

Kinetics of oxidation of glycylglycine by bromamine-T in acid medium

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Abstract. The kinetics of oxidation of a typical dipeptide glycylglycine (GG) by bromamine-T have been studied in HClO_4 medium at 40°C . The rate shows first-order dependence on $[\text{BAT}]_0$ and is fractional order in $[\text{GG}]_0$ which becomes independent of $[\text{substrate}]_0$ at higher $[\text{GG}]_0$. At $[\text{H}^+] > 0.02 \text{ mol dm}^{-3}$, the rate is inverse fractional in $[\text{H}^+]$ but is zero order at lower $[\text{H}^+]$ ($\leq 0.02 \text{ mol dm}^{-3}$). Variation in ionic strength or dielectric constant of the medium had no significant effect on the rate. The solvent-isotope effect was measured and $k'_{\text{H}_2\text{O}}/k'_{\text{D}_2\text{O}} = 1.45$. Proton inventory studies have been made. The reaction has been studied at different temperatures (308–323 K) and activation parameters have been computed.

Keywords. Diglycine; kinetics; oxidation; bromamine-T.

1. Introduction

The kinetics of oxidation of aminoacids by organic haloamines (Mahadevappa *et al* 1983, 1984) has been extensively investigated, but there is very little information in the literature on the oxidation kinetics of dipeptides. The kinetics of hydrolysis of glycylglycine (GG) has been reported (Akabori *et al* 1954; Hammel and Glasstone 1954). It was thought that it would be interesting to study the oxidation behaviour of aromatic sulphonylhaloamines towards a dipeptide, and thus we report here the kinetics of oxidation of the dipeptide of glycine, namely glycylglycine ($\text{NH}_2\text{CH}_2 \cdot \text{CONHCH}_2\text{COOH}$) by bromamine-T ($p\text{-CH}_3\text{C}_6\text{H}_4\text{SO}_2\text{NBrNa} \cdot 3\text{H}_2\text{O}$, BAT) in HClO_4 medium.

2. Experimental

Preparation of BAT and its purification has been reported in the literature (Nair and Indrasenan 1976; Rangappa *et al* 1981). Aqueous solutions of oxidant were standardized iodometrically and preserved in brown bottles. An aqueous solution of GG (Loba Chemie) was freshly prepared whenever required. All other chemicals were of analar grade. Triple-distilled water was used throughout.

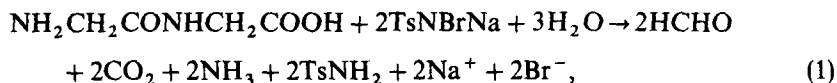
The reaction was carried out in glass-stoppered pyrex boiling tubes whose outer

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surface was coated black to eliminate photochemical effects. Requisite amounts of GG, HClO_4 , NaClO_4 and water (for total constant volume) were thermostatted at 40°C for thermal equilibrium. A measured amount of BAT solution, also thermostatted at 40°C , was added rapidly to the mixture in the boiling tube. The progress of the reaction was monitored at different intervals of time for the amount of unreacted BAT in a measured aliquot of reaction mixture by iodometry. The reaction was studied for about two half lives. The reaction was carried out under pseudo first-order conditions by using an excess (~ 10 -fold) of GG. The pseudo first-order rate constants, k' , were reproducible within $\pm 3\%$. Regression analysis of data was carried out on an EC-72 Statistical Calculator to obtain the regression coefficient r and standard deviation s of the points from the regression line.

2.1 Stoichiometry

The following stoichiometry was noted after the reaction mixture of GG, HClO_4 , NaClO_4 and BAT was equilibrated for 48 hours at 40°C .



(Ts = *p*- $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_2$ —).

The reaction product, (TsNH_2) , was detected (Mahadevappa and Gowda 1975) by paper chromatography. Benzyl alcohol saturated with water was used as the solvent with 0.5% vanillin in 1% HCl in ethanol as spray reagent ($R_f = 0.905$). Formaldehyde (Feigl 1956) was detected by the chromotropic acid test while ammonia and carbon-dioxide were identified through conventional tests.

