Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Journal of Molecular Catalysis A: Chemical 269 (2007) 225–233
JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL
www.elsevier.com/locate/molcata

N-Bromosuccinimide assisted oxidation of hydrophobic tetrapeptide sequences of elastin: A mechanistic study

N.S. Linge Gowda [a], M.N. Kumara [a], D. Channe Gowda [a], Kanchugara Koppal S. Rangappa [b,*], Netkal M. Made Gowda [c,**]

[a] Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570006, India
[b] Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland
[c] Department of Chemistry, Western Illinois University, 1 University Circle, Macomb, IL 61455, United States

Received 20 April 2006; received in revised form 10 January 2007; accepted 14 January 2007
Available online 18 January 2007

Abstract

The repeating sequences of elastin, glycyl–glycyl–alanyl–proline (GGAP) glycyl–glycyl–isoleucyl–proline (GGIP) and more hydrophobic glycyl–glycyl–phenylalanyl–proline (GGPP), were synthesized by classical solution phase methods and characterized. The kinetics of oxidation of tetrapeptides (TPs) and their constituent amino acids (AAs) by *N*-bromosuccinimide (NBS) was studied in the presence of perchlorate ions in acidic medium at 28 °C. The reaction was followed spectrophotometrically at $\lambda_{max} = 240$ nm. The reactions follow identical kinetics, being first order each in [NBS], [AA] and [TP]. No effect on the rate of [H^+], reduction product [succinimide] and ionic strength was observed. Effects of dielectric constant of the medium and the added anions such as chloride and perchlorate were studied. Activation parameters have been computed. The oxidation products of the reaction were isolated and characterized. The proposed mechanism is consistent with the experimental results. An apparent correlation was noted between the rate of oxidation and the hydrophobicity of AAs and TPs.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Amino acids; Tetra peptide sequences; Elastin; Oxidation; *N*-Bromosuccinimide

1. Introduction

Oxidative reactions play an important role in a variety of biochemical events ranging from normal metabolism to ageing and disease process [1,2]. Peptides and proteins represent major targets for modification in these reactions and the identification of sites and structures of modifications may lead to a mechanistic understanding and approaches for prevention. In this context, oxidation of α-amino acids is one of the well-documented biochemical processes. Several studies have been reported on the kinetics of *N*-bromosuccinimide (NBS) oxidation of various substrates in different media [3–8]. Extensive work has been reported on the kinetics of oxidation of amino acids and peptides with various metal ions and several other oxidants [9–11]. However, comparative studies on the kinetics of oxidation of amino acids and tetrapeptides by NBS have not been reported.

The cross-linked polytetrapeptide matrices based on the repeating amino acid sequences, Gly–Gly–Ala–Pro, Gly–Gly–Ile–Pro and Gly–Gly–Val–Pro were tested for cell adhesion promoting activity in both the absence and presence of fetal bovine serum [12]. The degree of cell attachments increases with the increase in hydrophobicity. In this context, it was thought to be interesting to investigate the hydrophobicity dependent oxidative behavior of NBS towards these tetrapeptides and their constituent amino acids.

Elastic protein-based polymers have their origins in repeating sequences of the mammalian protein elastin [13,14]. The first repeating sequence found in porcine elastin is $(Val^1–Pro^2–Gly^3–Gly^4)_n$, but this repeat has not been found to occur with $n > 2$ without substitution [15]. The monomers, oligomers and high polymers of these repeats have been synthesized and conformationally characterized [16]. These polymers have a number of medical and non-medical applications [17,18].

* Corresponding author. Tel.: +48 22 822 28 92; fax: +48 22 822 28 92.
** Corresponding author. Tel.: +1 309 298 1760; fax: +1 309 298 1760.
E-mail addresses: rangappaks@yahoo.com (K.K.S. Rangappa), GN-Made@wiu.edu (N.M. Made Gowda).

1381-1169/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcata.2007.01.019

The peptides were synthesized by classical solution phase methods [10]. The *tert*-butyloxycarbonyl (Boc) group was used for temporary N^{α}-protection and its removal was achieved with 4 M HCl in dioxane or trifluoroacetic acid. The C-terminal carboxyl group was protected by the benzyl ester and its removal was effected by hydrogenolysis using $HCOONH_4$–Pd/C (10%) [19]. All coupling reactions were achieved with isobutyl chloroformate. The protected peptides were purified by the crystallization and characterized by physical and analytical techniques [10]. The purity of the free peptides was checked by paper chromatography and HPLC.

2. Experimental

All the amino acids used except glycine were of L-configuration unless specified otherwise. All *tert*-butyloxycarbonyl (Boc) amino acids, amino acid derivatives and triflouroacetic acid (TFA) were purchased from Advanced Chem. Tech. (Louisville, KY, USA). Isobutylchloroformate and *N*-methylmorpholine (NMM) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All solvents and reagents were of analytical grade or were purified according to the procedure recommended for peptide synthesis. Thin-layer chromatography (TLC) was carried out on silica gel plates obtained from Whatman, with the following solvent systems: chloroform–methanol–acetic acid (90:10:3); Rf^1 and chloroform–methanol–acetic acid (85:15:3); Rf^2. The compounds on TLC plates were detected by UV light, spraying with ninhydrin or by chlorine–toludine. Paper chromatography was carried out on Whatman No. 1 chromatographic paper with the solvent system butanol–acetic acid–water (4:1:5, upper phase). The compounds on paper were detected by spraying with ninhydrin. Melting points (uncorrected) were determined with a Selaco Can. No. 103 melting-point apparatus. Elemental analyses were carried out by Mic Anal (Tucson, AZ, USA). Optical rotation was measured using a Perkin-Elmer Model 243 digital polarimeter. Amino acid analysis of the sample was performed on a Waters HPLC Pico-Tag analyzer using 6.0 M HCl containing 1% (v/v) phenol at 110 °C for 72 h in a sealed tube under vacuum from which the air had been removed using nitrogen. For each reaction the product analysis was carried out by gas chromatography (GC 15A, Shimadzu, Kyoto, Japan).

