration was varied at each temperature to Michealis-Menten kinetics (Fig 3). Activation parameters for the rate limiting step have been computed as follows:

Substrate	E_a (kJ mol^{-1})	$\Delta H^{\#}$ (kJ mol^{-1})	$\Delta G^{\#}$ (kJ mol^{-1})	$\Delta S^{\#}$ (KJ mol^{-1})
GG	26.15	23.59	104.3	-262
Glycine	19.0	16.44	110.2	-259

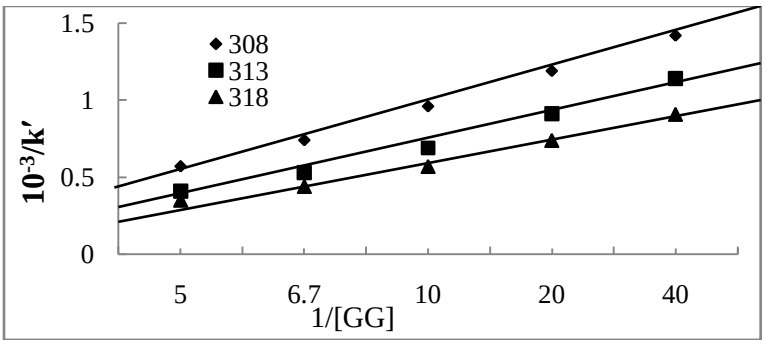


Fig 3. Plot 1/k′ versus 1/[GG] at rage 308-318K.

Absence of free radicals during the course of oxidation was confirmed when no polymerization was initiated with the addition of acrylonitrile solution to the reaction mixture. In aqueous solutions, Chloramine-T(CAT)[10] and its derivatives such as chloramine-B (CAB) and Bromamine-T act as electrolytes. Some of the important equilibria exhibited by CAT and CAB are represented by the following eqs.

$$ArSO_2NClNa \rightleftharpoons ArSO_2NCl^- + Na^+$$
$$ArSO_2NCl^- + H^+ \rightleftharpoons ArSO_2NHCl$$
$$2ArSO_2NHCl \rightleftharpoons ArSO_2NH_2 + ArSO_2NCl_2$$
$$ArSO_2NHCl^- + H^+ \rightleftharpoons ArSO_2NH_2Cl^+$$
$$ArSO_2NHCl + H_2O \rightleftharpoons ArSO_2NH_2 + HOCl$$
$$ArSO_2NCl_2 + H_2O \rightleftharpoons ArSO_2NHCl + HOCl$$
$$HOCl \rightleftharpoons H^+ + OCl^-$$
$$HOCl + H^+ \rightleftharpoons H_2OCl^+$$

CAT[11] exists in the following forms in aqueous medium viz., p-Me-Ph-SO_2NCl^-, (p-Me-Ph-$SO_2NH_2Cl)^+$, (p-Me-Ph-SO_2NCl_2), Cl^+, HOCl, and (H_2O^+Cl). In the present study, the possibility of ((p-Me-Ph-$SO_2NH_2Cl)^+$, Cl^+, H_2O^+Cl as the reactive species is ruled out, it is due to the rate decreases with increases in $[H^+]$ (table 2) in the oxidation of GG using Chloramine-T. ((p-Me-Ph-SO_2NCl_2) were to be the reactive species, the rate law would then predict a second order dependence of rate on (CAT) which is contrary to experimental observations. It suggests that Chloramine-T itself act as a reactive species in the oxidation of GG and glycine in aqueous medium.

Table 2. Effect of [$HClO_4$], [$NaClO_4$] and [p-TSA] on the rate of reaction, [CAT] =5 x 10^{-3} mol. dm^{-3} **;** [GG] = 2.5x 10^{-2} mol. dm^{-3}

[A] x 10^{-3} mol. dm^{-3}	A=[$NaClO_4$] 10^3 x k′ min^{-1}	A=[$HClO_4$] 10^3 x k′ min^{-1}	A=[p-TSA] 10^3 x k′ min^{-1}
00	6.4	6.4	6.4
1.0	6.7	5.8	6.3
1.5	6.6	4.6	6.3
2.0	6.8	3.9	6.5

Glycylglycine[12], like the amino acids, exists as zwitter ion, cation, anion and neutral molecule depending on the pH of the medium. Under the experimental conditions zwitterions form of glycylglycine is supposed to be the reactive species of glycylglycine, which gets further support from ionic strength effect. The inverse dependence of the reaction on $[H^+]$ suggests that the zwitter ion is reactive species taking part in the reaction. The absence of ionic strength effect on the rate limiting step. Based on the above observation, the mechanism of the reaction is outlined in Scheme 1:

$H_2N—CH_2—CONHCH_2COOH \rightleftharpoons H_3\overset{+}{N}—CH_2—CONHCH_2COO^-$ (X)

(X) + p-Me-$PhSO_2NHCl \overset{K}{\rightleftharpoons}$ X′

X′ $\xrightarrow[\text{Slow}]{k}$ X″

X″ + p-Me-$PhSO_2NHCl \rightleftharpoons$ Products (fast)