3. Results

At fixed [substrate] ($\gg [\text{BAT}]_0$), plots of $\log [\text{BAT}]$ versus time were linear indicating first-order dependence on $[\text{BAT}]_0$ (average $k' = 1.42 \pm 0.01 \times 10^{-4} \text{s}^{-1}$). The rate increased with increase in $[\text{GG}]_0$ and the plot of $\log k'$ versus $\log [\text{GG}]_0$ was linear (figure 1, $r = 0.9945$; $s = 0.02$) with a slope of 0.61 indicating fractional-order dependence of rate on $[\text{GG}]_0$.

At constant $[\text{BAT}]_0$ and $[\text{GG}]_0$, the rate was not affected at low $[\text{H}^+]$ ($0.005 - 0.03 \text{mol dm}^{-3}$) but retardation set in at $[\text{H}^+] = 0.03 - 0.08 \text{mol dm}^{-3}$ (table 1). Under the latter conditions, the plot of $\log k'$ versus $\log [\text{H}^+]$ (figure 1) was linear ($r = 0.9908$) with a negative slope of 0.86. In the low acid range ($[\text{H}^+] = 0.01 \text{mol dm}^{-3}$), variation of $[\text{GG}]_0$ had no effect on the rate (table 1).

Addition of the reaction product *p*-toluene sulphonamide, Cl^- or Br^- ions, or variation of ionic strength and dielectric constant of the medium had no effect on the rate (table 1).

The reaction was studied at different temperatures (308–323 K) and the activation parameters for the composite reaction were computed from the Arrhenius plot of $\log k'$ versus $1/T$ (table 2).

Solvent isotope studies in D_2O (99.4%) showed that the rate was further retarded in D_2O medium with $k'_{\text{D}_2\text{O}} = 0.98 \times 10^{-4} \text{s}^{-1}$ and $k'_{\text{H}_2\text{O}} = 1.42 \times 10^{-4} \text{s}^{-1}$ leading to a

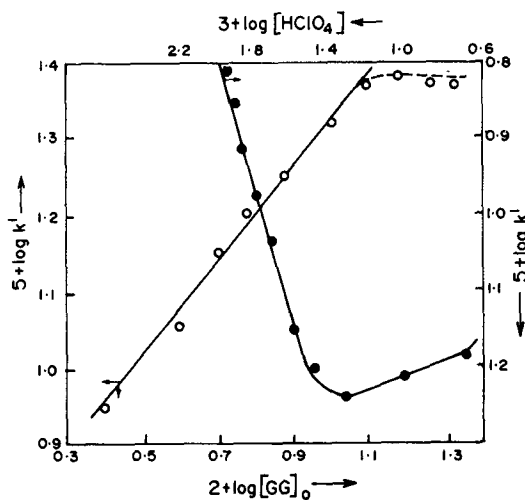


Figure 1. Plot of $\log k'$ vs $\log [GG]_0$ and $\log k'$ vs $\log [H^+]$. $[BAT]_0 = 0.005 \text{ mol dm}^{-3}$; $[HClO_4] = 0.04 \text{ mol dm}^{-3}$; $I = 0.5 \text{ mol dm}^{-3}$; temperature = 40°C .

Table 1. Effect of varying $[HClO_4]$ on the rate and the effect of $[GG]_0$ at low $[H^+]$.

