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Kinetics and mechanism of oxidation of some α-amino acids by pyridinium hydrobromide perbromide

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Oxidation of nine α-amino acids by pyridinium hydrobromide perbromide (PHPB) in aqueous acetic acid leads to the formation of the corresponding aldehydes. The reaction is first order with respect to PHPB. Michaelis-Menten type kinetics are observed with respect to some of the amino acids while other amino acids exhibit a second order dependence. The oxidation of perdeuterioglycine showed the absence of a kinetic isotope effect. The effect of solvent composition indicates that the reaction rate increases with an increase in the polarity of the medium. Addition of pyridinium hydrobromide and bromide ion have no effect on the rate of oxidation. The reaction is susceptible to both polar and steric effects of the substituents. It failed to induce polymerization of acrylonitrile. Suitable mechanisms have been proposed.

Pyridinium hydrobromide perbromide (PHPB) has been extensively used in organic synthesis as a mild and selective brominating agent[1,2] and as a dehydrogenating agent[3], but kinetic and mechanistic aspects[4,5] of its reactions have not been studied much. A study of the kinetics of oxidation of α-amino acids is important both from the mechanistic point of view and its bearing on the mechanism of amino acid metabolism. In this article we report the kinetics of oxidation of glycine (Gly), α-alanine (Ala), valine (Val), leucine (Leu), phenylalanine (Phe), isoleucine (Ile), 2-aminobutanoic acid (ABA), norleucine (NLE) and norvaline (NVA) by PHPB in aqueous acetic acid (50%, v/v).

Materials and Methods

All the amino acids were commercial products of highest degree of purity and were used as such. Perdeuterioglycine (ND_2CD_2COOD) was obtained from Sigma Chemicals (USA). PHPB was prepared by the reported method[6] and its purity checked by iodometric method.

The main products of the oxidation of amino acids were the corresponding carbonyl compounds and ammonia. The presence of ammonium ions in the reaction mixture was detected by the test with *p*-nitrobenzendiazonium chloride[7].

In a typical experiment, α-alanine (4.45 g, 0.05 mol) and PHPB (3.2 g, 0.01 mol) were made upto 100 ml in 50% (v/v) acetic acid-water. The mixture was allowed to stand for *ca.* 12 h in the dark to ensure completion of the reaction. It was then treated with an excess (250 ml) of saturated solution of 2,4-dinitrophenylhydrazine in 2 mol dm^{-3} HCl and kept in a refrigerator for *ca.* 10 h. The precipitated 2,4-dinitrophenylhyrazone (DNP) was filtered off, dried, weighed, recrystallized from ethanol and weighed again. The product was identical (mp and mixed mp) with an authentic sample of DNP of acetaldehyde. The yields of DNP before and after recrystallization wre 2.00 g (94%) and 1.85 g (87%), respectively. In similar experiments with other amino acids the yields of the DNP of the corresponding carbonyl compounds after recrystallization were 80-94%. The overall reaction may be represented by Eq.(1).

$$RCH(NH_2)COOH + PyHBr_3 + H_2O \rightarrow RCHO + PyHBr + 2HBr + NH_3 + CO_2 \quad \ldots(1)$$

Stoichiometry, with an excess of the reductant could not be studied because of the difficulty in measuring [reductant]. Stoichiometric determination under excess of PHPB (× 5 or greater than [aminoacid] in 1:1 (v/v) acetic acid-water mixture) showed that 2 mol of PHPB were consumed for 1 mol of the amino acid. This was because aldehydes, the initial products of the oxidation, were further oxidized to carboxylic acids.

Kinetic Measurements

Reaction's were carried out under pseudo-first order conditions by keeping a large excess (15-fold or greater) of the amino acid over PHPB. The reactions were carried out at a constant temperature (±0.1 K) and in flasks blackened from the outside to prevent any photochemical reaction. The reactions were

Table 1—Rate constants for the oxidation of α-alanine and leucine by PHPB at 308 K

10^3[PHPB] (mol dm^{-3})	[Alanine] (mol dm^{-3})	[Leucine] (mol dm^{-3})	$10^3 k_{obs}$ (s^{-1})	
			Ala	Leu
1.0	0.45	0.28	13.1	5.59
2.0	0.45	0.28	13.5	6.20
3.0	0.45	0.28	12.9	5.55
4.0	0.45	0.28	13.0	5.39
6.0	0.06	0.06	0.86	0.26
6.0	0.12	0.09	3.00	0.58
6.0	0.23	0.13	7.60	1.20
6.0	0.30	0.18	9.82	2.25
6.0	0.45	0.22	13.0	3.41
6.0	0.60	0.24	14.5	4.00
6.0	0.75	0.28	15.5	5.58

followed by monitoring the decrease in [PHPB] at 358 nm upto *ca.* 80% reaction. The solvent was 1:1 (v/v) acetic acid-water unless mentioned otherwise. The pseudo-first order rate constant, k_{obs}, was computed from the linear ($r > 0.990$) least squares plot of log [PHPB] versus time. Duplicate kinetic runs showed that the rates were reproducible to within ±3%.