2.1. *Boc–Phe–Pro–OBzl*

Boc–Phe–OH (13.3 g, 0.05 mol) dissolved in acetonitrile (100 mL) and cooled to 0 °C was added NMM (5.5 mL, 0.05 mol). The solution was cooled to -15 ± 1 °C and isobutylchloroformate (6.5 mL, 0.05 mol) was added under stirring while maintaining the temperature at -15 °C. After stirring the reaction mixture for 10 min at this temperature, a pre-cooled solution of HOBt (6.8 g, 0.05 mol) was added. The reaction mixture was stirred for an additional 10 min and a pre-cooled solution of HCl, H–Pro–OBzl (12.1 g, 0.05 mol) and NMM (5.5 mL, 0.05 mol) in DMF (120 mol) was added slowly. After 20 min, the pH of the solution was adjusted to 8 by the addition of NMM and the reaction mixture stirred overnight at room temperature. Acetonitrile was removed under reduced pressure and the residual DMF solution was poured into about 600 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 min. The precipitated peptide was filtered, washed with 1.0 M HCl followed by water and dried. The crude peptide was recrystallized from ether and petroleum ether to obtain 19.5 g of Boc–Phe–Pro–OBzl (yield, 86.5%).

2.2. *Boc–Gly–Phe–Pro–OBzl*

Boc–Phe–Pro–OBzl (13.6 g, 0.03 mol) was deblocked with 4N HCl/dioxane (10 mL/g of peptide) for 1.5 h. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether and dried (yield, 100%). The HCl·H–Phe–Pro–OBzl was neutralized with NMM (3.3 mL, 0.03 mol) and coupled to Boc–Gly (5.3 g, 0.03 mol) in acetonitrile (50 mL) and NMM (3.3 mL) using isobutylchloroformate (4.1 mL, 0.03 mol) and worked up the same as Boc–Phe–Pro–OBzl to obtain 13.4 g of Boc–Gly–Phe–Pro–OBzl (yield, 87.7%). The sample was recrystallized from ether/petroleum ether.

2.3. *Boc–Gly–Gly–Xaa–Pro–OBzl*

Xaa is Ala for Boc–Gly–Gly–Ala–Pro–OBzl, Ile for Boc–Gly–Gly–Ile–Pro–OBzl and Phe for Boc–Gly–Gly–Phe–Pro–OBzl. Each peptide (Boc–Gly–Ala–Pro–OBzl, Boc–Gly–Ile–Pro–OBzl, and Boc–Gly–Phe–Pro–OBzl, 0.02 mol) was deblocked with 4.0 M HCl/dioxane (10 mL/g of peptide) for 1.5 h. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether and dried (yield, 100%). The HCl·H–Gly–Xaa–Pro–OBzl was neutralized with NMM (2.2 mL, 0.02 mol) and coupled to Boc–Gly (3.5 g, 0.02 mol) in acetonitrile (35 mL) and NMM (2.2 mL, 0.02 mol) using isobutylchloroformate (2.6 mL, 0.02 mol) and worked up the same as Boc–Phe–Pro–OBzl to obtain Boc–Gly–Gly–Ala–Pro–OBzl, Boc–Gly–Gly–Ile–Pro–OBzl and Boc–Gly–Gly–Phe–Pro–OBzl. The sample was recrystallized from ether/petroleum ether.

2.4. *Boc-Gly–Gly–Xaa–Pro–OH*

Xaa is Ala for Boc–Gly–Gly–Ala–Pro–OH, Ile for Boc–Gly–Gly–Ile–Pro–OH and Phe for Boc–Gly–Gly–Phe–Pro–OH. Each peptide (Boc–Gly–Gly–Ala–Pro–OBzl, Boc–Gly–Gly–Ile–Pro–OBzl and Boc–Gly–Gly–Phe–Pro–OBzl, 0.01 mol) was hydrogenolysed in methanol (10 mL/g of peptides) using ammonium formate (2.0 equiv.) and 10% Pd–C (0.1 g/g of peptide) for 30 min at room temperature. The catalyst was filtered and washed with methanol. The combined filtrate was evaporated under reduced pressure and the residue taken in to $CHCl_3$, washed with water, and dried over Na_2SO_4. The solvent was removed under reduced pressure and triturated with ether, filtered, washed with ether and dried to obtain Boc–Gly–Gly–Ala–Pro–OH, Boc–Gly–Gly–Ile–Pro–OH, and Boc–Gly–Gly–Phe–Pro–OH.

2.5. *Gly–Gly–Xaa–Pro*

Xaa is Ala for Gly–Gly–Ala–Pro, Ile for Gly–Gly–Ile–Pro and Phe for –Gly–Gly–Phe–Pro. Each peptide (Boc–Gly–Gly–Ala–Pro–OH, Boc–Gly–Gly–Ile–Pro–OH and Boc–Gly–Gly–Phe–Pro–OH, 0.009 mol) was deblocked with TFA (10 mL/g of peptide) for 40 min. The solvent was removed under reduced pressure, triturated with ether, filtered, washed with ether to obtain TFA salts of Gly–Gly–Ala–Pro to Gly–Gly–Phe–Pro (Yield 100%).

