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Kinetic Analysis of Oxidation of Dipeptides by Sodium *N*-Bromobenzenesulfonamide in Acid Medium: a Mechanistic Approach

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The kinetics of the oxidation of five dipeptides (DPP) viz., glycylglycine (Gly-Gly), L-alanyl-L-alanine (Ala-Ala), L-valyl-L-valine (Val-Val), L-leucyl-L-leucine (Leu-Leu), and phenylglycyl-phenylglycine (Phg-Phg) by sodium *N*-bromobenzenesulfonamide or bromamine-B (BAB) in presence of $HClO_4$ was studied at 40 °C. The five reactions followed identical kinetics with a first-order dependence on [BAB] and fractional order in [DPP]. At $[H^+] > 0.04$ mol dm^{-3}, the rate was inverse fractional in $[H^+]$, but zero order at lower $[H^+]$ (≤ 0.04 mol dm^{-3}). A variation of the ionic strength or dielectric constant of the medium and the addition of halide ions and benzenesulfonamide had no effect on the rate of the reaction. Proton inventory studies were made in H_2O–D_2O mixtures for all five dipeptides. A Michaelis–Menten type mechanism has been suggested to explain the results. The decomposition and equilibrium constants were evaluated. The oxidation products were identified. The isokinetic temperature was 360 K, indicating the enthalpy to be a controlling factor. The rate of oxidation increased in the order Phg-Phg > Ala-Ala > Val-Val > Leu-Leu > Gly-Gly. The kinetics of oxidation of dipeptides was compared with those of their corresponding monomer amino acids, namely Phenylglycine, alanine, valine, leucine, and glycine. A general mechanism was proposed and the derived rate law are consistent with the observed kinetics.

The chemistry of *N*-haloarenesulfonamidates, generally known as organic haloamines, has evoked considerable interest, because they are sources of halogen cations, hypohalite species, and *N*-anions, which act both as bases and nucleophiles. The prominent members of this group are chloramine-T (CAT) and chloramine-B (CAB) and the mechanistic aspects of many of these reactions have been documented.[1–3] Bromamine-B (BAB), the bromine analogue of CAB, is gaining importance as a mild oxidant, and is found to be a better oxidizing agent than the chloro compound.[4] A review of the literature shows that kinetic studies using this reagent have been meager.[5–7]

Although extensive work has been reported on the kinetics of the oxidation of amino acids, using a variety of oxidants,[8–10] there is little information in the literature on the oxidation kinetics of dipeptides. Dipeptides are useful biomaterials in many analytical, biological, pharmaceutical, and synthetic applications. Glycylglycine, the first member of the dipeptide series, has been oxidized by manganese(Ⅲ) in an aqueous sulfuric acid medium[11] and bromamine-T in acid medium.[12] Also, its hydrolysis kinetics[13–14] has been reported. L-valyl-L-valine, L-alanyl-L-alanine, L-leucyl-L-leucine, and Phenylglycyl-Phenylglycine are other dipeptides. A literature survey shows that there is no report on the oxidation kinetics of these dipeptides. In view of this, we have taken up a systematic kinetic study of the oxidation of important dipeptides, namely, glycylglycine (Gly-Gly), L-valyl-L-valine (Val-Val), L-alanyl-L-alanine (Ala-Ala), L-leucyl-L-leucine (Leu-Leu), and phenylglycyl-phenylglycine (Phg-Phg) by bromamine-B ($C_6H_5SO_2NBrNa\cdot1.5\ H_2O$ or BAB) in an acid medium to explore the mechanistic aspects of these oxidations and also to understand the active form of BAB in an acid medium. Attempts have been made to asses the relative rates of oxidation of these dipeptides and to derive an isokinetic relationship with the computed activation parameters. Further, the rates of oxidation of these dipeptides were compared with their monomers by BAB under identical experimental conditions.

Experimental

Materials. Bromamine-B was prepared[15] by the partial debromination of dibromamine-B (DBB), which was obtained as follows. Pure chlorine was bubbled through an aqueous solution of chloramine-B (30 g in 560 mL of water) and liquid bromine (6 mL) was added dropwise from a burette with constant stirring. A yellow precipitate of DBB that formed was thoroughly washed with H_2O, filtered under suction, and dried in a vacuum desiccator. Dibromamine-B (31.5 g) was digested in small batches with constant stirring in 50 mL of 4 mol dm^{-3} NaOH. The mass was cooled in ice and filtered under suction, and the product was dried over anhydrous calcium chloride.

The purity of BAB was checked iodometrically through its active bromine content and the compound was further characterized by its ^{13}C FT-NMR spectrum (obtained on a Bruker WH 270-MHz nuclear magnetic resonance spectrometer) with D_2O as a solvent and TMS as the internal standard (ppm relative to TMS) 143.38 (C-1, carbon attached to S atom), 134.30 (C-4, para to hetero atom), 131.26 (C-2,6), and 129.31 (C-3,5). An aqueous solution of BAB was standardized iodometrically and preserved in brown bottles to prevent its photochemical deterioration.

