Kinetics of electrophilic bromine transfer from *N*-bromosuccinimide to amines and amino acids

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The kinetics of the reactions of glycine, sarcosine, 2-aminoisobutyric acid, proline and pyrrolidine with *N*-bromosuccinimide (NBS) have been studied and it has been found that the rates of formation of the *N*-bromoamino products are proportional to the NBS and amino substrate concentrations; that they decrease linearly with increasing H^+ concentration in the $-\log [H^+]$ interval 8.50 to 4.00; and that they are independent of ionic strength and the concentration and nature of the buffer. The second order reaction rate constant increases with the basicity of the amino substrate, ranging from 2.7×10^6 dm^3 mol^{-1} s^{-1} for the formation of *N*-bromoglycine to 4.74×10^7 dm^3 mol^{-1} s^{-1} for the formation of *N*-bromopyrrolidine (I = 0.1 mol dm^{-3}). The activation enthalpies measured for these reactions are small and typical of fast processes while the activation entropies are large and negative and indicative of a highly solvated transition state.

On the basis of these observations, we propose that the *N*-bromo compounds form *via* a concerted reaction in which the unprotonated amino group of the substrate attacks the NBS bromine, the overall result being 'Br^+' transfer from the nitrogen atom of NBS to the nitrogen atom of the substrate.

Introduction

N-Bromosuccinimide (NBS) is a strong oxidizing agent that has found numerous applications in both synthetic and analytical chemistry, due mainly to its high selectivity.[1] It has been used to brominate a wide variety of organic compounds, and for the oxidation of amines and amino acids.[1–4] In these latter reactions, amines are converted into the corresponding aldehydes,[1] while amino acids form an unstable *N*-bromoamino acid, which subsequently decarboxylates to give the corresponding aldehyde or nitrile, depending on the reaction conditions.

Recent kinetic studies on the oxidation reactions of amino acids with *N*-bromosuccinimide[5] and *N*-chlorosuccinimide[6] have led to the proposal of a reaction mechanism in which an acyl hypohalite ($RCHNH_2COOX$, X = Cl or Br) forms initially, and then decomposes to an aldehyde. This mechanism implies nucleophilic attack on the halogen atom by the carboxy group, which is behaviour rather different to that seen in analogous reactions in which hypochlorite or hypobromite is the halogenating agent. In these reactions, the amino nitrogen of the amine or amino acid is the reactive centre, and the *N*-halogenoamino compound is the major reaction product.[7–9] This product forms in two steps whose markedly different rates allow them to be studied independently. In the first, the bromine is transferred to the nitrogen of the amino compound in 10–100 ms[7] (indeed, in our own experiments, this reaction between glycine and hypobromite was complete within 10 ms, and was consequently difficult to follow using stopped-flow techniques); the second is a much slower decomposition reaction involving loss of a carboxy group from amino acids.[10,11]

In order to clarify whether the reaction between amino acids and NBS takes place at the amine or at the carboxylate group, and with a view to establishing definitive mechanisms for the reactions of amines and amino acids with NBS, we have carried out kinetic and spectroscopic studies of these reactions.

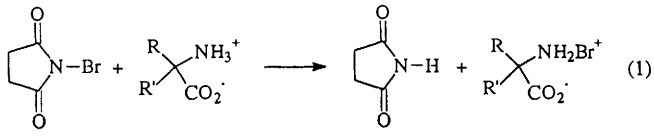
Experimental

Materials

Succinimide and *N*-bromosuccinimide (both purchased from Merck) were recrystallized;[12] pyrrolidine (Aldrich) was redistilled. Glycine (Carlo Erba), sarcosine and 2-aminoisobutyric acid (Merck) and proline (Sigma) were used as supplied. A 0.01 mol dm^{-3} NBS stock solution was freshly prepared each day (by dissolving 0.178 g of NBS in double-distilled water and making the solution up to 100 cm^3) and stored in a UV-opaque bottle. Buffers (boric acid–sodium borate, sodium dihydrogen phosphate–disodium hydrogen phosphate, acetic acid–sodium acetate and citric acid–sodium citrate), 2 mol dm^{-3} perchloric acid, 2 mol dm^{-3} sodium hydroxide and 2 mol dm^{-3} sodium perchlorate—which was used to maintain constant ionic strength (at 0.1 mol dm^{-3} unless otherwise stated)—were prepared directly from Merck or Aldrich products of the highest available purity.