Results

The rate laws and other experimental data were obtained for all the nine amino acids studied. Since the results are similar, only representative data are reported here.

The reaction is of first order with respect to PHPB. The order of reaction with respect to amino acid is more than one but less than two in the case of gly, ala, ABA, NLE, NVA and phe whereas the order with respect to Val, Leu and Ile is two. The rate constants of the oxidation of Ala and Leu are recorded in (Table 1). Plots of $1/k_{obs}$ for Gly, Ala, ABA, NLE, NVA and Phe against 1/[Amino acid]^2 is linear ($r > 0.990$) with an intercept on the rate ordinate. Thus Michaelis-Menten type kinetics are observed with respect to these amino acids. This leads to the postulation of the following overall mechanism (Eqs 2 and 3) and rate law (Eq.4).

$$2 \text{ Amino acid} + \text{PHPB} \overset{K}{\rightleftharpoons} [\text{Complex}] \quad \ldots (2)$$

$$[\text{Complex}] \xrightarrow{k_2} \text{Products} \quad \ldots (3)$$

$$\text{Rate} = k_2 K [\text{Amino acid}]^2 (\text{PHPB}]/ (1 + K [\text{Amino acid}]^2) \quad \ldots (4)$$

It is proposed that the oxidation of valine, leucine and isoleucine also follow similar mechanism. It is suggested that in their case, equilibrium constants have very small values. Thus in these cases K[amino acid]^2 << 1 and no Michaelis - Menten type kinetics are observed but a second order dependence on the reductant is obtained. The variation in [substrate] was studied at different temperatures and the values of K and k_2 were evaluated from the double reciprocal plots. The thermodynamic parameters of the complex formation and activation parameters of the decomposition of the complexes were calculated from the values of K and k_2 respectively at different temperatures (Tables 2 and 3). The addition of pyridinium bromide or bromide ion has no effect on the rate.

To ascertain the importance of the cleavage of the α-C-H bond in the rate determining step, the oxidation of perdeuterioglycine was studied. Under the conditions [PHPB] = 0.006 mol dm^{-3} [Gly] = 0.2 mol dm^{-3} and T = 318 K, k_{obs} (H)/k_{obs} (D) = 1.05 showed the absence of kinetic isotope effect.

The rates of oxidation of amino acids were determined in solvents containing different amounts of water and acetic acid. The value of k_{obs} decreased as the acetic acid content of the solvent increased. For example at 308 K when [PHPB] = 0.006 mol dm^{-3} and [Ala] = 0.6 mol dm^{-3}, $10^3 k_{obs}$ decreased from 47.0 to 5.33 when % ACOH was increased from 20 to 70. In other words the rate increased with an increase in the polarity of the solvent.

The oxidation of the amino acids by PHPB in the atmosphere of nitrogen failed to induce polymerisation of acrylonitrile. In control experiments, with the amino acid being absent, acrylonitrile was not oxidized by PHPB. Thus a free radical mechanism is unlikely.

Discussion

Activation enthalpies and entropies of the oxidation of the amino acids are linearly related (r^2 = 0.9972). The isokinetic temperature computed from $\Delta H^{\ddagger}$ versus $\Delta S^{\ddagger}$ plot is 389 ± 3 K. The correlation was tested and found genuine (r^2 = 0.9994) by applying Exner's criterion[9]. The value of isokinetic temperature calculated by Exner's method is 387 ± 2 K. The linear isokinetic relation indicates that all the amino acids are oxidized by the same mechanism and the changes in the rate are governed by changes in both enthalpy and entropy of activation.

