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Oxidation of a Dipeptide by N-Bromophthalimide in Aqueous Acetic Acid Medium and Comparison with Monomer Glycine: A Kinetic and Mechanistic Study

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The oxidation reactions of dipeptide, glycylglycine have been carried out with N-bromophthalimide in presence of mercuric acetate. The kinetics revealed first order dependence on N-bromophthalimide and fractional order dependence on glycylglycine. Michaelis-Menten type mechanism was proposed. Thermodynamic parameters have been evaluated. The oxidation reactions were compared to that of the monomer glycine.

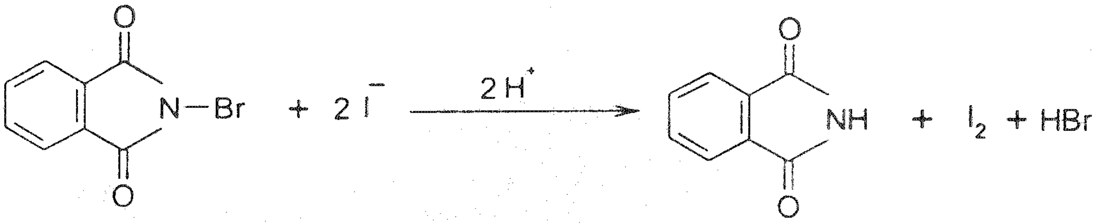
Key Words: N-Bromophthalimide, Glycylglycine, Mercuric acetate, Glycine.

INTRODUCTION

Oxidation reactions of peptides and proteins play an important role in various biochemical events ranging from normal metabolism to ageing and disease processes[1]. The oxidation of dipeptides has been studied by various oxidants[3-5]. The present study reports the oxidation of glycylglycine (GG) by N-bromophthalimide (NBP) in aqueous acetic acid medium.

EXPERIMENTAL

Chromatographically pure glycylglycine was used. NBP was obtained from Aldrich and used as such. All other chemicals were of analytical grade. The oxidation reactions of glycylglycine were conducted under the conditions [NBP] < [Glycylglycine] in 10% (v/v) acetic acid medium in the presence of excess of $[Hg(OAc)_2]$. The progress of the reaction was monitored by estimating the unreacted [NBP] at different intervals of time. The NBP content was estimated iodometrically[3] using a 1% solution of freshly prepared starch (GR) as an indicator. The concentration of NBP was calculated using the following stoichiometric equation:



Stoichiometry and Product Analysis

Under the conditions [NBP] ≫ [Glycylglycine] and in presence of excess of $Hg(OAc)_2$, the reaction was allowed to go to completion. The unreacted [NBP] was estimated and it indicated that one mole of glycylglycine needed two moles of NBP to get oxidized and the stoichiometric equation is given as

$$NH_2CH_2CONHCH_2COOH + 2NBP + 3H_2O \longrightarrow 2HCHO + 2NHP + 2CO_2 + 2NH_3 + 2HBr$$

Under the conditions of [NBP] ≫ [glycylglycine], the products obtained by the oxidation of glycylglycine (GG) were formaldehyde, NH_3 and CO_2. Formaldehyde was detected by chromotropic acid method[4], while ammonia was identified by Nessler's reagent[6] and CO_2 was identified by gas evolution apparatus.

RESULTS AND DISCUSSION

The plots of log a/(a-x) *vs.* time where a and (a − x) correspond to the concentrations of NBP at zero time and at time 't' were found to be linear passing through the origin, indicating first order dependence of rate on [NBP]. From the slopes of such plots pseudo-first order rate constants (k′) were evaluated (Table-1). The plot of log k′ *vs.* log [GG] was linear with $n = 0.21$ ($r = 0.96$), indicating fractional order dependence of rate on [GG]. The Lineweaver Burks plots of 1/k′ *vs.* 1/[GG] were linear with positive slopes and intercepts on y-axis ($r = 0.98$), indicating the formation of a complex prior to rate limiting step (Fig. 1). The addition of phthalimide, the reduced product of NBP, had no effect on the rate of the reaction (Table-2). The rate of oxidation decreased with increase in $[H_2SO_4]$ at constant [GG] (Table-2). The rate of oxidation of glycylglycine was not affected with increase in ionic strength of the medium (Table-2).