2.6. *Preparation of solutions of the oxidant and the substrates*

An aqueous solution of NBS was prepared fresh each day from a GRS Merck reagent and its strength was checked by the Iodometric method [20]. Aqueous solutions of AAs and TPs of known concentrations were prepared. All other reagents were of analytical grade. Double distilled water was used throughout the investigation.

2.7. *Kinetic measurements*

Mixtures of solutions containing the requisite amount of substrate, perchloric acid (to maintain a known acid concentration), succinimide and water (to keep the total volume constant) were placed in stoppered boiling tubes. The mixture was thermally equilibrated in a water bath at 28 °C. To the solution in this tube was added an aliquot of pre-equilibrated NBS stock solution to give a known overall concentration. The progress of the reaction was monitored for two half-lives by measuring the absorbance of the unreacted NBS at 240 nm using a spectrochem Elico SL 150 UV–Vis spectrophotometer. The reaction mixture was quenched appropriately; plots of log (absorbance) versus time were linear. The rate constants, k_{obs}, calculated from these plots were reproducible to within ±3% error.

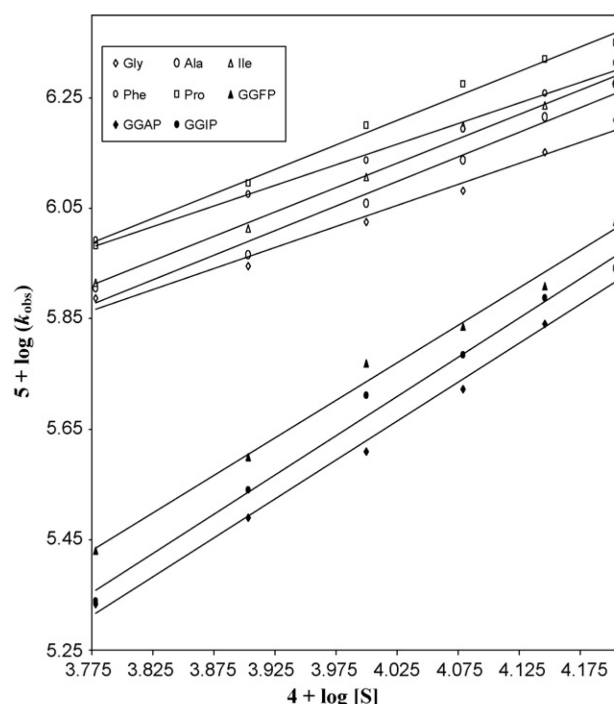


Fig. 1. Effect of [substrate] on the reaction rate.

3. Results

3.1. *Dependence of the rate on [NBS], [AA] and [TP]*

All kinetic runs were performed under pseudo-first-order conditions with $[AA]_0 \gg [NBS]_0$ and $[TP]_0 \gg [NBS]_0$. Plots of log [NBS] versus time, which were linear with slopes 1.05, 0.99 and 1.08 for over 75% of the reaction, showed a first-order dependence of the rate on [NBS] (Table 1). At constant $[NBS]_0$, $[succinimide]_0$, $[HClO_4]_0$ and temperature, the rate increased with increase in $[AA]_0$ or $[TP]_0$ (Table 1). Plots of log k_{obs} versus log $[AA]_0$ and log k_{obs} versus log $[TP]_0$ (Fig. 1) were linear with slopes 1.01, 0.98, and 1.10 for glycyl–glycyl–alanyl–proline,

Table 1
Effect of varying reactant concentration on the reaction rate with $[HClO_4] = 0.01$ mol dm^{-3}; [succinimide] = 0.1 mol dm^{-3}; [Hg $(CH_3COO)_2$] = 0.001 mol dm^{-3}; $T = 301$ K

[NBS] × 10^6 (mol dm^{-3})	[S] × 10^4 (mol dm^{-3})	$k_{obs} \times 10^5$ (s^{-1})							
		Gly	Ala	Ile	Phe	Pro	GGAP	GGIP	GGFP
0.6	1.0	10.61	11.64	12.76	13.68	15.88	4.07	5.15	5.85
0.8	1.0	10.66	11.62	12.77	13.72	15.70	4.05	5.13	5.86
1.0	1.0	10.61	11.61	12.79	13.75	15.88	4.01	5.18	5.85
1.4	1.0	10.24	11.58	12.89	13.69	15.20	4.04	5.16	5.84
1.6	1.0	10.10	11.48	12.85	13.68	15.45	4.03	5.14	5.83
0.6	0.6	07.73	08.05	08.24	9.81	09.59	2.15	2.18	2.69
0.6	0.8	08.18	09.23	10.33	11.90	12.47	3.09	3.46	3.98
0.6	1.0	10.61	11.64	12.79	13.70	15.88	4.07	5.15	5.87
0.6	1.2	12.07	13.72	15.86	15.62	18.82	5.28	6.08	6.84
0.6	1.4	14.20	16.39	17.25	18.16	20.86	6.92	7.71	8.12
0.6	1.6	16.24	17.85	19.15	20.06	22.36	8.03	8.79	9.48

Table 2
Effect of varying dielectric constant on the reaction rate, with [NBS] = 1.0×10^{-6} mol dm^{-3}; [S] = 1.0×10^{-4} mol dm^{-3}; [Hg $(CH_3COO)_2$ = 0.001 mol dm^{-3}; [Succinimide] = 0.1 mol dm^{-3}; [$HClO_4$] = 0.01 mol dm^{-3}; T = 301 K

MeOH (%, v/v)	Dielectric constant (D)	$k_{obs} \times 10^5$ (s^{-1})							
		Gly	Ala	Ile	Phe	Pro	GGAP	GGIP	GGFP
0	76.73	10.61	11.64	12.79	13.70	15.88	4.07	5.15	5.87
10	72.37	12.47	12.39	14.25	16.51	18.51	7.15	8.10	8.23
20	67.48	15.88	14.48	16.63	18.06	20.06	11.03	12.03	13.11
30	62.71	17.54	15.85	18.1	20.11	23.11	15.74	16.54	17.78
40	58.06	20.06	17.85	20.25	23.05	26.05	19.63	20.06	21.25

glycyl–glycyl–isoleucyl–proline, and glycyl–glycyl–phenylalanyl–proline, respectively.

