Asian Journal of Chemistry; Vol. 24, No. 10 (2012), 4671-4674

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

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

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(*Received*: 23 August 2011; *Accepted*: 11 May 2012) AJC-11465

The oxidation reactions of dipeptide glycylglycine (GG) have been carried out with *N*-bromosuccinimide in presence of mercuric acetate. The kinetics revealed first order dependence on *N*-bromosuccinimide 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.

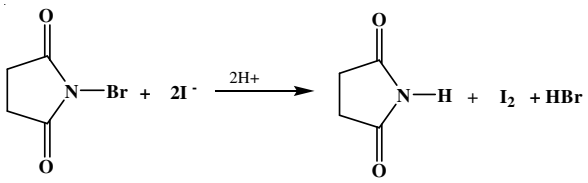
Key Words: *N*-Bromosuccinimide, Glycylglycine, Mercuric acetate, Glycine.

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]. Peptides and proteins represent major targets for modification in these reactions and for identification of sites which may lead to mechanistic understanding and approaches for prevention. Glycylglycine is a typical dipeptide which is the first member of dipeptide series. It undergoes oxidation by two different routes. The first route is through C-C bond cleavage resulting in decarboxylation and the second one is through N-H bond cleavage. The oxidation of dipeptides has been studied by various oxidants[2-8]. The present study reports the oxidation of glycylglycine by *N*-bromosuccinimide in aqueous acetic acid medium.

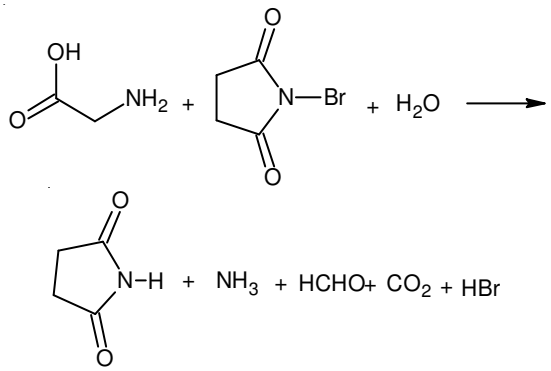
EXPERIMENTAL

Chromatographically pure glycylglycine (GG) was used. *N*-bromosuccinimide (NBS) 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 [NBS] < [GG] in 10 % (v/v) acetic acid medium in the presence of excess of mercuric acetate. The progress of the reaction was monitored by estimating the unreacted [NBS] at different intervals of time. The *N*-bromosuccinimide content was estimated by iodometrically[9] using a 1 % solution of freshly prepared starch as an indicator. The concentration of *N*-bromosuccinimide was calculated using the following stoichiometric equation[10].

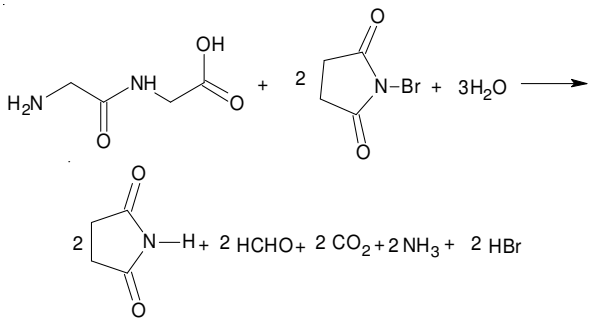


Stoichiometry and product analysis: Under the conditions [NBS] >> [Glycylglycine] in presence of excess of mercuric acetate the reaction was allowed to go to completion. The unreacted [NBS] was estimated and it was found that, one mole of oxidant was sufficient to oxidize one mole of glycine and two moles of oxidant was sufficient to oxidize one mole of glycylglycine. Based on these results, the following stoichiometric equations are suggested.

Stoichiometric equation for glycine:



Stoichiometric equation for glycylglycine:



Under the conditions of [NBS] >> [GG], the products obtained by the oxidation of glycylglycine were formaldehyde, ammonia and carbon dioxide. Formaldehyde was detected by Chromo tropic acid method[11], while ammonia was identified by Nessler's reagent[12] and carbon dioxide was detected by gas evolution apparatus.