Chromatographically pure Gly-Gly and Ala-Ala (Merck), Val-Val and Leu-Leu (Bachem AG, Switzerland) were used as received. Phg-Phg was prepared[16] by following the standard procedure, and was confirmed by its NMR data. Phg-Phg was prepared by employing a Boc group for N-protection and methyl ester as a C-protecting group. The hydrolysis of the methyl ester followed by removal of Boc group gave the dipeptide. Aqueous solutions of dipeptides were prepared and employed. All other chemicals were of analytical grade. Heavy water (99.4%) employed for solvent isotope studies was supplied by the Bhabha Atomic Research Centre, Mumbai, India. The ionic strength (I) of the system was maintained at a constant high value (0.5 mol dm^{-3}) using a concentrated solution of sodium perchlorate. The permittivity of the reaction mixture was altered by the addition of methanol in varying proportions (v/v), and the values of the permittivity (dielectric constant) of methanol-water mixtures reported in the literature[17] were employed. Triply distilled water was used in preparing all aqueous solutions throughout the studies. A regression analysis of the experimental data was carried out on an EC-75 statistical calculator to obtain the regression coefficient, (r).

Kinetic Measurements. Kinetic runs were performed under pseudo first-order conditions with a known excess of the [substrate] over [oxidant] at 40 °C. The reaction was carried out in stoppered pyrex boiling tubes whose outer surfaces were coated black to eliminate any photochemical effects. For each run, requisite amounts of solutions of the substrate, acid, $\mathrm{NaClO_4}$, and the water (to maintain a constant total volume) were introduced into the tube and thermostated at 40 ± 0.1 °C until thermal equilibrium was attained. A measured amount of BAB solution, also thermostated at the same temperature, was added rapidly to the above mentioned mixture to initiate the reaction. The mixture was periodically shaken to ensure uniform concentration, and the progress of the reaction was monitored by an iodometric determination of unreacted BAB in a measured aliquot of the reaction mixture at different time intervals. The reaction was followed for more than two half-lives. The pseudo-first order rate constants (k'), calculated from linear plots of log [BAB] versus time, were reproducible within ±3%.

Stoichiometry and Product Analysis. Varying ratios of ([oxidant] $\gg$ [substrate]) in the presence of 0.04 mol dm^{-3} $\mathrm{HClO_4}$ were equilibrated at 40 °C for 48 h. An iodometric determination of the unconsumed BAB indicated that two moles of oxidant were needed per mole of dipeptide to give the corresponding aldehyde. The observed stoichiometry is

$$\mathrm{NH_2CHRCONHCHRCOOH + 2PhSO_2NBrNa + 3H_2O \rightarrow 2RCHO + 2CO_2 + 2NH_3 + 2PhSO_2NH_2 + 2Na^+ + 2Br^-} \quad (1)$$

where R = -H for Gly-Gly, $\mathrm{-CH_3}$ for Ala-Ala, $\mathrm{-CH(CH_3)_2}$ for Val-Val, $\mathrm{CH_2CH(CH_3)_2}$ for Leu-Leu, and - $\mathrm{C_6H_5}$ for Phg- Phg.

The reaction mixture was extracted with ether and the corresponding obtained aldehydes (RCHO, oxidation products of dipeptides) were detected by the formation of their 2,4-dinitrophenylhydrazone derivatives.[18] The hydrazone derivatives were characterized by their IR spectra. Formaldehyde, acetaldehyde, isobutyraldehyde, Isovaleraldehyde, and benzaldehyde are the oxidation products of Gly-Gly, Ala-Ala, Val-Val, Leu-Leu, Phg-Phg and the melting points[18] of the corresponding 2,4-DNP hydrazone derivatives are 164 °C, 168 °C, 186 °C, 122 °C, and 235 °C, respectively. Benzenesulfonamide ($\mathrm{PhSO_2NH_2}$, the reduction product of BAB) was identified[19] by TLC using pet. ether–$\mathrm{CHCl_3}$–1-butanol (2:2:1 v/v) as a solvent system and iodine as a spray reagent ($R_f = 0.88$). $\mathrm{NH_3}$ and $\mathrm{CO_2}$ were identified by conventional tests. Ammonia was quantitatively estimated[20] by the micro-kjeldahl procedure.

Results

The kinetics of the oxidation of dipeptides (DPP) by BAB was investigated at several initial concentrations of the reactants in a $\mathrm{HClO_4}$ medium. The same oxidation behavior was observed for all five dipeptides.