TABLE-1
COMPARISON OF RATE OF OXIDATION OF GLYCYLGLYCINE WITH THAT OF GLYCINE

$[NBP] = 1 \times 10^{-3}$ mol dm^{-3} $[Hg(OAc)_2] = 2 \times 10^{-3}$ mol dm^{-3}
[HOAc] = 10% (v/v) Temp. = 303 K

$10^2 \times$ [GG] (mol dm^{-3})	$10^4 \times k'$ (s^{-1})	$10^2 \times$ [glycine] (mol dm^{-3})	$10^4 \times k'$ (s^{-1})
1.00	1.35	1.00	5.41
2.00	1.85	2.00	5.94
3.00	2.09	3.00	6.17
4.00	2.14	4.00	6.43
5.00	2.74	5.00	7.59

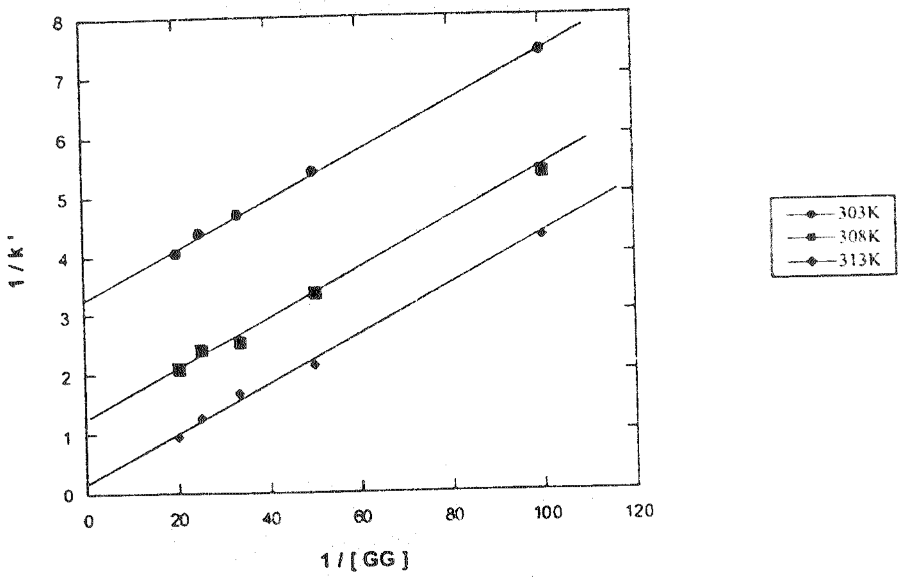


Fig. 1. Effect of [GG] on k′ in NBP-glycylglycine (GG) reaction at different temperatures

TABLE-2
EFFECT OF IONIC STRENGTH, MERCURIC ACETATE, PHTHALIMIDE (NHP) AND H^+ ON k′ IN THE OXIDATION OF GLYCYLGLYCINE BY NBP AQUEOUS ACETIC ACID MEDIUM

10^2 [A] (mol dm^{-3})	A = [KCl] $10^4 \times k'$ (s^{-1})	A = [NHP] $10^4 \times k'$ (s^{-1})	A = [$HgOAc)_2$] $10^4 \times k'$ (s^{-1})	A = [H^+] $10^4 \times k'$ (s^{-1})
1.00	1.30	1.40	1.36	1.34
2.00	1.25	1.86	1.35	1.30
3.00	1.17	1.93	1.28	1.26
4.00	1.10	2.00	1.24	1.16
5.00	1.00	2.17	1.10	1.12

The reaction rate was found to decrease with increase in acetic acid content in the reaction medium (Table-3) and plot of log k′ *vs.* 1/D and log k′ *vs.* (D − 1)/(2D + 1) were found to be linear (Figs. 2 and 3) with negative and positive slopes, respectively supporting the dipole-dipole reaction. The addition of olefinic monomers such as acrylamide and acrylonitrile to the reaction mixture did not induce polymerization indicating the absence of free radicals as intermediates during the course of the reaction. Comparison of rates of oxidations of glycylglycine (GG) and glycine revealed that the rate of oxidation of GG is faster than glycine.