RESULTS AND DISCUSSION

All kinetic runs were performed under pseudo first order conditions with [GG]>> [NBS]. The plots of log (a/a-x) *vs.* time, were found to be linear, passing through origin, (where, 'a' corresponds to the concentration of *N*-bromosuccinimide at zero time and (a-x) corresponds to the concentration of *N*-bromosuccinimide after time 't') indicating first order dependence of rate on concentration of *N*-bromosuccinimide. From the slopes of such plots pseudo-first order rate constants (k') were evaluated (Fig. 1).

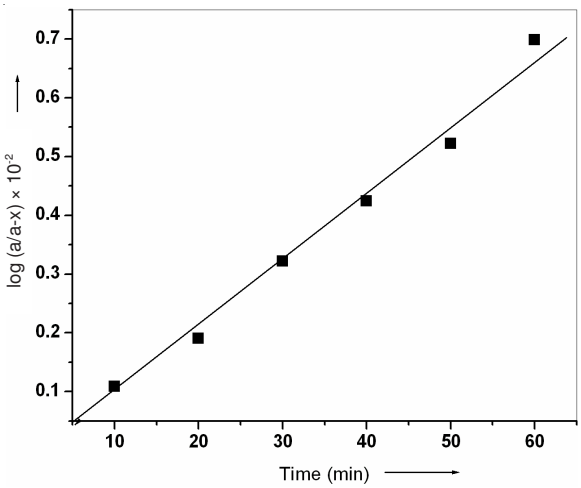


Fig. 1. Oxidation of glycylglycine by *N*-bromosuccinimide. [GG] = 1 × 10^{-2} mol dm^{-3}, [NBS] = 1 × 10^{-3} mol dm^{-3}, [$Hg(OAc)_2$] = 2 × 10^{-3}, [AcOH] = 10 % (v/v), T = 308 K

The plot of log k' *vs.* log [GG] (Fig. 2) was linear with n = 0.2 (r = 0.89) indicating fractional order dependence of rate on [GG]. Comparison of rates of oxidations of glycylglycine and glycine revealed that the rate of oxidation of glycine is faster than glycylglycine (Table-1).

To determine the activation parameters, the reactions were carried out at different temperatures. Using the Arrhenius equation the activation energies (E_a) were calculated and from these values thermodynamic parameters were evaluated (Table-2).

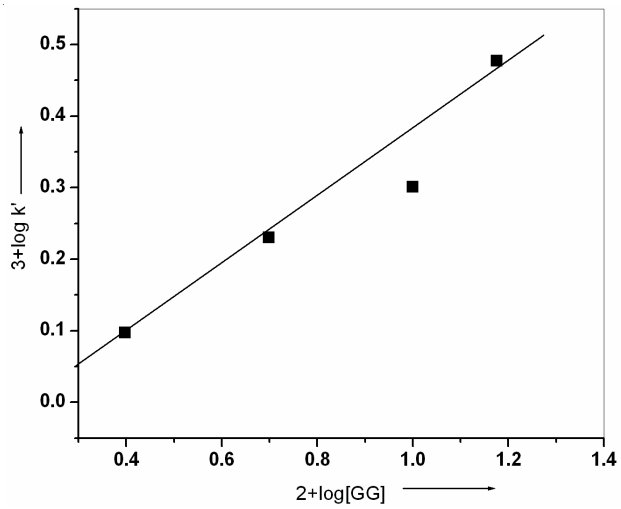


Fig. 2. Effect of [GG] on k' in NBS-glycylglycine reaction; [GG] = 1 × 10^{-2} mol dm^{-3}, [NBS] =1 × 10^{-3} mol dm^{-3}, [$Hg(OAc)_2$] = 2 × 10^{-3}, [AcOH] = 10 % (v/v), T = 308 K

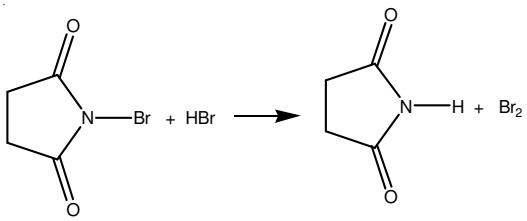
TABLE-1
COMPARISON OF RATE OF OXIDATION OF GLYCYLGLY-CINE WITH THAT OF GLYCINE
[NBS] = 1 × 10^{-3} mol dm^{-3}, [$Hg(OAc)_2$] = 2 × 10^{-3} mol dm^{-3}, [AcOH] =10 % (v/v), Temperature = 303 K