Scheme-1

Then rate is,

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

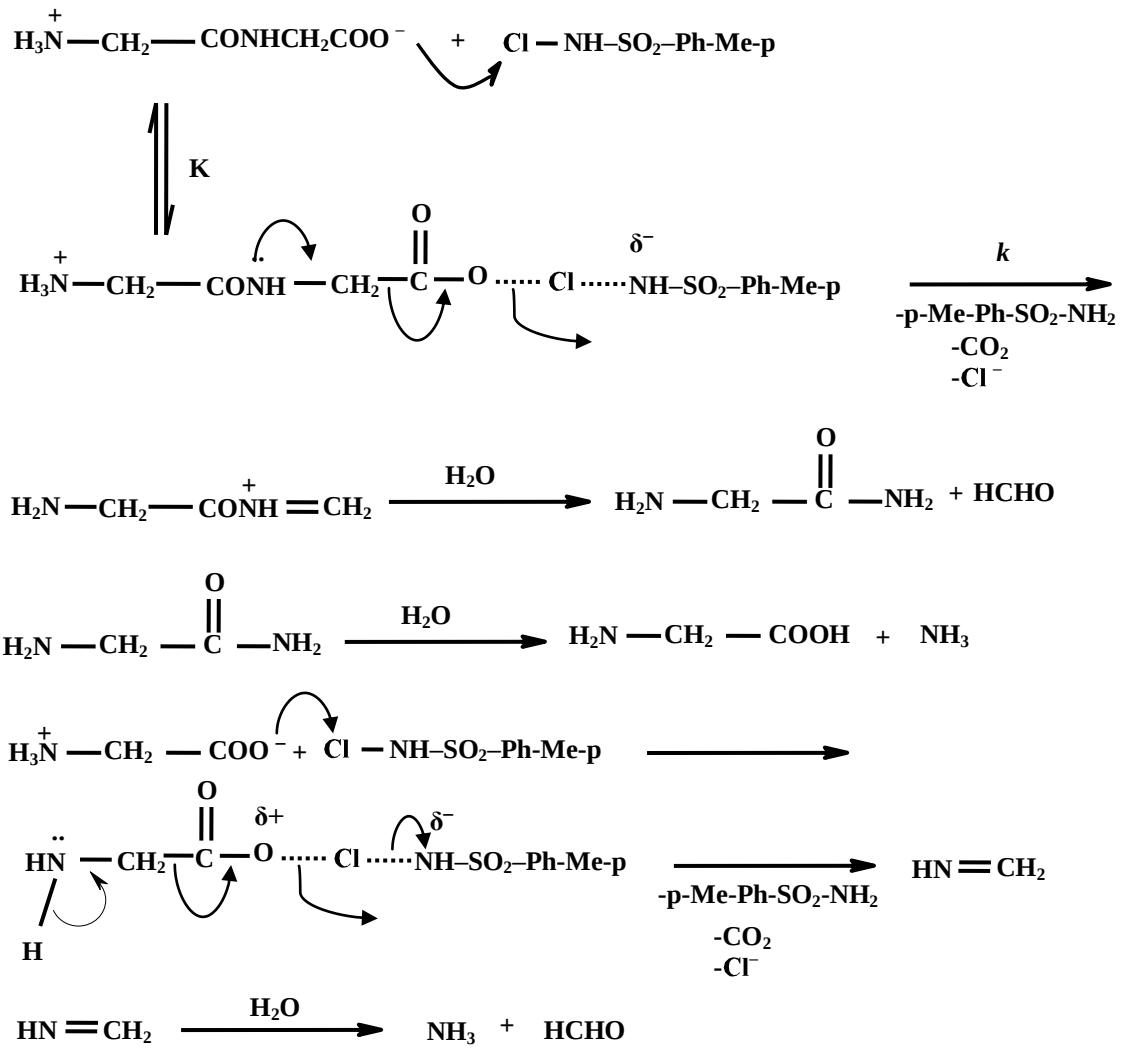
$$\frac{rate}{[CAT]} = \frac{kK[GG]}{1+K[GG]} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Reciprocal of equation (2) yields

$$\frac{[CAT]}{rate} = \frac{1}{kK[GG]} + \frac{1}{k} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

A plot of 1/k′ versus 1/[GG] is linear (r=0.97) and from the intercept of the reciprocal plot, the value of k was computed. The rate of reaction was fractional order in $(GG)_0$ and so Michealis –Menten[13] type kinetics were followed by varying the $[substrate]_0$ over the range of temperature (308-318K) and the values of 10^4k was computed as 5.58, 7.14 and 9.91 min^{-1} at 308, 313 and 318K respectively.

The rate of oxidation of GG by CAT was compared with that of glycine by CAT under identical experimental conditions and it was found that the rate of oxidation of GG is shows slightly slower than glycine (table 3). The difference of reaction rates may be due to the (i) increased distance between the functional groups, which result in weaker electrostatic effects (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 glycylglycine is expected to be slower than the monomer.



Scheme-2

Table 3. Comparison of rates of oxidation of GG and Gly by CAT: [CAT]= 5 x 10^3 mol dm^{-3}, Temp = 308 K

10^2 x [GG] (mol dm^{-3})	k′x 10^3 min^{-1}	10^2 × [Glycine] (mol-dm^{-3})	10^3 × k′(min^{-1})
2.50	6.4	2.5	10.0
5.00	8.4	5.0	13.1
10.00	10.4	10.0	16.5
15.00	14.1	15.0	19.8
20.00	17.0	20.0	21.9

Scheme 2 depicts the probable mode of oxidation of GG by CAT in aqueous medium over a range of temperature (308-318K). The Zwitter ion of GG is attacked at the carboxylate end by halogen species yielding an intermediate, X′. The intermediate, X′ with the liberation of CO_2, Cl^- and $TsNH_2$ forms another intermediate, X″ which ultimately gives the products observed.

APPLICATIONS

This report is helpful for the study of oxidation of amino acids, peptides and proteins with a radical and non radical reactive species as well as an exploration of the significance of reactive species in the atmosphere, disinfection process and environment remediation.

CONCLUSIONS

The rate of oxidation of glycine and glycylglycine by CAT were compared under identical experimental conditions and it was found that the rate of oxidation of glycylglycine is slower than that of glycine. The change is due to the increased distance between the functional groups and consequently weaker electrostatic effects[14]. Hence, the oxidation of glycylglycine is expected to be slower than that of glycine.

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