3.2. Dependence of the rate on [acid]

Kinetic measurements were performed in $HClO_4$–$NaClO_4$ solutions of different [H^+]. The effective [H^+] used was evaluated using the calibration curve of [$HClO_4$] versus [H^+]. An increase in [H^+] (from 0.010 to 1.0 M) had no effect on the rate.

3.3. Dependence of the rate on the reduction product and added salts

The effect on the rate of varying concentrations of succinimide (which is the reduction product of the oxidant, NBS) was investigated. An increase in [succinimide] (from 0.01 to 0.5 M) had no effect on the rate. This indicated that the product is not involved in a pre-equilibrium with the oxidant. Similarly, the effect of anions Cl^- (from 0.01 to 0.5 M) and $ClO_4{}^-$ (from 0.01 to 1.0 M) on the rate was insignificant.

3.4. Effect of solvent composition

The dielectric constant (D) of the reaction medium was varied by changing the solvent composition with added MeOH (0.0–40%). The rate increased with increase in MeOH content (Table 2). The plots of log k_{obs} versus $1/D$ were linear ($r > 0.998$, $s \leq 0.01$) with positive slopes (Fig. 2). Measurements of rate constants for the oxidation by NBS were done both in the presence and the absence of each substrate (AA or TP) and the rate constants were taken for the calculation of effective k_{obs}. The rate of oxidation of MeOH by NBS in the absence of AA or TP was negligible under the conditions used.

3.5. Activation parameters

To determine the activation parameters, the reactions were carried out at different temperatures (293–313 K, Table 3). Arrhenius plots of log k_{obs} versus $1/T$ (Fig. 3), which were linear with slopes 1.05, 1.10 and 1.08 used to calculate activation energies (E_a). Based on these values, the activation parameters ($\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta G^{\ddagger}$) along with the frequency factor (log A) were evaluated (Table 4).

3.6. Test for free radicals

Addition of reaction mixture to aqueous acrylamide monomer solutions did not initiate polymerization, indicating the absence of *in situ* formation of free radical species in the reaction sequence.

3.7. Reaction stoichiometry

Reaction mixtures containing AA or TP (0.001 M), perchloric acid (0.1 M) and excess NBS (0.01 M) were kept for 24 h at 25 °C. The unconsumed NBS was then determined to calculate the stoichiometric ratios. One mole of oxidant was sufficient to oxidize one mole of glycine, alanine, phenylalanine and isoleucine and two moles for proline, whereas, five moles of oxidant was required to oxidize one mole of TP leading to the formation of products, aldehydes, carbon dioxide, ammonia and

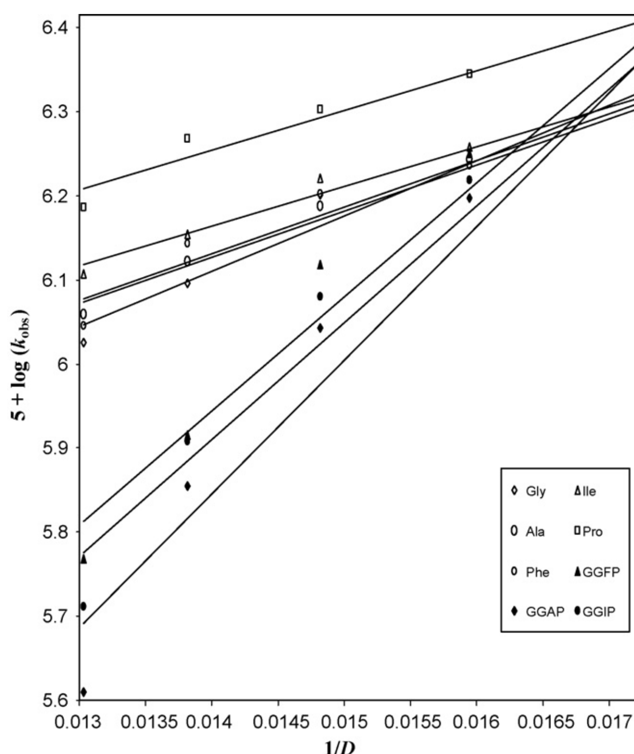


Fig. 2. Effect of dielectric constant (D) on the reaction rate.