10^2 × [GG] (mol dm^{-3})	10^3 × k' (s^{-1})	10^2 × [Glycine] (mol dm^{-3})	10^3 × k' (s^{-1})
2.5	2.66	2.5	6.81
5.0	2.87	5.0	8.12
10.0	3.00	10.0	9.54
15.0	3.17	15.0	11.76
20.0	3.20	20.0	13.01

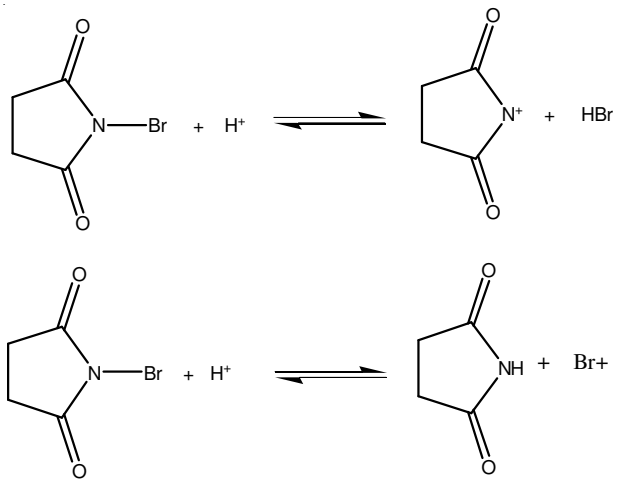
TABLE-2
ACTIVATION PARAMETERS FOR THE OXIDATION OF GLYCYLGLYCINE
[NBS] = 1 × 10^{-3} mol dm^{-3} ; [$Hg(OAc)_2$] = 2 × 10^{-3} mol dm^{-3}; [GG] = 1 × 10^{-2} mol dm^{-3}; [AcOH] = 10 % (v/v)

Temp. (K)	$\Delta H^{\#}$ (KJ mol^{-1})	$\Delta G^{\#}$ (KJ mol^{-1})	$\Delta S^{\#}$ (J mol^{-1} K^{-1})
308	45.3	76.26	-0.10051
313	45.26	76.79	-0.1007
318	45.22	77.47	-0.1014

The results of the oxidation of glycine and glycylglycine revealed that, the reactions have identical kinetics and thus appear to have common mechanism. Insignificant effect of mercuric acetate on reaction rate rules out it's involvement in *N*-bromosuccinimide oxidation and acts only as a scavenger[13,14] for any Br^- formed in the reaction. It suppresses completely the oxidation by Br_2, which would have been formed by the interaction of HBr and *N*-bromosuccinimide as follows.



Mercuric acetate thus ensures the oxidation purely through *N*-bromosuccinimide. *N*-Bromosuccinimide is known to exist in acidic media in the following equilibriums.



The *N*-bromosuccinimide itself or protonated *N*-bromosuccinimide, *i.e.* N^+BSH or Br^+ may be the possible oxidizing species in acidic media. In the presence of mercuric acetate protonated form of NBS, *i.e.* N^+BSH has been considered as a reactive species[15] of NBS in acidic media.

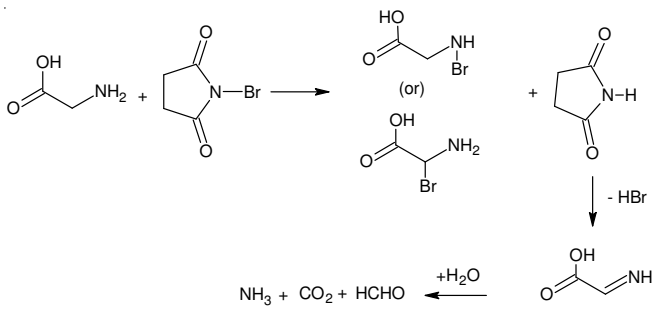
In the present investigation, the active species may be *N*-bromosuccinimide or N^+BSH. At constant ionic strength increase of H^+ did not affect the rate. Hence N^+BSH does not participate in the rate-determining step. All these factors indicate that *N*-bromosuccinimide is the only possible reactive species taking part in the reaction. In the light of the experimental results, a suitable mechanism has been proposed.