$I = 0.5 \text{ mol dm}^{-3}$; temperature = 40°C .

| $10^3 [BAT]_0$ (mol dm^{-3}) | $10^2 [GG]_0$ (mol dm^{-3}) | $10^2 [HClO_4]$ (mol dm^{-3}) | $10^4 k'$ (s^{-1}) |
|--|---|---|-------------------------------|
| 5.0 | 5.0 | 1.0 | 1.62 |
| 5.0 | 5.0 | 1.5 | 1.50 |
| 5.0 | 5.0 | 2.0 | 1.75 |
| 5.0 | 5.0 | 3.0 | 1.60 |
| 5.0 | 5.0 | 4.0 | 1.42 |
| 5.0 | 5.0 | 5.0 | 1.08 |
| 5.0 | 5.0 | 6.0 | 0.94 |
| 5.0 | 5.0 | 7.0 | 0.81 |
| 5.0 | 5.0 | 8.0 | 0.71 |
| 5.0 | 0.5 | 1.0 | 1.43 |
| 5.0 | 1.0 | 1.0 | 1.45 |
| 5.0 | 2.0 | 1.0 | 1.55 |
| 5.0 | 7.5 | 1.0 | 1.70 |
| 5.0 | 10.0 | 1.0 | 1.69 |
| 5.0 ^a | 5.0 | 4.0 | 1.46 |
| 5.0 ^b | 5.0 | 4.0 | 1.42 |
| 5.0 ^c | 5.0 | 4.0 | 1.40 |
| 5.0 ^d | 5.0 | 4.0 | 1.35 |
| 5.0 ^e | 5.0 | 4.0 | 1.45 |
| 5.0 ^f | 5.0 | 4.0 | 1.40 |

In presence of: ^a $(Cl^-) = 0.05 \text{ mol dm}^{-3}$; ^b $[Br^-] = 0.01 \text{ mol dm}^{-3}$; ^cPTS = $0.006 \text{ mol dm}^{-3}$; ^d $I = 1.0 \text{ mol dm}^{-3}$; ^e40% methanol; ^f20% methanol.

Table 2. Thermodynamic parameters for the oxidation of GG by BAT in HClO_4 medium at 40°C , (a) for the composite reaction and (b) for the rate-limiting step.

| Parameters | (a) $\log k'$ vs $1/T$ | (b) $\log k_3$ vs $1/T$ |
|---|---------------------------|----------------------------|
| E_a (kJ mol $^{-1}$) | 79.3 | 74.3 |
| ΔH^\ddagger (kJ mol $^{-1}$) | 76.7 | 71.7 |
| ΔG^\ddagger (kJ mol $^{-1}$) | 100.1 ± 0.1 | 97.4 ± 0.1 |
| ΔS^\ddagger (KJ $^{-1}$ mol $^{-1}$) | -74.1 ± 0.1 | -81.6 ± 0.1 |
| $\log A$ | 9.0 | 8.6 |

Table 3. Proton-inventory plot for the oxidation of GG by BAT in H_2O - D_2O mixtures.

| n | $10^4 k'_n$ (s $^{-1}$) |
|-------|--------------------------|
| 0.000 | 1.42 |
| 0.248 | 1.32 |
| 0.496 | 1.20 |
| 0.744 | 1.04 |
| 0.871 | 0.98 |

Conditions are as in table 1

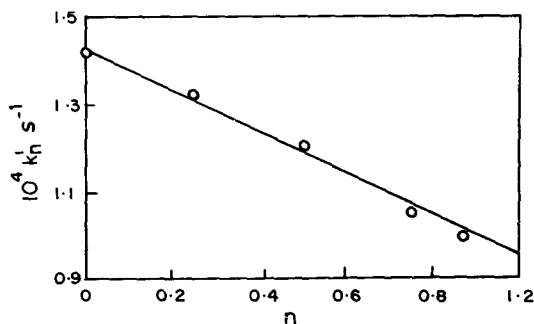


Figure 2. Plot of k'_n vs n . $[\text{BAT}]_0 = 0.005 \text{ mol dm}^{-3}$; $[\text{GG}]_0 = 0.05 \text{ mol dm}^{-3}$; $[\text{HClO}_4] = 0.04 \text{ mol dm}^{-3}$; $I = 0.5 \text{ mol dm}^{-3}$; temperature = 40°C .