Kinetic experiments

On mixing solutions of NBS and amino acid, the *N*-bromoamino acid is rapidly formed in accordance with eqn. (1).



(1)

Spectra of the reaction mixture at different times show that an intense absorption band develops at 290 nm in the course of the reaction. On the basis of published information for other *N*-bromo compounds,[7,13] this absorption band is assigned to the formation of the *N*-bromoamino acid.

Kinetic experiments were carried out in an Applied Photophysics DX.17MV Sequential Stopped-flow Spectrofluorimeter. The temperature of the solutions in the syringes and cell of the stopped-flow device was kept constant (±0.1 °C) by means of water circulating *via* a thermostatted bath.

The reaction kinetics were studied using the isolation method, the concentration of the substrate being at least ten times that of the NBS (typically 2–6 × 10^{-4} mol dm^{-3}). The observed first order rate constants (k'_{obs}) were obtained by non-linear regression of the absorbance–time data using a program supplied by Applied Photophysics, and are the mean values for 6–10 kinetic runs. The treatment of errors used

Table 1 Influence of acid concentration on the rate constant for formation of *N*-bromoglycine; [NBS] = 4×10^{-4} mol dm^{-3}, [Gly] = 4×10^{-3} mol dm^{-3}, I = 0.1 mol dm^{-3} ($NaClO_4$), T = 298 K

$-\log [H^+]$	k_{obs}/s^{-1}	$-\log [H^+]$	k_{obs}/s^{-1}
Boric acid–borate		Dihydrogen phosphate–hydrogen phosphate	
8.57	1900 ± 200	7.97	440 ± 10
8.44	1030 ± 70	7.65	146 ± 2
8.38	980 ± 60	7.25	50.2 ± 0.6
8.16	690 ± 40	7.07	33.8 ± 0.3
8.14	510 ± 20	6.82	19.7 ± 0.5
		6.48	9.7 ± 0.2
		6.17	5.7 ± 0.1
Acetic acid–acetate		Citric acid–citrate	
5.75	2.96 ± 0.04	4.04	0.071 ± 0.001
5.62	2.03 ± 0.04	4.22	0.097 ± 0.002
5.19	0.82 ± 0.01	4.66	0.246 ± 0.001
4.55	0.190 ± 0.003	5.20	0.778 ± 0.005
4.21	0.086 ± 0.005	6.09	4.92 ± 0.03
		5.03	0.553 ± 0.006

standard statistical procedures.[14] Observed reaction rate constants, k'_{obs}, were corrected using eqn. (2) to take mixing

$$k_{obs} = (1/k'_{obs} - 1/k_{mix})^{-1} \quad (2)$$

into account,[15] where k_{mix} is the first order mixing rate constant, which for the stopped-flow device used in this study is 1700 s^{-1}. Corrections for rate constants with values less than 100 s^{-1} are small (< 5%) but, for higher values, this systematic error significantly exceeds the random uncertainty of a single determination, and so correction is essential.

pH was measured using a Crison pH meter equipped with an Ingold combined glass electrode. Actual $-\log [H^+]$ values were calculated using an empirical equation obtained by monitoring electrode response during a titration between standard solutions of NaOH and $HClO_4$ at 0.1 mol dm^{-3} ionic strength and 25 °C.