Table 3
Temperature dependence of the oxidation of substrate with $[NBS] = 1.0 \times 10^{-6}$ mol dm^{-3}; $[S] = 1.0 \times 10^{-4}$ mol dm^{-3}; [Succinimide] = 0.1 mol dm^{-3}; $[H^+] = 0.01$ mol dm^{-3}

Temperature (K)	$(1/T)$ $(\times 10^{-3}$ K$^{-1})$	$k_{obs} \times 10^5$ (s^{-1})							
		Gly	Ala	Ile	Phe	Pro	GGAP	GGIP	GGFP
295	3.389	5.89	08.46	8.41	8.68	9.12	1.92	2.45	2.54
298	3.355	7.86	10.48	10.23	10.81	12.02	2.95	3.48	3.67
301	3.322	10.61	11.64	12.79	13.70	15.88	4.07	5.15	5.87
304	3.289	13.54	14.46	15.67	16.19	20.64	5.14	6.35	7.50
307	3.257	16.47	16.23	18.10	20.44	25.47	6.64	8.89	9.79

Table 4
Activation parameters for the oxidation of substrate by NBS, with $[NBS] = 1.0 \times 10^{-5}$ mol dm^{-3}; $[S] = 1.0 \times 10^{-4}$ mol dm^{-3}; [Succinimide] = 0.1 mol dm^{-3}; $[HClO_4] = 0.01$ mol dm^{-3}

Substrates	E_a (kJ mol^{-1})	$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta G^{\ddagger}$ (kJ mol^{-1})	$\log A$
Gly	64.78	62.27	−114.7	96.82	7.243
Ile	48.25	45.75	−167.9	96.29	4.570
Phe	70.25	67.75	−93.40	95.87	8.358
Ala	50.38	49.24	−122.8	94.90	6.397
Pro	68.94	65.50	−91.98	96.96	7.984
GGAP	73.45	70.94	−93.63	99.13	8.344
GGIP	74.97	72.47	−86.60	98.54	8.712
GGFP	79.38	76.87	−71.62	98.43	9.493

succinimide. Based on these results, the following stoichiometric Eqs. (a)–(c) are suggested:

- A general stoichiometric equation for four amino acids; glycine (Gly), alanine (Ala), isoleucine (Ile) and phenylalanine (Phe)

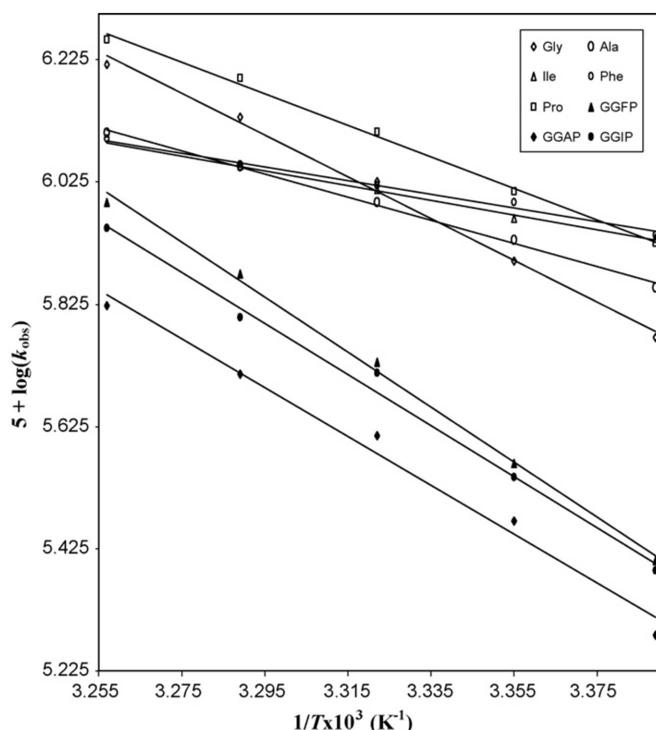
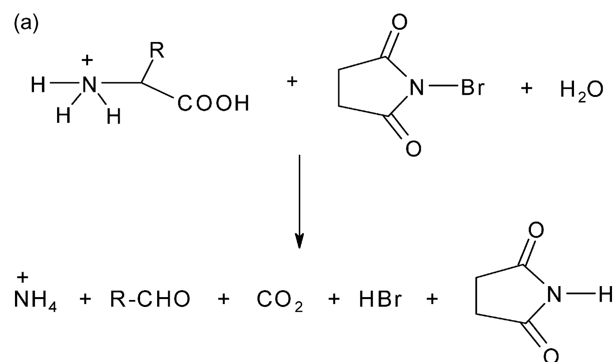
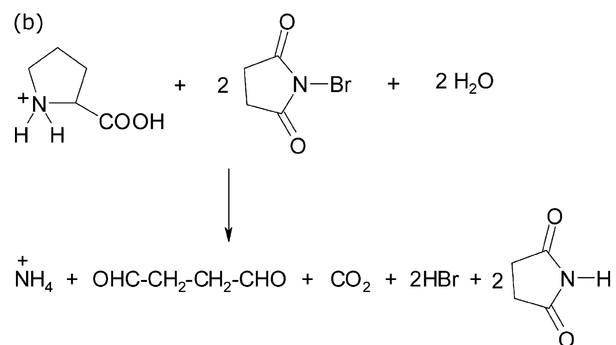


Fig. 3. Effect of temperature on the reaction rate.

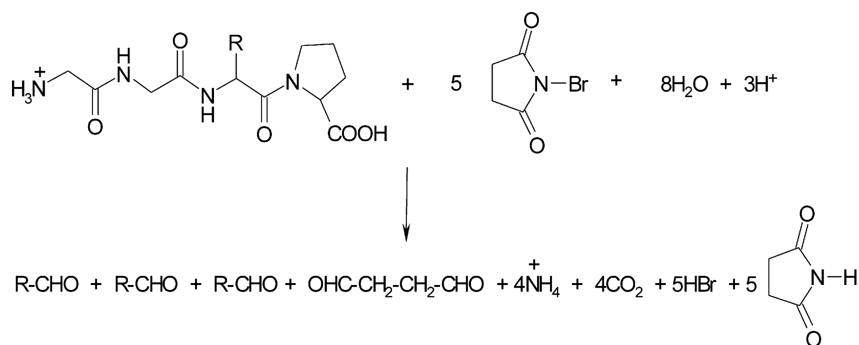


where $R = –H$ for glycine; $R = –CH_3$ for alanine; $R = –CH_2C_6H_5$ for phenylalanine; $R = –CH(CH_3)CH_2CH_3$ for isoleucine.