Reactive Species and Mechanism

N-Bromophthalimide exists in the following forms in acidic solutions, *viz.*, NBP, $(NBPH)^+$, Br^+, HOBr and (H_2O^+Br). The possibility of $(NBPH)^+$, Br^+, H_2O^+Br as the reactive species is ruled out in the present investigation as the observed rate decreases with increases in [H^+] (Table-2). If HOBr were to be the reactive species, rate should have exhibited an inverse dependence on [phthalimide], which was also not observed. As the reactions were conducted in the presence of excess of $Hg(OAc)_2$, which eliminates bromide ion *vis* complexation, thus any possible oxidation due to Br_2 is eliminated. Therefore, free NBP itself is thought to be the active oxidizing species.

TABLE-3

EFFECT OF SOLVENT ON k′ IN THE OXIDATION OF GLYCYLGLYCINE BY NBP IN AQUEOUS ACETIC ACID MEDIUM

$[NBP] = 1 \times 10^{-3}$ mol dm^{-3} ; $[GG] = 1 \times 10^{-2}$ mol dm^{-3}
$[Hg(OAc)_2] = 2 \times 10^{-3}$ mol dm^{-3} ; Temp = 303 K

AcOH % (v/v)	Dielectric constant (D)	$10^3/D$	$10^2(D-1)/2D+1$	$10^5 \times k'$ (s^{-1})	5 + log k′
10	64.2	15.5	48.8	13.50	1.130
20	58.2	17.2	48.7	12.10	1.083
30	52.2	19.2	48.6	11.40	1.057
40	45.5	22.0	48.4	10.50	1.021
50	39.0	25.6	48.1	9.21	0.964

Glycylglycine exists as zwitterion, cation, anion and neutral molecule depending on the pH of the medium[4, 5].

$$H_2N\text{—}CH_2CONH\text{—}CH_2COOH \rightleftharpoons \overset{+}{N}H_3\text{—}CH_2\text{—}CONHCH_2COO^-$$

$$\rightleftharpoons \overset{+}{N}H_3CH_2CONHCH_2COOH \rightleftharpoons NH_2CH_2CONHCH_2COO^-$$

Under the experimental conditions (pH = 4), neutral glycylglycine is supposed to be the reactive species of glycylglycine, which gets further support from ionic strength effect and solvent effect which suggest that the reaction is dipole-dipole type. The plots of log k′ *vs.* 1/D and log k′ *vs.* (D − 1)/(2D + 1) were found to be linear (Figs. 2 and 3) with a negative and positive slope, respectively. Addition of olefinic monomer such as acrylamide or acrylonitrile did not induce polymerization confirming the absence of free radicals in the reaction mixture.