Under pseudo-first order conditions of [substrate] $\gg$ [oxidant] at constant [DPP], [$\mathrm{HClO_4}$] and temperature, plots of log [BAB] versus time were linear ($r > 0.9926$) indicating a first-order dependence of the reaction rate on [BAB]. The rate constant (k') was not affected by a change in [BAB], confirming the first-order dependence of the rate on [BAB] (Table 1). The values of k' increased with an increase in [DPP] (Table 1). Plots of log k' versus log [DPP] are linear ($r > 0.9922$) with fractional slopes (0.42–0.58), indicating a fractional order dependence on [DPP]. Furthermore, plots of k' versus [DPP] are linear ($r > 0.9812$) with a y-intercept, confirming the fractional order dependence on [DPP]. The order in [DPP] becomes zero as [DPP] is increases further (> 0.15 mol dm^{-3}), obeying

Table 1. Effect of Variation of Reactant Concentrations on the Rate of Reaction, [$\mathrm{HClO_4}$] = 4.0×10^{-2} mol dm^{-3}; I = 0.5 mol dm^{-3} ; temp = 313 K

10^4 [BAB]	10^2 [DPP]	10^4 k'/s^{-1}				
mol dm^{-3}	mol dm^{-3}	Gly-Gly	Leu-Leu	Val-Val	Ala-Ala	Phg-Phg
2.0	4.0	2.50	7.75	9.45	11.9	13.3
4.0	4.0	2.60	7.70	9.50	11.8	13.4
6.0	4.0	2.55	7.60	9.48	11.9	13.2
8.0	4.0	2.68	7.80	9.52	11.7	13.3
10.0	4.0	2.52	7.65	9.56	11.9	13.4
12.0	4.0	2.64	7.84	9.50	11.8	13.2
4.0	0.5	0.92	2.84	2.92	4.10	5.45
4.0	1.0	1.40	3.96	4.40	5.92	7.50
4.0	2.0	1.90	5.50	6.55	7.55	10.3
4.0	4.0	2.60	7.70	9.50	11.8	13.4
4.0	8.0	3.68	10.5	14.1	18.0	16.8
4.0	12.0	4.35	12.4	18.2	23.1	20.2
4.0	15.0	4.40	12.5	18.1	23.0	20.3
4.0	20.0	4.38	12.6	18.2	23.2	20.2
4.0	25.0	4.30	12.5	18.1	23.1	20.4
4.0	30.0	4.45	12.4	18.3	23.2	20.3

Table 2. Effect of Variation of Acid Concentration on the Rate of Reaction, [BAB] = 4.0×10^{-4} mol dm^{-3}; [DPP] = 4.0×10^{-2} mol dm^{-3} ; I = 0.5 mol dm^{-3}; temp = 313 K

10^2 [$HClO_4$]	$10^4 k'$/s^{-1}				
mol dm^{-3}	Gly-Gly	Leu-Leu	Val-Val	Ala-Ala	Phg-Phg
0.5	2.50	7.75	9.42	11.9	13.2
1.0	2.55	7.58	9.55	11.8	13.3
2.0	2.62	7.65	9.50	11.8	13.4
3.0	2.68	7.55	9.58	11.7	13.2
4.0	2.60	7.70	9.50	11.8	13.4
5.0	2.22	6.82	8.10	10.1	11.5
6.0	1.94	5.90	7.25	8.86	10.4
8.0	1.55	5.08	6.10	7.30	8.70
12.0	1.20	3.85	4.54	5.42	6.60

Table 3. Proton Inventory Studies for the Oxidation of Dipeptides in H_2O–D_2O Mixtures, [BAB] = 4.0×10^{-4} mol dm^{-3}; [DPP] = 4.0×10^{-2} mol dm^{-3}; [$HClO_4$] = 4.0×10^{-2} mol dm^{-3}; I = 0.5 mol dm^{-3}; temp = 313 K

Atom fraction of deuterium (n)	$10^4 k'_n$/s^{-1}				
	Gly-Gly	Leu-Leu	Val-Val	Ala-Ala	Phg-Phg
0.000	2.60	7.70	9.50	11.8	13.4
0.248	2.25	6.65	8.40	10.1	11.8
0.496	1.90	5.88	7.10	9.80	10.6
0.744	1.75	5.40	6.31	7.92	9.89
0.992	1.55	5.08	5.80	7.10	9.10

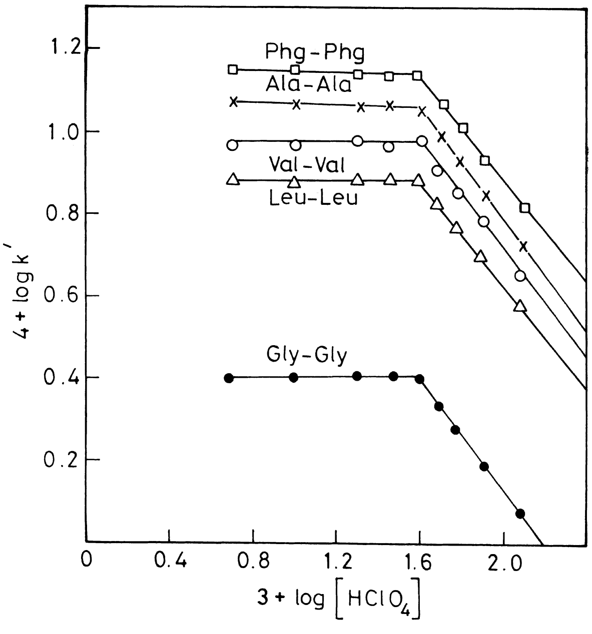


Fig. 1. Plots of log k' versus log [$HClO_4$]. Experimental conditions are as in Table 2.