- A stoichiometric equation for Proline (Pro)



- A general stoichiometric equation for three tetrapeptides; glycyl–glycyl–alanyl–proline (GGAP), glycyl–glycyl–isoleucyl–proline (GGIP) and glycyl–glycyl–phenylalanyl –proline (GGPP)



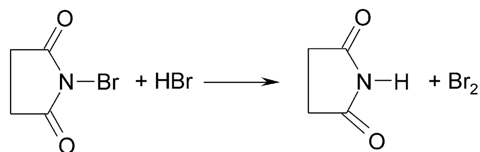
where R = –CH$_2$–C$_6$H$_5$ for GGFP; R = –CH3 for GGAP; R = –CH(CH$_3$)–CH$_2$–CH$_3$ for GGIP.

3.8. Product analysis

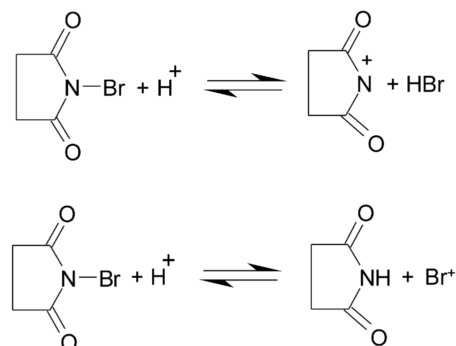
After the reaction was completed, the reaction products were extracted with diethyl ether and subjected to column chromatography on silica gel (60–200 mesh) using gradient elution (dichloromethane to chloroform). Rf[1]: CHCl$_3$–MeOH–HOAC (95:5:3); Rf[2]: CHCl$_3$–MeOH–HOAC (90:10:3); Rf[3]: CHCl$_3$–MeOH–HOAC (85:15:3). Aldehydes were determined qualitatively by gas chromatography. The retention values of formaldehyde, acetaldehyde, 2-methyl butyraldehyde, phenyl acetaldehyde and succinaldehyde are 6.0, 5.14, 27.4, 31.09 and 31.9, respectively, which are identical with those for authentic samples. Ammonia and CO_2 were detected by conventional tests.

4. Discussion

The results of the oxidation of amino acids and tetrapeptides, recorded here, have revealed that the reactions have identical kinetics, and thus appear to have common mechanism. Insignificant effect of mercuric acetate on reaction rate rules out its involvement in NBS oxidation and acts only as a scavenger [21,22] 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 NBS as follows:



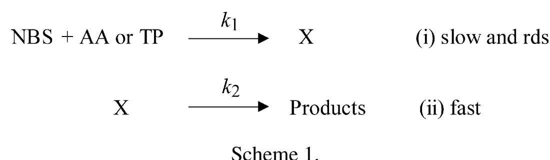
Mercuric acetate thus ensures the oxidation purely through NBS. NBS is known to exist in acidic media in the following equilibria:



The possible oxidizing species of NBS in aqueous acidic solutions are: NBS itself, protonated NBS (i.e., N$^+$BSH), and Br^+. In the presence of mercuric acetate protonated form of NBS, i.e. N$^+$BSH has been considered [23] as its reactive species in acidic medium.

In the present investigation, it was found that the added succinimide has a negligible effect on the rate of the reaction. This categorically excludes Br^+ as the oxidizing species. Hence, the active species may be NBS or N$^+$BSH. The order of the reaction with respect to [H$^+$] is zero, and hence, N$^+$BSH does not participate in the rate-determining step. All these factors indicate that NBS is the only possible oxidant species taking part in the reaction. In the light of the experimental results, a suitable mechanism has been proposed due to the difference in the free energies of product and reactants.

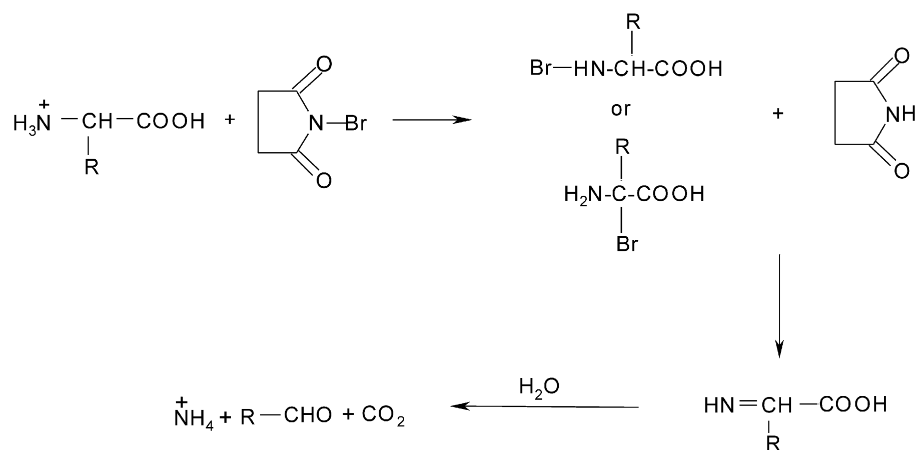
The following Scheme 1 accounts for the observed experimental results for AA and TP:



Scheme 1.