Results and discussion

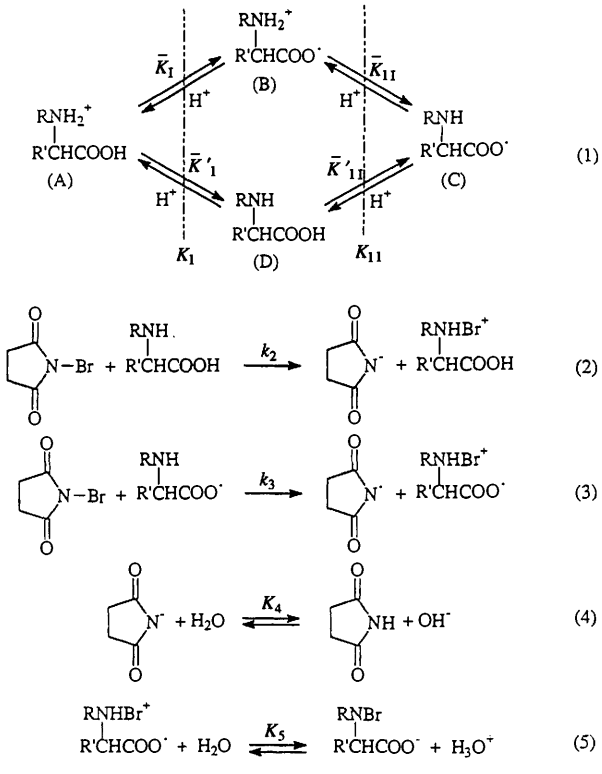
The rate of formation of *N*-bromoglycine at constant ionic strength (0.1 mol dm^{-3}) was studied in the four buffer solutions ($-\log [H^+]$ 4.0–8.5). The values of k_{obs} obtained decreased with $-\log [H^+]$ (Table 1). The effect of the buffer concentration on the reaction rate was studied using acetic acid–acetate buffer solutions of different concentrations. The rate constant did not vary with the buffer concentration, and was also found to be independent of the nature of the buffer.

At $-\log [H^+]$ values below 4.00, decomposition of the *N*-bromoamino acid competes effectively with its formation, which hampered independent study of the two processes.

The plot of $\log k_{obs}$ against $-\log [H^+]$ is a straight line (Fig. 1) with a slope of 0.96 ± 0.01 (r = 0.9983). Thus, the observed rate constant is linearly related to the reciprocal acid concentration, and the rate law has order −1 with respect to acid concentration. To explain this behaviour we propose a mechanism in which the amino acid species that reacts with the NBS is not protonated at the amino group (Scheme 1). Since the only reactive species being considered are (C) and (D), as seen in Scheme 1, the rate equation for the reaction is given by eqn. (3).

$$v = k_2[D][NBS] + k_3[C][NBS] \quad (3)$$

The protonation equilibria of the amino acid, where K_I and K_{II} are the macroscopic constants for the loss of the first and second protons, respectively, are shown in step (1) of Scheme 1. The values of the microscopic constants are not known, but can



Scheme 1

be estimated from K_I and K_{II} by assuming the microscopic constant $\bar{K}_{I'}$ to be equal to the acidity constant (K_e) for the corresponding amino acid ester.

The concentrations of (C) and (D) can thus be expressed as functions of the acidity and the total concentration of the amino acid [AA], obtaining $[C] = K_IK_{II}[AA]/([H^+]^2 + K_I[H^+] + K_IK_{II})$ and $[D] = K_e[AA][H^+]/([H^+]^2 + K_I[H^+] + K_IK_{II})$. Since, under the conditions used $K_I \gg [H^+]$ and $[H^+] \gg K_{II}$, these expressions simplify to $[C] = K_{II}[AA]/[H^+]$ and $[D] = K_e[AA]/K_I$, which when substituted into eqn. (3) give eqn. (4). The observed rate constant should therefore be given by eqn. (5), which represents a linear relationship between k_{obs} and the

$$v = (k_2K_e[AA]/K_I + k_3K_{II}[AA]/[H^+])[NBS] \quad (4)$$

$$k_{obs} = k_2K_e[AA]/K_I + k_3K_{II}[AA]/[H^+] \quad (5)$$

reciprocal acid concentration, with slope $k_3K_{II}[AA]$, and y-intercept $k_2K_e[AA]/K_1$. Plotting k_{obs} against $1/[H^+]$ for the data in Table 1 gave a straight line without a significant intercept, thus indicating that the term containing k_2 is much smaller than the one containing k_3, and that the reaction takes place mainly through species (C). The expression for observed rate constant therefore simplifies to eqn. (6) according to which