Michaelis–Menten kinetics (Table 1).

At constant [BAB] and [DPP], the rate was not affected at low [H^+] (5.0×10^{-3}–4.0×10^{-2} mol dm^{-3}), but retardation set in at [H^+] = 4.0×10^{-2}–12.0×10^{-2} mol dm^{-3} (Table 2). Under the latter conditions, plots of log k' versus log [H^+] (Fig. 1) were linear ($r > 0.9968$) with negative fractional slopes (0.64–0.68). In the low acid range ([H^+] = 1.0×10^{-2} mol dm^{-3}), the variation of [DPP] had no effect on the rate (values are not reported).

When the ionic strength of the solution was varied by adding $NaClO_4$ (0.1–0.8 mol dm^{-3}), the rate was unaffected. The addition of reaction product, benzenesulfonamide ($PhSO_2NH_2$; 2.0×10^{-4}–2.0×10^{-3} mol dm^{-3}) and Cl^- or Br^- ions in the form of NaCl or NaBr (5.0×10^{-4}–5.0×10^{-3} mol dm^{-3}) had a negligible effect on the rate. The rate was not significantly altered on varying the dielectric constant of the medium by adding methanol (5–30% v/v). Studies of the reaction rate in a D_2O medium for Gly-Gly, Leu-Leu, Val-Val, Ala-Ala, and Phg-Phg, revealed that while $k'(H_2O) = 2.60 \times 10^{-4}$ s^{-1}, 7.70×10^{-4} s^{-1}, 9.50×10^{-4} s^{-1}, 11.8×10^{-4} s^{-1}, 13.4×10^{-4} s^{-1}, and $k'(D_2O) = 1.55 \times 10^{-4}$ s^{-1}, 5.08×10^{-4} s^{-1}, 5.80×10^{-4} s^{-1}, 7.10×10^{-4} s^{-1}, 9.10×10^{-4} s^{-1}, respectively. The solvent isotope effect, $k'(H_2O)/k'(D_2O)$ = 1.68, 1.51, 1.64, 1.66, and 1.47 for the five dipeptides. Proton inventory studies were made in H_2O–D_2O mixtures for all five dipeptides; the results are given in Table 3. Corresponding proton inventory plots for the rate constant (k'_n) in a solvent mixture of deuterium atoms fraction (n) are given in Fig. 2.

The reaction was studied at different temperatures (303–323 K). From Arrhenius plots of log k' versus 1/T ($r > 0.9855$), composite activation parameters were calculated. These results are summarized in Table 4. The addition of the reaction mixture to acrylamide in a nitrogen atmosphere failed to initiate polymerization of the latter, showing the absence of free-radical species.

Discussion

Because organic haloamines have similar chemical properties,[21–23] it is expected that similar equilibria exist in solutions of these compounds. Bromamine-B, which is analogous to chloramine-T and chloramine-B, behaves like a strong electro-

Table 4. Temperature Dependence and Activation Parameters for the Oxidation of Dipeptides by BAB in $HClO_4$ Medium, [BAB] = 4.0×10^{-4} mol dm^{-3}; [DPP] = 4.0×10^{-2} mol dm^{-3}; [$HClO_4$] = 4.0×10^{-2} mol dm^{-3}; I = 0.5 mol dm^{-3}

Temperature	$10^4k'/s^{-1}$				
K	Gly-Gly	Leu-Leu	Val-Val	Ala-Ala	Phg-Phg
303	0.82	2.51	3.25	4.85	5.50
308	1.41	4.30	5.33	7.25	8.72
313	2.60	7.70	9.50	11.8	13.4
318	4.62	13.4	16.1	19.0	18.9
323	8.30	24.2	26.8	27.8	27.9
E_a/kJ mol^{-1}	94.7	86.2	82.1	71.1	64.8
$\Delta H^{\neq}$/kJ mol^{-1}	92.1	83.6	79.5	68.5	62.2
$\Delta S^{\neq}$/J K^{-1} mol^{-1}	−19.8	−38.1	−49.4	−82.6	−102
$\Delta G^{\neq}$/kJ mol^{-1}	98.3	95.5	94.9	94.4	94.1