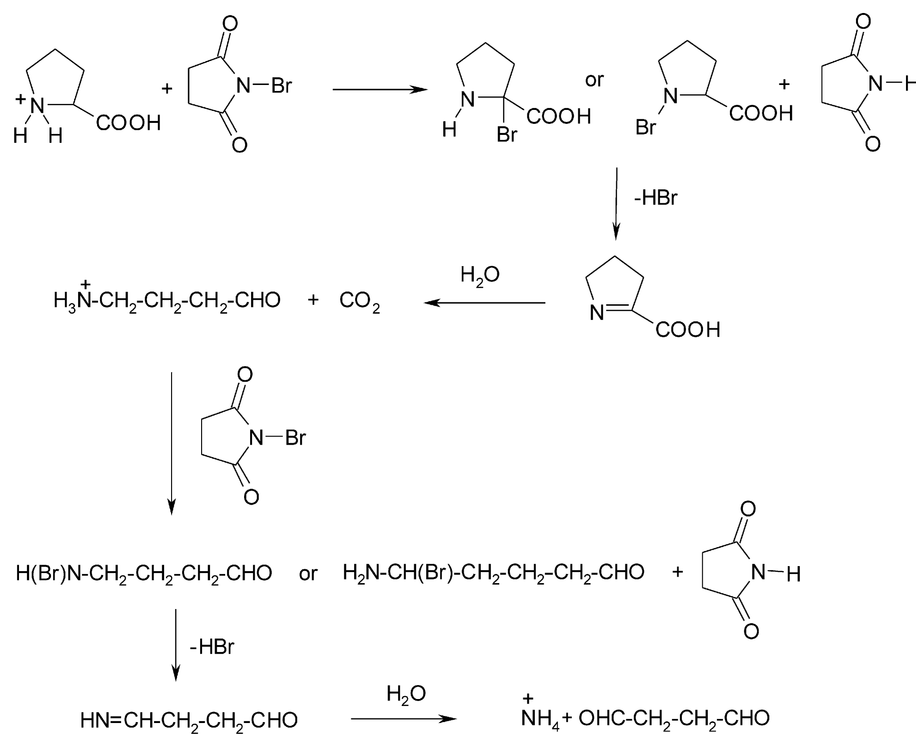
Hence, Rate = k_1 [NBS] [AA or TP]

- Mechanism for amino acids for glycine (Gly), alanine (Ala), isoleucine (Ile) and phenylalanine (Phe)

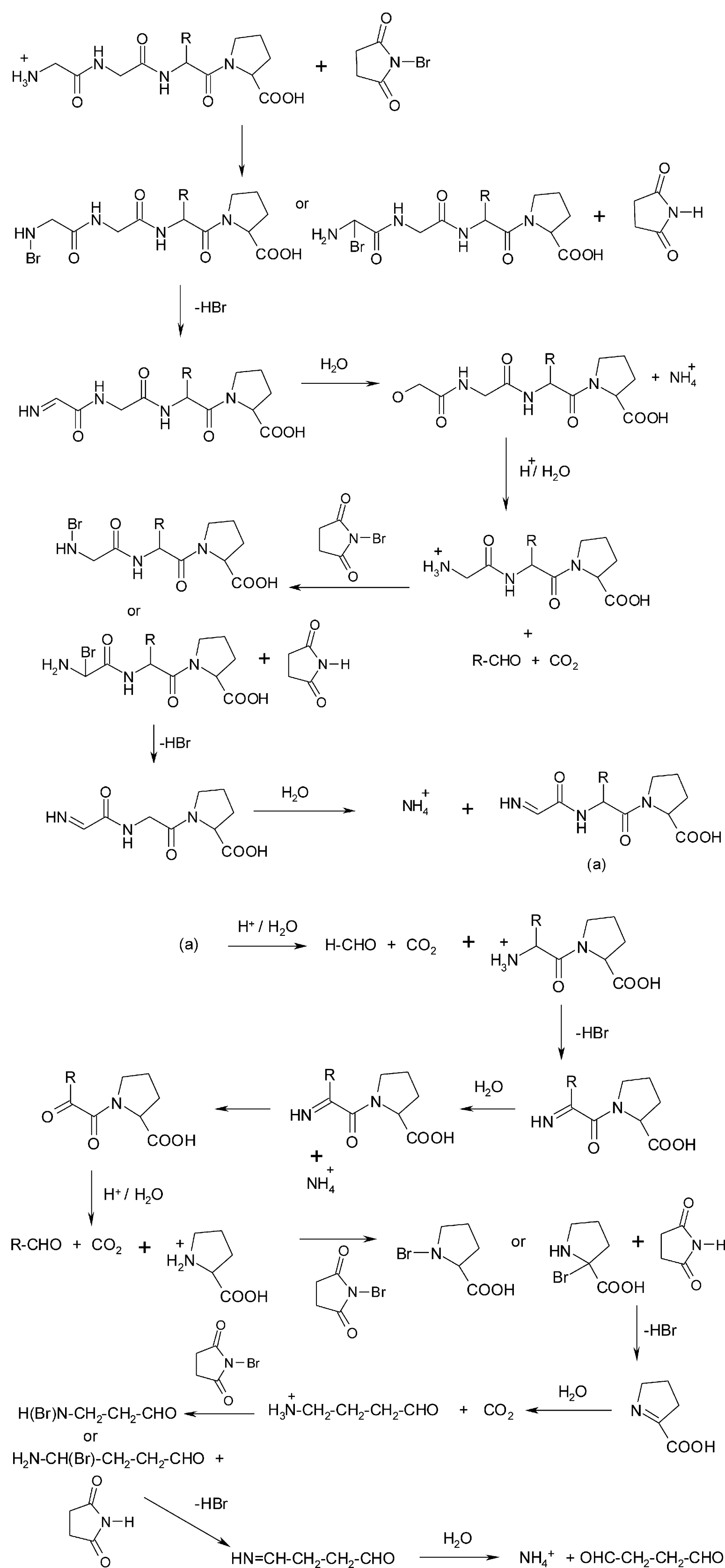


where R = –H for glycine; R = –CH_3 for alanine; R = –$CH_2C_6H_5$ for phenylalanine; R = [–$CH(CH_3)CH_2CH_3$] for isoleucine.