$$NBS + [S] \underset{}{\overset{K}{\rightleftharpoons}} X \qquad (1)$$

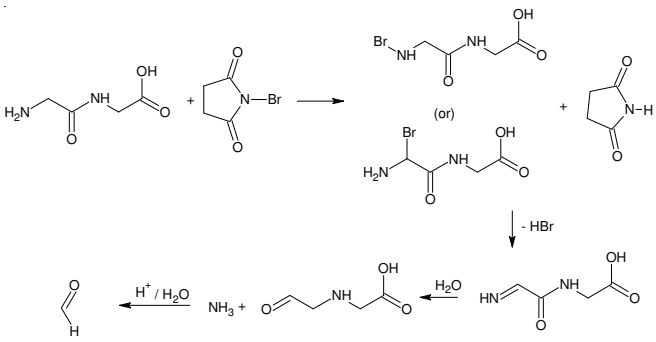
$$X \xrightarrow[k]{n.H_2O} \text{Products} \qquad (2)$$

where, [S] = glycine (or) glycylglycine.

Mechanism for oxidation of glycine:



Mechanism for oxidation of glycylglycine:



It is observed that in the oxidation of glycylglycine by *N*-bromosuccinimide, the increase in rate of reaction was negligible with the increase in ionic strength, dielectric constant.

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

$$\text{Rate} = \frac{-d[NBS]}{dt} = \frac{kK[\text{glycy lg lycine}][NBS]}{1 + K[\text{glycy lg lycine}]} \qquad (1)$$

$$\frac{\text{Rate}}{[NBS]} = \frac{kK[\text{glycy lg lycine}]}{1 + K[\text{glycy lg lycine}]} \qquad (2)$$

Since, rate/NBS = k_{obs} (or) k'; equation (2) may be transformed to:

$$k' = \frac{kK[\text{glycy lg lycine}]}{1 + K[\text{glycy lg lycine}]} \qquad (3)$$

Taking reciprocals on both sides for equation (3) leads to:

$$\frac{1}{k'} = \frac{1}{kK[\text{glycy lg lycine}]} + \frac{1}{k} \qquad (4)$$

From equation (4), a plot of 1/ k' *versus* 1/[glycylglycine] should be a straight line with positive intercept on y-axis. Which were obtained experimentally (Fig. 3), thus supporting the proposed mechanism. Further, it is also possible to calculate the value of k from the corresponding intercept. Also, from the ratio of intercept to slope of the line, it is possible to evaluate the value of K. The numerical values of k and K are determined as 4.115×10^{-3} S^{-1} and 5.86 respectively.

Comparison of rates of glycylglycine -NBS reaction with glycine -NBS reactions revealed that the rate of oxidation is approximately three times faster in case of glycine (Table-1). The difference of reaction rates may be due to, (i) the 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.

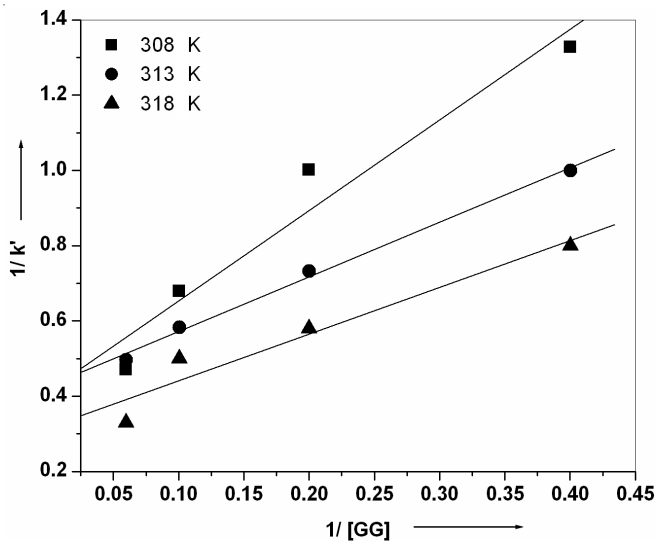


Fig. 3. Effect of [GG] on k' in NBS-glycylglycine reaction at different temperatures

Conclusion

The rates of oxidation of glycine and glycylglycine by *N*-bromosuccinimide 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[16]. Hence, the oxidation of glycylglycine is expected to be slower than that of glycine.

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