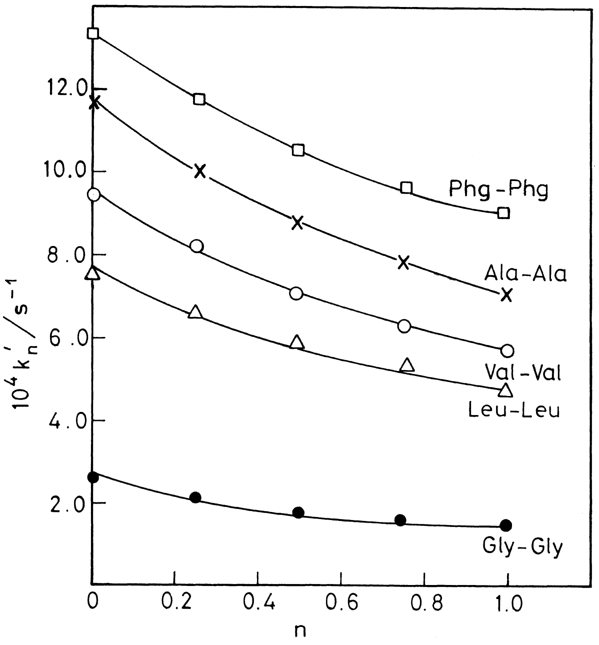


Fig. 2. Proton inventory plots for the oxidation of dipeptides by BAB in H_2O–D_2O mixtures. Experimental conditions are as in Table 3.

lyte in both acidic and alkaline media. In general, BAB undergoes a two-electron change in its reactions. The oxidation potential of BAB/$PhSO_2NH_2$ is pH dependent and decreases with an increase in the pH of the medium (1.4 V at pH 0.65 and 0.50 V at pH 12.0). In acid solutions, BAB exists in the following equilibria:

$$PhSO_2NBrNa \rightleftharpoons PhSO_2NBr^- + Na^+ \tag{2}$$

$$PhSO_2NBr^- + H^+ \xrightleftharpoons{K_a} PhSO_2NHBr, \quad K_a = 1.5 \times 10^{-5} \text{ at } 25\,°C \tag{3}$$

$$2PhSO_2NHBr \xrightleftharpoons{K_d} PhSO_2NH_2 + PhSO_2NBr_2, \quad K_d = 0.113 \text{ at } 25\,°C \tag{4}$$

$$PhSO_2NBr_2 + H_2O \xrightleftharpoons{K_h} PhSO_2NHBr + HOBr \tag{5}$$

$$PhSO_2NHBr + H_2O \xrightleftharpoons{K'_h} PhSO_2NH_2 + HOBr, \quad K'_h = 1.5 \times 10^{-5} \text{ at } 25\,°C \tag{6}$$

$$HOBr \xrightleftharpoons{K_a} H^+ + OBr^-, \; K_a = 2.0 \times 10^{-9} \text{ at } 25\,°C \tag{7}$$

$$HOBr + H^+ \rightleftharpoons H_2OBr^+ \tag{8}$$

Therefore, the probable oxidizing species in an acid solution of BAB are $PhSO_2NHBr$, $PhSO_2NBr_2$, HOBr, and H_2OBr^+. The experimental rate law for the oxidation of dipeptides by BAB in $HClO_4$ at 40 °C is found to be

$$\text{Rate} = k[\text{BAB}]\,[\text{DPP}]^x\,[H^+]^{-y}, \tag{9}$$

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

The first-order dependence of the rate on [BAB] and the addition of $PhSO_2NH_2$ having no effect on the reaction rate indicate that $PhSO_2NBr_2$ and HOBr may not be the reactive species ((4) and (6)); further these species were present in very low concentrations[21] under the experimental conditions employed. The absence of any ionic- strength effect indicates the involvement of a neutral species in the rate-limiting step (r.l.s.). Hence, the effective oxidizing species could be the conjugate acid, $PhSO_2NHBr$.

Glycylglycine exists[24] in the following equilibrium in acidic solutions:

$$\underset{\text{cation } (SH^+)}{{}^+NH_3CH_2CONHCH_2COOH} \rightleftharpoons \underset{\text{zwitterion } (S°)}{{}^+NH_3CH_2CONHCH_2COO^-} + H^+ \tag{10}$$

The same equilibria also exist for other dipeptides. The inverse dependence of the reaction on the acidity suggests that there is an equilibrium between the zwitterion (S°) and the protonated form (SH^+), and that it is the zwitterion which takes part in the reaction.