$$k_{obs} = k_3K_{II}[AA]/[H^+] \quad (6)$$

the reaction is first order with respect to the amino acid. Experiments examining the influence of amino acid concentration on the rates of formation of *N*-bromoglycine and also other *N*-bromoamino acids and *N*-bromopyrrolidine (which were studied in order to compare substituent effects), confirmed this to be the case: for all these compounds, straight lines with approximately unit slope were obtained when log ($k_{obs}[H^+]$) was regressed on log [AA]. For glycine, for example, this plot for the data in Table 2 had slope 0.9 ± 0.1 (r = 0.9895), and the corresponding plot of $k_{obs}[H^+]$ against [Gly] had slope (7.5 ± 0.6) × 10^4 (r = 0.9904), from which k_3 for the reaction between glycine and NBS was calculated to be (2.7 ± 0.2) × 10^6 dm^3 mol^{-1} s^{-1} using the value of K_{II} at 0.1 mol dm^{-3} ionic strength (Table 3).[16]

The influence of the ionic strength on the observed rate constant was studied by varying the concentration of $NaClO_4$,

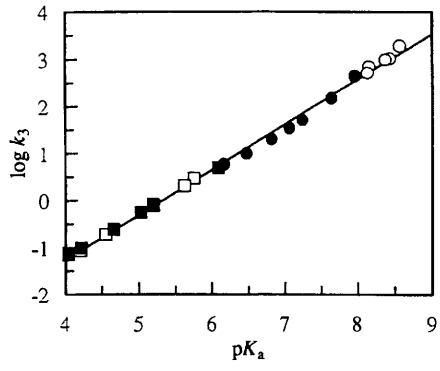


Fig. 1 Logarithmic plot showing the influence of acid concentration on the rate constant for formation of *N*-Br-glycine. I = 0.1 mol dm^{-3} ($NaClO_4$), T = 298 K. Buffer solutions: ○, boric acid–borate; ●, dihydrogen phosphate–hydrogen phosphate; □, acetic acid–acetate and ■, citric acid–citrate.

Table 2 Influence of glycine concentration on the reaction rate; [NBS] = 2 × 10^{-4} mol dm^{-3}, I = 0.1 mol dm^{-3} ($NaClO_4$), T = 298 K

−log [H$^+$]	[Gly]/mol dm^{-3}	k_{obs}/s^{-1}
7.29	0.002	40 ± 0.6
7.32	0.005	78 ± 1
7.31	0.010	139 ± 5
7.36	0.015	290 ± 10
7.29	0.020	290 ± 20

with all the other reaction conditions kept constant. The reaction rate was found to be independent of ionic strength in the range 0.02–0.50 mol dm^{-3}, thus indicating that at least one of the species involved in the rate-controlling step is molecular.

The influence of succinimide concentration on the reaction rate was studied at −log [H$^+$] = 7.22 (phosphate buffer) and constant ionic strength (I = 0.1 mol dm^{-3}). A tenfold increase in the succinimide concentration (0.002–0.02 mol dm^{-3}) had no effect on the reaction rate. This observation, coupled with the fact that the reaction of glycine with hypobromous acid is much faster than its reaction with NBS, rules out a mechanism in which the effective brominating agent is hypobromous acid derived from NBS hydrolysis; it also indicates that the rate limiting step of *N*-bromoglycine formation is irreversible.

Varying the the initial NBS concentration had no effect on the observed rate constant. The absorbance at t = ∞ obtained in these experiments was used to calculate the molar absorptivity of the *N*-bromoglycine product at 290 nm, giving a value of 372 ± 6 dm^3 mol^{-1} cm^{-1}, which is in good agreement with the values reported for similar *N*-bromoamino compounds.[7,11] Moreover this band is also present in the spectra of pyrrolidine (an amine without a carboxy group) and NBS mixtures, while the spectra of organic acyl hypobromites[17,18] are rather different from the spectra obtained in our experiments. There is therefore no spectrophotometric evidence for the formation of an acyl hypobromite intermediate.