- An oxidation mechanism for proline (Pro)



- A general oxidation mechanism for three tetrapeptides by NBS



where R = –CH$_2$–C$_6$H$_5$ for GGFP; R = –CH$_3$ for GGAP; R = –CH(CH$_3$)–CH$_2$–CH3 for GGIP.

5. Conclusions

The rates of oxidation of amino acids (AAs) and tetrapeptides (TPs) by NBS were compared under identical experimental conditions. It was found that the rate of oxidation of TPs was slower than that of the corresponding free AAs or monomers. The change is due to the increased distance between the functional groups and resultant weaker electronic effects. Furthermore, an apparent correlation was noted between the rate of oxidation and the hydrophobicity [24] of those peptide sequences where increased hydrophobicity resulted in an increased rate of oxidation. The most hydrophobic tetrapeptide, Gly–Gly–Phe–Pro, oxidized at a faster rate than the less hydrophobic tetrapeptide, Gly–Gly–Ala–Pro or Gly–Gly–Ile–Pro. The probable reason for the increased oxidation rate for the more hydrophobic peptides is that the carboxylic groups are more destabilized enhancing the rate of formation of a transition state with NBS which in turn increases the oxidation rate.

Acknowledgements

One of us (K.S.R.) is grateful to the INSA-PAS (2005–2006) exchange programme and UGC-SAP (Phase-I)/DST-FIST programmes, Government of India, New Delhi, for financial support.

References

[1] E.R. Stadtman, Science 257 (1992) 1220.
[2] B.S. Berlett, E.R. Stadtman, J. Biol. Chem. 272 (1997) 20313.
[3] K.T. Itoh, S. Hashimoto, Y. Masaki, Synthesis (2003) 2289.
[4] X.-F. Sun, R.-C. Sun, J. Sci. Food Agric. 84 (2004) 800.
[5] C. Karunakaran, C. Ganapathi, J. Phys. Org. Chem. 3 (2004) 235.
[6] S. Kameshwar, J.N. Tiwari, S.P. Mushran, Int. J. Chem. Kinet. 10 (2004) 995.
[7] S. Sivakamasundari, R. Ganeshan, Int. J. Chem. Kinet. 12 (2004) 837.
[8] V.B. Sharma, S.L. Jain, J. Mol. Catal. 227 (2005) 47.
[9] N. Kambo, N. Grover, S.K. Upadyay, J. Ind. Chem. Soc. 79 (2002) 939.
[10] D. Channegowda, B.K. Kempegowda, K.S. Rangappa, J. Phys. Org. Chem. 14 (2001) 716.
[11] M.N. Kumara, D. Channegowda, K.S. Rangappa, J. Chem. Sci. 116 (2004) 49.
[12] K.U. Prasad, M. Iqbal, D.W. Urry, Int. J. Peptide Protein Res. 25 (1985) 408.
[13] L.B. Sandberg, J.G. Leslie, C.T. Leach, V.L. Torres, A.R. Smith, D.W. Smith, Pathol. Biol. 33 (1985) 266.
[14] H. Yeh, N. Ornstein-Goldstein, Z. Indik, P. Sheppard, N. Anderson, J.C. Rosenbloom, G. Cicila, K. Yoon, J. Rosenbloom, Collagen Relat. Res. 7 (1987) 235.
[15] L.B. Sandberg, N.T. Soskel, J.B. Leslie, N. Engl. J. Med. 304 (1981) 566.
[16] D.W. Urry, M.M. Long, CKC Crit. Rev. Biochem. 4 (1976) 1.
[17] D.W. Urry, A. Niuol, D.C. Gowda, L.D. MeKee, T. Williams, U.B. Olsen, B.A. Cox, in: C.G. Gebelein (Ed.), Proceedings of the Biotechnological Polymers: Medical, Pharmaceutical and Industrial Applications Conference, Technomic Publishing Co. Inc., Atlanta, Georgia, 1993, p. 82.
[18] D.W. Urry, D.T. McPhcrson, J. Xu, H. Daniell, C. Guda, D. Channegowda, N. Jing, T.M. Parker, Polymeric Materials Encyclopedia, 9, CRC Press, New York, Boca Katon, FL, 1996, p. 7263.
[19] M.K. Anwer, A.F. Spaiola, Synthesis (1980) 929.
[20] M.Z. Barakat, A.M.P. Wahab, Anal. Chem. 26 (1973).
[21] G. Gopalakrishnan, J.L. Hogg, J. Org. Chem. 50 (1985) 1206.
[22] P.F. Kruse, K.L. Grist, T.A. Mecoy, Anal. Chem. 26 (1954) 1319.
[23] N. Venkatasubramanian, V. Thyagarajan, Can. J. Chem. 47 (1969) 694.
[24] D.W. Urry, D. Channegowda, Biopolymers 32 (1992) 1243.