Based on the preceding discussion and experimental observations, Scheme 1 is proposed to explain the reaction mechanism for the oxidation of dipeptides by BAB in a $HClO_4$ medium:

Here, X and X′ represent the complex intermediate species,

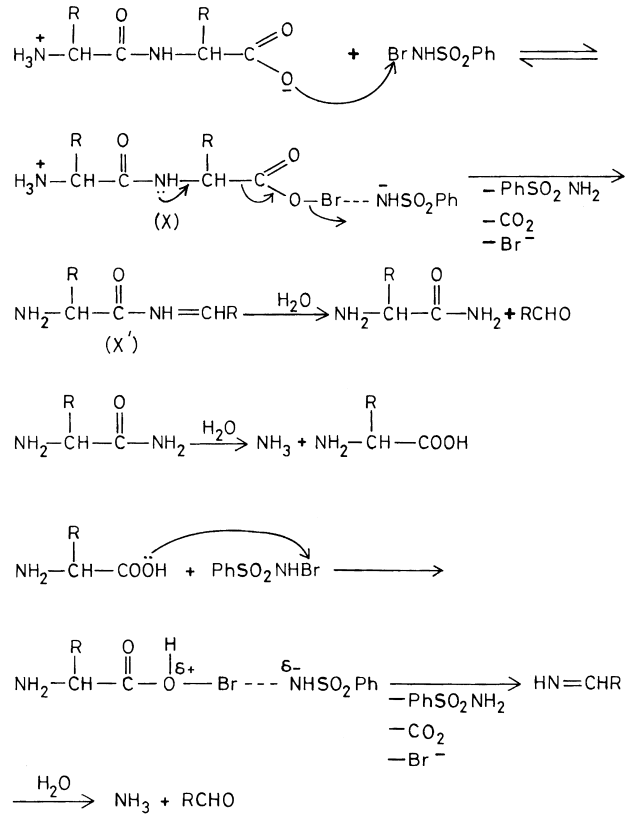
$$SH^+ \overset{K_1}{\rightleftharpoons} S^\circ + H^+ \qquad \text{(i) fast}$$

$$S^\circ + PhSO_2NHBr \overset{K_2}{\rightleftharpoons} X \qquad \text{(ii) fast}$$

$$X \xrightarrow{k_3} X' \qquad \text{(iii) slow and r.l.s}$$

$$X' + PhSO_2NHBr \xrightarrow{k_4} \text{Products} \qquad \text{(iv) fast}$$

Scheme 1.



Scheme 2.

whose structures are shown in Scheme 2, which depict the probable mode of oxidation of dipeptides by BAB in $HClO_4$ medium. Dipeptides are not expected to undergo hydrolysis before oxidation, since drastic reaction conditions ($>$ 2 mol dm^{-3} HCl and high temperature) are needed to effect the hydrolysis[25] of a dipeptide bond. Martin[26] has studied the mechanism of the hydrolysis of glycylglycine in aqueous acetic acid–perchloric acid mixtures at 120 °C. Synge[27] has used equal volumes of acetic acid and 10 mol dm^{-3} HCl to effect hydrolysis. Hence, dipeptides are oxidized rather than hydrolyzed under the conditions maintained in the present study.

If $[BAB]_t$ is the total concentration of BAB, then $[BAB]_t = [PhSO_2NHBr] + [X]$, from which

$$[X] = \frac{K_1K_2[BAB]_t[SH^+]}{[H^+] + K_1K_2[SH^+]}. \tag{11}$$

The rate of the reaction is given by

$$\frac{-d[BAB]}{dt} = k_3[X] = \frac{K_1K_2k_3[BAB]_t[SH^+]}{[H^+] + K_1K_2[SH^+]}. \tag{12}$$

Equation 12 is in the agreement with the experimental results indicating a first-order dependence of the rate on [BAB]. Fractional and inverse fractional orders in [DPP] and $[H^+]$, respectively, are observed. At higher [DPP], $[H^+] < K_1K_2[SH^+]$, so that the reaction becomes independent of [DPP], as has been observed experimentally (Table 1). In the limiting case at low $[H^+]$, the reaction is independent of $[H^+]$ and [DPP], as in evident from Eq. 12.

If k' is the observed rate constant, Eq. 12 can be rearranged as

$$\frac{1}{k'} = \frac{[H^+]}{K_1K_2k_3[SH^+]} + \frac{1}{k_3}. \tag{13}$$

Double reciprocal plots of $1/k'$ versus $1/[SH^+]$ (or 1/[DPP]) were linear ($r > 0.9804$), and from the intercept and slope of these plots the values of k_3 and K_1K_2 were calculated using Eq. 13 for each dipeptide at 313 K. Because the rate was fractional order in [DPP], Michaelis–Menten kinetics[28] were adopted to study the effect of [DPP] on the rate at different temperatures (303–323 K). The decomposition constant (k_3) for the rate-limiting step, and the K_1K_2 values were calculated using Eq. 13 for each dipeptide at different temperatures. The activation parameters for the rate-limiting step (step (iii) in Scheme 1) were evaluated using Arrhenius plots ($r > 0.9918$) of log k_3 versus 1/T. These data are presented in Tables 5 and 6.