The results of experiments examining the influence of the reaction temperature on the reaction rate are shown in Table 4. By plotting ln (k_3K_{II}/T) against $1/T$, the overall activation enthalpy and entropy of reaction ($\Delta H^{\ddagger}_{reac}$ and $\Delta S^{\ddagger}_{reac}$ respectively) were obtained, in accordance with eqn. (7). Since

$$\ln(k_3K_{II}/T) = \ln k_B/h + \Delta S^{\ddagger}_{reac}/R - \Delta H^{\ddagger}_{reac}/RT \quad (7)$$

$\Delta S^{\ddagger}_{reac} = \Delta S^{\circ}_{II} + \Delta S^{\ddagger}_3$ and $\Delta H^{\ddagger}_{reac} = \Delta H^{\circ}_{II} + \Delta H^{\ddagger}_3$, and values of ΔS°_{II} and ΔH°_{II} (corresponding to the protonation equilibrium of the amino group) are tabulated in the literature[16] the activation parameters ($\Delta S^{\ddagger}_3$ and $\Delta H^{\ddagger}_3$) for the bromine transfer process [reaction(3) of Scheme 1] can also be obtained (Table 5).

On the basis of the results obtained in the experimental section we propose the mechanism shown in Scheme 1 for the reaction of NBS with amino acids or amines. Specifically, a

Table 4 Influence of temperature on the rate of formation of the studied *N*-bromo compounds

	$k_3K_{II}/10^{-4}$s^{-1}			
T/K	Gly	Sar	Aib	Pro
288	2.78 ± 0.03	1.58 ± 0.01	3.78 ± 0.02	2.20 ± 0.02
293	4.21 ± 0.03	2.39 ± 0.02	6.00 ± 0.03	3.35 ± 0.01
298	6.87 ± 0.08	3.81 ± 0.03	9.40 ± 0.02	5.24 ± 0.04
303	11.1 ± 0.2	6.49 ± 0.07	15.6 ± 0.2	7.64 ± 0.07
308	18.1 ± 0.4	10.4 ± 0.1	25.0 ± 0.5	12.6 ± 0.1

Table 3 Values of pK_{II} and k_3 for the amine and amino acids studied

Amino acid (AA)	pK_{II} (25 °C, I = 0.1 mol dm^{-3})[a]	k_3/dm^3 mol^{-1} s^{-1}
Glycine (Gly)	9.56	2.7 × 10^6
Sarcosine (Sar)	9.97	3.56 × 10^6
2-Aminoisobutyric acid (Aib)	10.10	1.18 × 10^7
Proline (Pro)	10.47	1.55 × 10^7
Pyrrolidine (Pyr)	11.20	4.74 × 10^7

[a] pK_a values from ref. 16.

Table 5 Activation parameters for the key steps shown in Scheme 1 [a]

AA	$\Delta S^{\ddagger}_{reac}$	$\Delta H^{\ddagger}_{reac}$	ΔS°_{II}	ΔH°_{II}	$\Delta S^{\ddagger}_{3}$	$\Delta H^{\ddagger}_{3}$	$\Delta G^{\ddagger}_{3}$
Gly	−80.3 ± 0.8	67 ± 1	−38.5	44.4	−42	23	36
Sar	−83 ± 7	68 ± 1	−57.7	41.0	−25	27	34
Aib	−77 ± 5	67 ± 1	−35.1	47.7	−42	19	32
Pro	−103 ± 6	62 ± 2	−58.6	43.3	−44	19	32

[a] Values of ΔS in J mol^{-1} K^{-1} and values of ΔH and ΔG in kJ mol^{-1}.

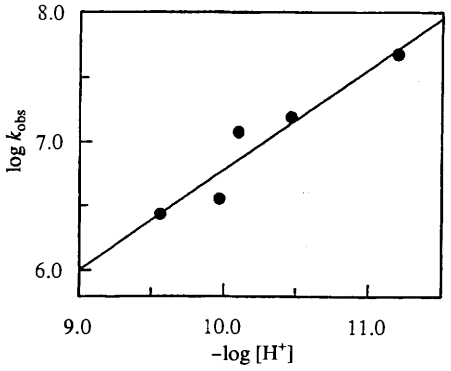
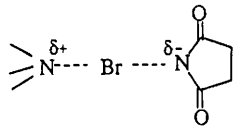