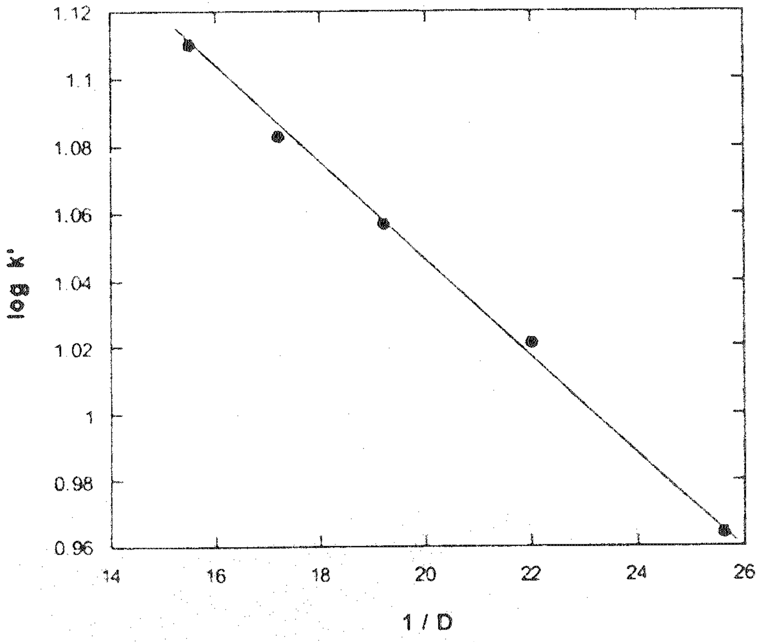


Fig. 2. Effect of Solvent-Amis plot

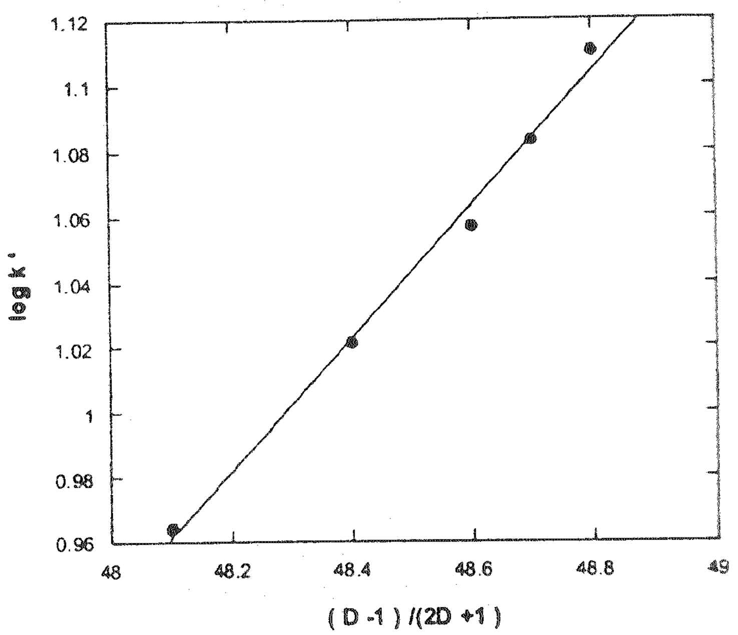
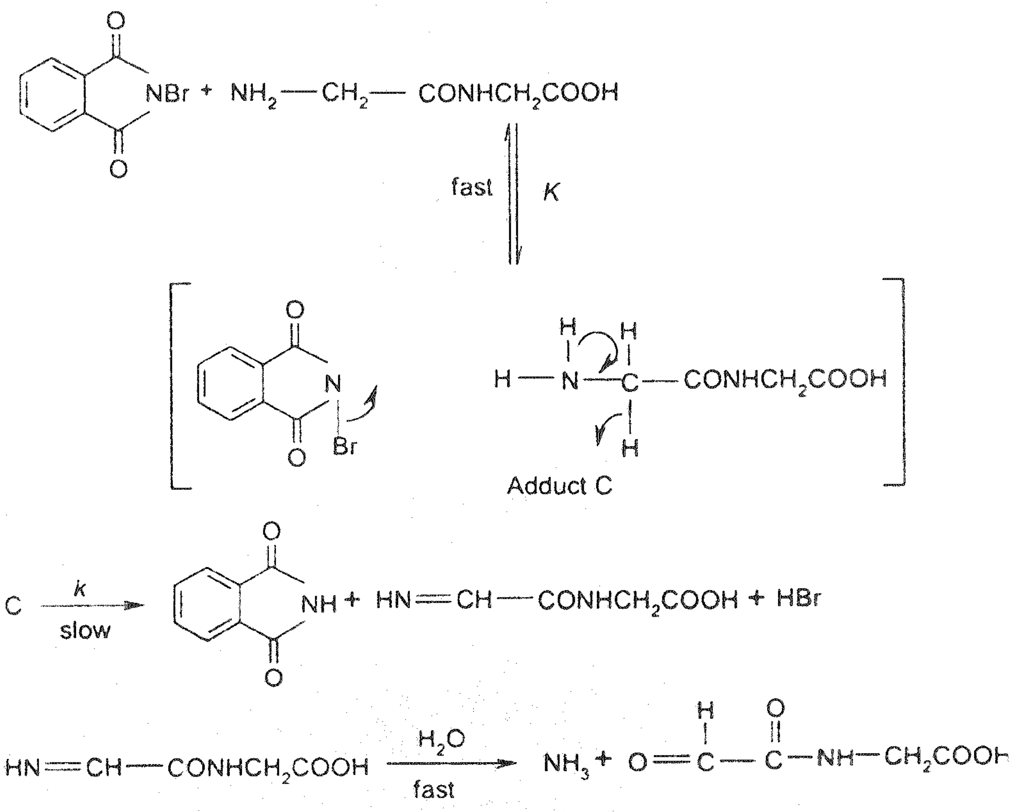
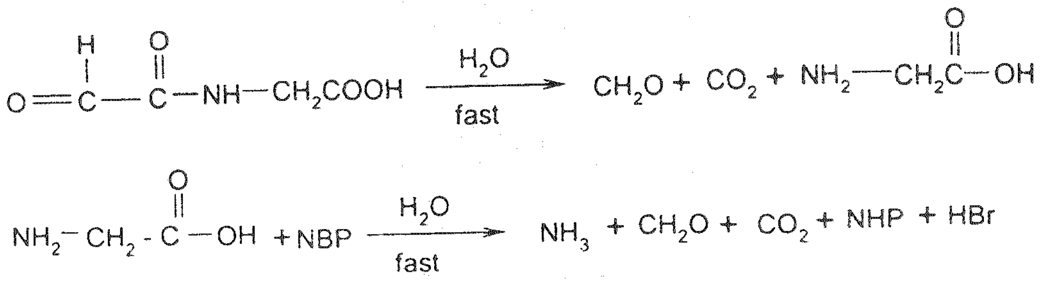