The proposed mechanism is supported by the following experimental facts:

The effect of varying the solvent composition on the reac-

Table 5. Values of Decomposition Constant (k_3) at Different Temperatures and Activation Parameters for the Rate-Limiting Step, [BAB] = 4.0 × 10^{-4} mol dm^{-3}; $[HClO_4]$ = 4.0 × 10^{-2} mol dm^{-3}; I = 0.5 mol dm^{-3}

Temperature	$10^4\ k_3/s^{-1}$				
K	Gly-Gly	Leu-Leu	Val-Val	Ala-Ala	Phg-Phg
303	1.54	4.45	5.00	7.69	14.0
308	2.50	7.30	9.09	12.5	19.5
313	4.35	11.8	14.3	22.2	28.7
318	6.66	17.7	20.0	30.3	39.6
323	11.1	28.2	38.4	45.5	52.0
E_a/kJ mol^{-1}	76.6	79.4	73.2	69.5	52.9
$\Delta H^{\neq}$/kJ mol^{-1}	74.0	76.7	70.6	66.9	50.2
$\Delta S^{\neq}$/J K^{-1} mol^{-1}	−73.6	−56.4	−74.5	−83.4	−134
$\Delta G^{\neq}$/kJ mol^{-1}	97.0	94.4	93.9	93.0	92.1

Table 6. Values of Product of Equilibrium Constant (K_1K_2) at Different Temperatures, [BAB] $= 4.0 \times 10^{-4}$ mol dm^{-3}; [$HClO_4$] $= 4.0 \times 10^{-2}$ mol dm^{-3}; I = 0.5 mol dm^{-3}

Temperature	K_1K_2				
K	Gly-Gly	Leu-Leu	Val-Val	Ala-Ala	Phg-Phg
303	1.6	1.9	1.7	2.0	1.7
308	1.6	1.7	1.6	1.6	1.8
313	2.0	2.1	2.0	1.7	1.6
318	2.0	2.0	2.0	1.9	1.9
323	2.0	1.9	1.7	1.8	2.0

tion kinetics has been described in detail in well-known monographs on chemical kinetics. For the limiting case of zero-angle of approach between two dipoles or an ion-dipole system, Amis[29] has shown that a plot of log k' versus 1/D gives a straight line with a negative slope for a 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 a varying dielectric constant on the rate in the present cases cannot be explained by the Amis theory. Applying the Born equation, Laidler[30] has proposed the following equation for a dipole–dipole interaction:

$$\mathrm{In}k' = \mathrm{In}k_{\mathrm{o}} + \frac{3}{8kT}(2/D - 1)\left[\frac{\mu_{\mathrm{A}}^2}{r_{\mathrm{A}}^3} + \frac{\mu_{\mathrm{B}}^2}{r_{\mathrm{B}}^3} - \frac{\mu_{\neq}^2}{r_{\neq}^3}\right]. \quad (14)$$

Here, k_{o} is the rate constant in a medium of infinite dielectric constant and μ and r refer to the dipole moment and radii of reactants A and B and the activated complex. It can be seen from Eq. 14 that the rates should be higher in a medium of lower dielectric constant when $r_{\neq}^3 > r_{\mathrm{A}}^3 + r_{\mathrm{B}}^3$, indicating that the extent of charge dispersal in the transition state is different. On the other hand, $r_{\neq}^3 \approx r_{\mathrm{A}}^3 + r_{\mathrm{B}}^3$ implies the absence of a dielectric effect of the solvent on rate, as was observed in the present investigations.

The proposed mechanism is supported by a decrease in rate in D_2O medium. For acid-catalyzed reactions involving a fast pre-equilibrium proton transfer, $k'(D_2O)/k'(H_2O) > 1$, since D_3O^+ is a stronger acid[31] than H_3O^+. The reverse holds for reactions involving retardation by the H^+ ion. Hence, the proposed mechanism is also supported by the decrease in rate in D_2O medium, indicating retardation by H^+. Proton inventory studies in H_2O–D_2O mixtures could throw light on the nature of the transition state. The dependence of the rate constant (k'_n) on n, the atom fraction of deuterium in a solvent mixture of D_2O and H_2O, is given[32,33] by a form of the Gross–Butler equation, as in

$$k'_{\mathrm{o}}/k'_{\mathrm{n}} = \frac{\overset{\mathrm{TS}}{\pi}(1 - n + n\varnothing_i)}{\overset{\mathrm{RS}}{\pi}(1 - n + n\varnothing_j)}, \quad (15)$$

where $\varnothing_i$ and $\varnothing_j$ are the isotope fractionation factors for isotopically exchangeable hydrogen sites in the transition state (TS) and in the reactant state (RS), respectively. Equation 15 allows one to calculate the fractionation factor of TS if the reactant fractionation factors are known. However, the curvature of the proton inventory plot could reflect the number of exchangeable protons in the reaction.[32] Plots of k'_n versus n (Fig. 2) for all five dipeptides in the present case are curves. These in comparison[34] with the standard curves indicate the involvement of a single proton or H–D exchange in the reaction sequence. Hence, the participation of the H^+ ion in the formation of transition state is inferred.