Fig. 2 Brønsted-type plot for 'Br^+' transfer between NBS and the amino compounds studied

nucleophilic attack on the bromine atom of NBS by the nitrogen atom of the amino group is proposed which results in 'Br^+' transfer between the two nitrogen atoms. This reaction can thus be considered to be a halogenophilic or X-philic reaction,[19] in which nucleophilic substitution takes place at a bromine atom. The overall process can be represented by the general equation, eqn. (8), where X is, *e.g.* NO,[20] Cl [8,9] or Br,[7]

$$Z^- + X - Y \longrightarrow Z - X + Y^- \qquad (8)$$

Z^- is a nucleophile and Y^- is a leaving group. In our particular case, the reaction can be considered to be a concerted nucleophilic displacement of the Y^- group from the halogenating agent by the nitrogenous compound. Since the reaction takes place between the unprotonated amino group of the substrate and a neutral molecule of NBS, a separation of charges must occur in the transition state. Although from this study we cannot predict the degree of bromine transfer in the transition state, the high and negative values of the entropy of activation ($\Delta S^{\ddagger}_{3}$) suggest that the transition state is highly solvated,[20,21] which may be because charge separation is taking place.



Suggested transition state

The magnitudes of the rate constants, k_3 (Table 3), indicate that these reactions are not diffusion controlled. Thus the rates of reaction of NBS with a series of nitrogenous compounds should be directly related to their nucleophilicities. Since nitrogen nucleophilicity toward 'X^+' can be expected to parallel nitrogen basicity, a linear free energy relationship of the form in eqn. (9) may be used to correlate the rates of *N*-bromination

$$\log k_3 = \log k_0 + pK_a \qquad (9)$$

(k_3) with the basicities of the nitrogenous substrates (K_{II}). In the plot of eqn. (9) in Fig. 2, which is analogous to a Brønsted plot, log k_3 increases linearly with the pK_a of the amino group, the slope of this straight line (β) being 0.8 ± 0.1 (r = 0.954). The fact that the result for pyrrolidine, which lacks a carboxy group, also lies on this line provides further evidence against a mechanism involving an acyl hypobromite intermediate. Moreover, analogous positive correlations between the reaction rate and the basicity and the amino group have been observed for *N*-halogenation of amines or amino acids by hypobromite [7] or hypochlorite,[9] which are considered to proceed *via* a mechanism involving direct transfer of the halogen from the hypohalite-ion oxygen to the amino group.

Snyder and Margerum [21] have observed that a similar linear relationship between substrate nucleophilicity and the rate constant for chlorine transfer from chloramine to amines, amino acids and peptides approaches a limiting value for amino compounds more basic than ammonia. Since this limit was ten times lower than the diffusion control limit, these authors suggested that nucleophilic attack on the chlorine by the amino group led to an intermediate which decomposes to the *N*-chloro product at a rate independent of amine nucleophilicity. The fact that no such limiting value of k_3 was observed in this work further supports a mechanism involving direct transfer of the 'Br^+' from NBS to the amino group of the substrate.

As can be seen from the above reactions in which the rate limiting step is nucleophilic attack, a linear relationship exits between the logarithm of the reaction rate and the pK_a of the amino group. However, these studies have covered only a narrow pK_a interval. In a recent study examining nitrosation of a series of nucleophilic nitrogen compounds covering 8 pK_a units, it was concluded that the basicity of nitrogen nucleophiles is not a good measure of their reactivity, especially when the nucleophiles being compared are structurally very different.[20]

Conclusions

Acyl hypobromite intermediates were not formed, even in small amounts, during bromine transfer from NBS to the amino acids studied, since there was no spectrophotometric evidence in support of their existence, and no evidence of any catalytic effect due to the carboxy group when the rates of *N*-bromination of amino acids were compared with that of pyrrolidine. Moreover, the fact that (*i*) the reaction of glycine with NBS was slower than its reaction with hypobromous acid; (*ii*) there is a linear relationship between the logarithm of the rate of formation of the *N*-bromo products and the pK_a (albeit over a narrow range) of the amino substrates and (*iii*) this relationship did not reach a limiting value below the diffusion control limit, are all in keeping with a mechanism in which the unprotonated amino group of the substrate attacks the NBS bromine in a concerted reaction, the overall result being 'Br^+' transfer from the nitrogen atom of NBS to the nitrogen atom of the substrate.

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