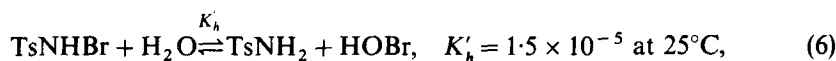
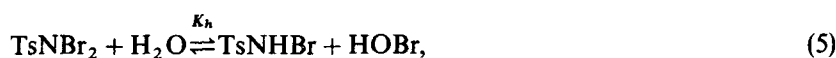
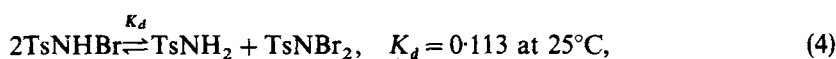
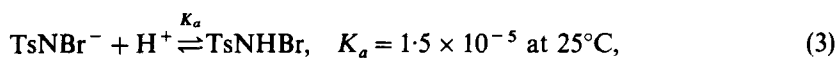
solvent isotope effect, $k'_{\text{H}_2\text{O}}/k'_{\text{D}_2\text{O}} = 1.45$. Proton inventory studies were made using different atom fractions 'n' of deuterium (table 3, figure 2, $r = 0.9964$).

The absence of free radicals during the course of reaction was indicated when no polymerization occurred on the addition of acrylamide solution to the reaction mixture.

4. Discussion

Bromamine-T is the bromine analogue of chloramine-T (CAT) and exhibits similar equilibria in aqueous acidic and basic solutions (Bishop and Jennings 1958). In acid

solutions, BAT exists in the following equilibria:



Therefore, the probable reactive species in acid solution of BAT are TsNHBr, TsNBr₂, HOBr and H₂OBr⁺.

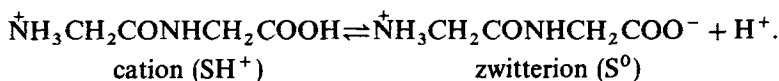
The experimental rate law for the oxidation of GG by BAT in HClO₄ at 40°C is found to be

$$\text{Rate} = k[\text{BAT}]_0[\text{GG}]_0^x[\text{H}^+]^{-y}, \quad (9)$$

where $x = y = 0$ in lower $[\text{H}^+]$ ranges and x and y are fractional at higher $[\text{H}^+]$. The fractional order in $[\text{GG}]_0$ tends to become zero at $[\text{GG}]_0 > 0.15 \text{ mol dm}^{-3}$.

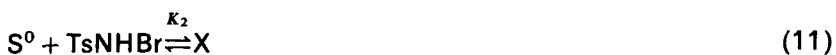
The first-order dependence of rate on $[\text{BAT}]_0$ and the addition of TsNH₂ having no effect on the reaction rate indicate that TsNBr₂ and HOBr may not be the reactive species ((4) and (6)), and further, that these species are present in very low concentrations (Bishop and Jennings 1958) at the experimental conditions employed. The absence of ionic strength effects indicates the involvement of a neutral species in the rate-limiting step. Hence, the effective oxidizing species could be the conjugate acid, TsNHBr.

Glycylglycine (Fieser and Fieser 1958) exists in the following equilibrium in acidic solutions:



The inverse dependence of the reaction on acidity suggests that there is an equilibrium between the zwitterion (S⁰) and the protonated form (SH⁺) and that it is the zwitterion which takes part in the reaction.

Based on experimental observations, scheme 1 is proposed to explain the reaction mechanism.



Scheme 1.

Glycylglycine is not expected to undergo hydrolysis before oxidation since drastic reaction conditions ($> 2\text{NHCl}$ and high temperature) are needed to effect hydrolysis (Haurowitz 1950) of a dipeptide bond. Martin (1955) has studied the mechanism of hydrolysis of GG in aqueous acetic acid–perchloric acid mixtures at 120°C . Synge (1945) has used equal volumes of glacial acetic acid and 10NHCl to effect hydrolysis. Hence, GG is oxidized rather than hydrolysed at the conditions maintained in the present study.