From an inspection of the rate data, the rate of oxidation of dipeptides follows the order Phg-Phg > Ala-Ala > Val-Val > Leu-Leu > Gly-Gly. In the case of Phg-Phg, the formation of the X′ intermediate by ready decarboxylation is favoured due to resonance stabilization and extended conjugation. In the case of Ala-Ala, Val-Val and Leu-Leu, the presence of alkyl groups due to an inductive effect (+*I*) makes Ala-Ala, Val-Val and Leu-Leu react more readily with the oxidant. However, a steric interaction between the alkyl chain and the oxidant greatly affects the rate of decarboxylation; thus, the order has been found to be Ala-Ala > Val-Val > Leu-Leu. In the case of Gly-Gly, because the +*I* and resonance effects are at a minimum, it is found to be least reactive among of all the dipeptides used, and the overall reactivity of these dipeptides has been found to be Phg-Phg > Ala-Ala > Val-Val > Leu-Leu > Gly-Gly.

It can be seen from Table 4 that the activation energy is highest for the slowest reaction, and vice versa, indicating that the reaction is enthalpy-controlled. Further, the values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ can be correlated linearly ($r = 0.9912$), resulting in an isokinetic relationship. From the slope, the value of the isokinetic temperature (β) is 360 K, which is much higher than the experimental temperature, 313 K. The relationship was proved to be genuine through the Exner criterion[35] by plotting log $k'_{(313\ \mathrm{K})}$ versus log $k'_{(323\ \mathrm{K})}$,which gives a straight line ($r = 0.9950$). The value of β was calculated from the equation $\beta = T_1(1\text{–}q)/(T_1/T_2)-q$, where q is the slope of the Exner plot; β was found to be 366 K, thus proving that the reaction is enthalpy-controlled. The moderate energy of activation supports the proposed mechanism. The negative $\Delta S^{\neq}$ values point towards the formation of a more ordered activated complex, and the near constancy of $\Delta G^{\neq}$ shows the operation of similar mechanisms for the oxidation of these dipeptides by BAB.

The zero effect of halide ions on the rate indicates that no interhalogen or free bromine is formed. The variation of the ionic strength of the medium does not alter the rate, indicating that a neutral species is involved in the rate-limiting step. The reduction product of the oxidant, benzenesulfonamide, does not influence the rate, showing that it is not involved in any pre-equilibrium. These observations are also in conformity with the proposed mechanism.

It was thought worthwhile to compare the rate of oxidation of these dipeptides with the oxidation of their monomers (phenylglycine, alanine, valine, leucine, and glycine) under

Table 7. Temperature Dependence and Activation Parameters for the Oxidation of Amino Acids by BAB in $HClO_4$ medium, [BAB] = 4.0×10^{-4} mol dm^{-3}; [Amino Acid] = 4.0×10^{-2} mol dm^{-3}; [$HClO_4$] = 4.0×10^{-2} mol dm^{-3}; I = 0.5 mol dm^{-3}

Temperature	$10^3k'$/s^{-1}				
K	Glycine	Leucine	Valine	Alanine	Phenylglycine
303	1.03	3.70	4.65	6.05	8.82
308	1.62	5.30	7.35	8.25	11.1
313	2.50	8.25	10.5	12.0	15.7
318	3.95	12.2	15.9	16.1	19.4
323	6.45	18.1	21.6	23.9	23.8
E_a/kJ mol^{-1}	74.1	65.7	61.3	53.8	41.8
$\Delta H^{\neq}$/kJ mol^{-1}	71.5	63.0	58.7	51.8	39.2
$\Delta S^{\neq}$/J K^{-1} mol^{-1}	−66.9	−83.9	−95.8	−112	−155
$\Delta G^{\neq}$/kJ mol^{-1}	92.5	89.3	88.7	86.1	87.6

identical experimental conditions. The rate of oxidation of amino acids increased in the order phenylglycine > alanine > valine > leucine > glycine (Table 7), while in the case of dipeptides the order was found to be Phg-Phg > Ala-Ala > Val-Val > Leu-Leu > Gly-Gly. It was also found that the amino acids are nearly 10-times faster than the dipeptides. The change in each case can be ascribed to the increased distance between the functional groups and consequently weaker electrostatic effects. In the case of dipeptides, a lone pair on nitrogen [Scheme 2, (X)] is involved in resonance with the carbonyl group. Therefore, its nucleophilic character will decrease and, hence, the rate decreases, where as in the case of monomer amino acids there is no decrease in the nucleophilic character and, hence, the rate is much faster compared to dipeptides. The decrease in the rate for dipeptides compared to their monomers may also be due to decreased ionization of the COOH group, which is evidenced by an increase in the pK_1 values (e.g. glycine pK_1 = 2.4; Gly-Gly pK_1 = 3.4). Since the availability of the COOH group for this reaction is trivial to determine the rate, the rate concurrently decreases due to a lower concentration of the acidity, resulting in a decrease of the dissociation ability of the COOH group and, hence, a decrease in the rate of the reaction in the case of dipeptides. The same argument also holds good for other four dipeptides.

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