Fig. 3. Solvent effect: Kirkwood plot

The unit order dependence of rate on [NBP] and fractional order dependence on [GG] suggest that the reaction might occur *via* a fast pre-equilibrium step leading to the formation of an adduct which dissociates in the slow rate-determining step to give final products. On the basis of observed kinetic results, products of oxidation and above discussion, the mechanism of oxidation of glycylglycine by NBP may be written as given in **Scheme-1.**



Scheme-1 (Contd.)



Scheme-1

At constant ionic strength the rate law for the above mechanism can be written as

$$\text{Rate} = \frac{-d[\text{NBP}]}{dt} = \frac{kK[\text{Glycylglycine}][\text{NBP}]}{1 + K[\text{Glycylglycine}]} \qquad (1)$$

$$\frac{\text{Rate}}{[\text{NBP}]} = \frac{kK\,[\text{Glycylglycine}]}{1 + K[\text{Glycylglycine}]} \qquad (2)$$

Reciprocal of eqn. (2) yields

$$\frac{[\text{NBP}]}{\text{rate}} = \frac{1}{kK[\text{Glycylglycine}]} + \frac{1}{k} \qquad (3)$$

According to eqn. (3), Lineweaver Burk's plots of [NBP]/rate *vs.* 1/[GG] should be linear with positive slope and intercept on Y-axis. This has been observed in the present investigation (Fig. 1), supporting the proposed mechanism. From the intercepts and slopes of these plots at various temperatures k and K were calculated from which the activation parameters were calculated.

Comparison of rates of glycylglycine-NBP reaction with glycine-NBP reactions revealed that the rate of oxidation is *ca.* 4 times faster in case of glycine. 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 with pK_1 3.2 and pK_2 8.2 is weaker both as an acid and as a base when compared to glycine with pK_1 2.4 and pK_2 9.8. Thus, the oxidation of dipeptide glycylglycine is expected to be slower than the monomer, which is observed in the present study.

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