If $[\text{BAT}]_t$ is the total concentration, then $[\text{BAT}]_t = [\text{TsNHBr}] + [\text{X}]$ from which,

$$[\text{X}] = \frac{K_1 K_2 [\text{BAT}]_t [\text{SH}^+]}{[\text{H}^+] + K_1 K_2 [\text{SH}^+]}$$

The rate of reaction is given by

$$-\frac{d[\text{BAT}]}{dt} = k_3 [\text{X}] = \frac{k_3 K_1 K_2 [\text{BAT}]_t [\text{SH}^+]}{[\text{H}^+] + K_1 K_2 [\text{SH}^+]} \quad (14)$$

Equation (14) is in agreement with the experimental results wherein a first-order dependence of rate on $[\text{BAT}]_0$, fractional and inverse fractional orders in $[\text{GG}]_0$ and $[\text{H}^+]$ respectively are observed. At higher $[\text{GG}]_0$, the inequality $[\text{H}^+] \ll K_1 K_2 [\text{SH}^+]$ holds good so that the reaction becomes independent of $[\text{GG}]_0$ and this has been observed experimentally (figure 1). In the limiting case, at low $[\text{H}^+]$, the reaction is independent of $[\text{H}^+]$ and $[\text{GG}]_0$, as is evident from (14).

If k' is the observed rate constant, equation (14) is rearranged into,

$$\frac{1}{k'} = \frac{[\text{H}^+]}{k_3 K_1 K_2 [\text{SH}^+]} + \frac{1}{k_3} \quad (15)$$

A plot of $1/k'$ vs $1/[\text{GG}]_0$ is linear and from the intercept of the double reciprocal plot, the value of k_3 could be computed. Since a fractional order in $[\text{GG}]_0$ was observed, the substrate concentration was varied at different temperatures and k_3 was calculated at each temperature from the double reciprocal plots (figure 3, $r > 0.9980$):

| | | | | |
|----------------------------|------|------|------|------|
| Temperature (K) | 308 | 313 | 318 | 323 |
| $10^4 k_3 (\text{s}^{-1})$ | 2.52 | 3.75 | 6.39 | 9.44 |

The activation parameters for the rate limiting step were determined from a plot of $\log k_3$ versus $1/T$ (table 2).

The effect of varying solvent composition on the reaction kinetics has been described in detail in the well-known monographs on chemical kinetics. For the limiting case of zero-angle of approach between two dipoles or an ion-dipole system, Amis (1966) has shown that a plot of $\log k'$ vs $1/D$ gives a straight line with a negative slope for reaction between a negative ion and a dipole or between two dipoles, while a positive slope results for a positive ion-dipole interaction. The total absence of the effect of varying dielectric constant on rate in the present case cannot be explained by the Amis theory. Applying the Born equation, Laidler (1973) has proposed the following equation for a dipole–dipole reaction:

$$\ln k' = \ln k_0 + \frac{3}{8kT} \left(\frac{2}{D} - 1 \right) \left[\frac{\mu_A^2}{r_A^3} + \frac{\mu_B^2}{r_B^3} - \frac{\mu_{\ddagger}^2}{r_{\ddagger}^3} \right] \quad (16)$$

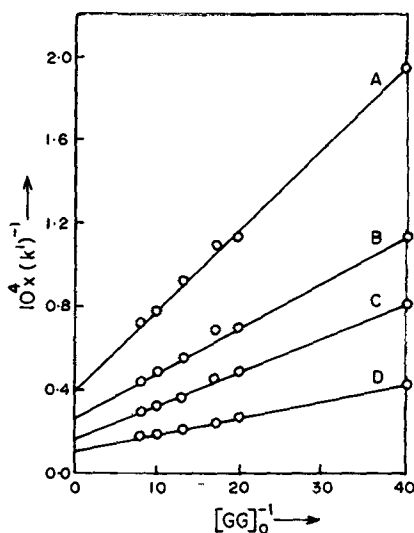


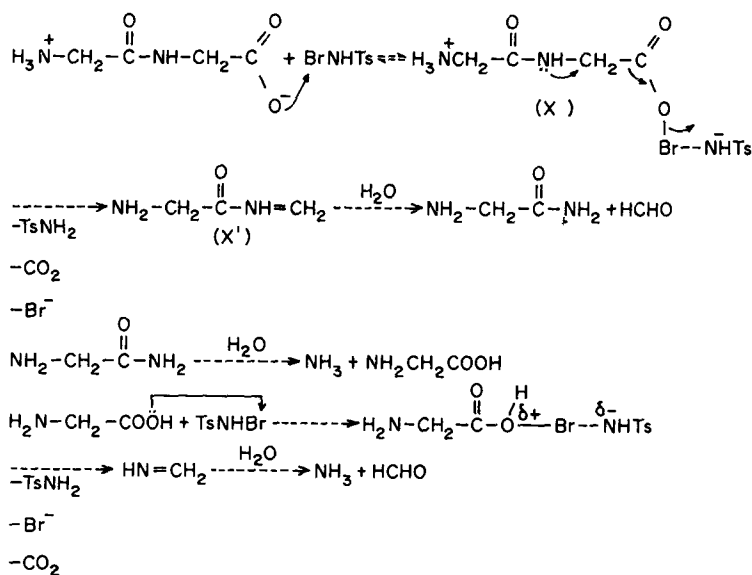
Figure 3. Plot of $1/k'$ vs $1/[GG]_0$ at (A) 308, (B) 313, (C) 318, (D) 323 K. $[BAT]_0 = 0.005 \text{ mol dm}^{-3}$; $[HClO_4] = 0.04 \text{ mol dm}^{-3}$; $I = 0.5 \text{ mol dm}^{-3}$.

Here k_0 is the rate constant in a medium of infinite dielectric constant, while μ represents the dipole moment, r refers to the radii of the reactants and activated complex. It is seen that the rate should be greater in a medium of lower dielectric constant when $r_+^3 > r_A^3 + r_B^2$. On the other hand, $r_+^3 \cong r_A^3 + r_B^3$ implies the absence of dielectric effect of solvent on the rate, as was found in the present case.

The proposed mechanism is also supported by the observed solvent isotope effect since the rate is retarded in D_2O medium leading to a solvent isotope effect, $k'_{H_2O}/k'_{D_2O} = 1.45$. For acid-catalysed reactions, involving a fast pre-equilibrium proton transfer, $k'_{D_2O}/k'_{H_2O} > 1$, since D_3O^+ is a stronger acid than H_3O^+ (Collins and Bowman 1970). But for reactions retarded by H^+ ions, this ratio becomes less than unity as has been observed in the present study. Proton inventory studies are expected to throw light on the nature of the transition state as the number of exchangeable protons during the reaction can be ascertained. A plot of k'_n vs n , the atom fraction of deuterium (figure 2) was found to be fairly linear ($r = 0.9964$), indicating a single proton exchange (Issacs 1987) during the formation of the transition state. The fairly high negative ΔS^\ddagger value indicates the presence of a rigid transition state.

A probable mode of oxidation of GG by BAT is given in scheme 2. The zwitterion of GG is involved in an electrophilic attack by the halogen of the oxidation species at the carboxylate end of GG, which yields the intermediate (x). With the liberation of CO_2 , Br^- and $TsNH_2$, the intermediate x' is formed, from which, the ultimate products are obtained as depicted in scheme 2.

It was also interesting to compare the rate of oxidation of GG with that of glycine under the same experimental conditions. It was found that the rate of oxidation of glycine was nearly ten times faster than that of the dipeptide. Glycylglycine (pK_1 3.2 and pK_2 8.2) is weaker both as an acid and as a base (Fieser and Fieser 1958) when compared to glycine (pK_1 2.4 and pK_2 9.8). The change in each case can be ascribed to the increased distance between the functional groups and consequently weaker



Scheme 2.

electrostatic effects. Hence oxidation of the dipeptide is expected to be slower than the monomer.

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