In solutions, PHPB may undergo reactions (5) and (6).

$$PyH^+Br_3^- \rightleftharpoons Br_2 + PyH^+Br^- \quad \ldots (5)$$

$$PyH^+Br_3^- \rightleftharpoons Br_3^- + PyH^+ \quad \ldots (6)$$

Table 2—Formation constants and thermodynamic parameters of amino acid-PHPB complex

Subst.	K (dm^3 mol^{-1})			$\triangle H$ (kJmol^{-1})	$\triangle S$ (Jmol^{-1}K^{-1})	$\triangle G$ (kJmol^{-1})
	308 K	318 K	328 K			
H	9.63	5.00	2.60	−57.4±0.7	−160±2.3	−9.9±0.6
Me	14.6	7.06	3.50	−62.4±0.5	−173±1.6	−11.1±0.4
$PhCH_2$	6.22	3.16	1.75	−55.7±0.8	−158±2.6	−8.7±0.7
ABA	14.3	7.20	3.83	−57.8±0.4	−158±1.3	−10.9±0.3
NLE	13.8	6.40	3.10	−65.2±0.4	−182±1.1	−11.0±0.3
NVA	13.2	6.00	3.18	−61.0±1.3	−169±3.9	−10.8±1.0

Table 3—Rates of decomposition of the complexes and the activation parameters

Subst.	$10^3 k_2$ (s^{-1})			$\triangle H^{\ddagger}$ (kJmol^{-1})	$\triangle S^{\ddagger}$ (Jmol^{-1}K^{-1})	$\triangle G^{\ddagger}$ (kJmol^{-1})
	308 K	318 K	328 K			
H	1.71	3.59	7.78	61.1±1.3	−101±4.2	90.9±1.0
Me	17.3	26.3	39.7	31.9±0.5	−176±1.5	84.2±0.4
$PhCH_2$	20.0	30.0	46.1	32.4±0.8	−173±2.6	83.9±0.6
Et	23.0	33.0	48.0	28.4±0.6	−185±2.0	83.4±0.5
Bu	35.3	48.0	66.0	23.8±0.5	−197±1.7	82.2±0.4
Pr	33.5	45.5	63.5	24.3±0.8	−195±2.5	82.3±0.6
			$10^3 k_2$ (dm^{6}mol^{-2}s^{-1})			
Pr^i	43.5	56.4	74.4	20.0±0.6	−207±1.8	81.5±0.4
Bu^i	70.0	85.0	108	15.7±0.9	−217±3.0	80.2±0.7
Bu^s	103	117	139	10.1±0.9	−232±2.8	79.1±0.7

The probable oxidizing species in a solution of PHPB are PHPBs itself, tribromide ion or molecular bromine. However, strict first order dependence on PHPB and the absence of any effect of pyridinium bromide rule out both bromine and tribromide ion as the reactive oxidizing species. Hence PHPB itself must be the reactive oxidizing species in the reaction.

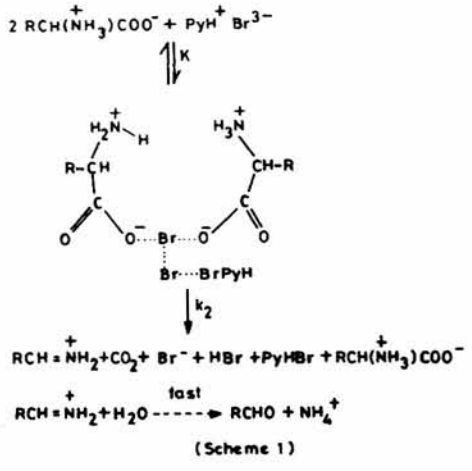
The absence of a kinetic isotope effect indicates that the α-C-H bond is not cleaved in the rate determining step.

In aqueous solutions, amino acids are known to exist in the zwitter-ionic form (Eq.7).

$$RCH(NH_2)COOH \rightleftharpoons RCH(\overset{+}{N}H_3)COO^- \quad \ldots (7)$$

The formation of the zwitter ions is facilitated by the increased polarity of the solvent due to better solvation of the ionic species. Therefore, the fact that the rate increses with an increase in the polarity of solution suggests that the zwitter ionic form is the reactive species.

From rate law (Eq.4), it is apparent that a 1:2 complex of PHPB and amino acid is formed in the pre-equilibria, which then decomposes in the slow step to give products. With the present data, it is not possible to state definitely the nature of the intermediate complex. Heasley *et al.*[10] have postulated the formation of an intermediate π-complex in the reaction of alkenes with PHPB. A similar complex may be formed by the interaction of non-bonded pairs of electrons of the carboxylic oxygen and PHPB. The formation of a moderately stable intermediate is supported by the observed values of thermodynamic parameters (Table 3). The complex formation is favoured by the enthalpy term but there is loss of entropy indicating the formation of a rigid structure. The following mechanism accounts for all the observed data (Scheme 1).



(Scheme 1)

The observed negative entropy of activation also supports a polar transition state. As the charge separation takes place, the two charged ends become highly solvated. This results in an immobilization of a large number of solvent molecules, reflected in the loss of entropy.

Correlation of structure and reactivity

The formation constant, K, of the PHPB-amino acid complexes do not vary much with the nature of the substituents. Similar observations have earlier been recorded in the oxidation of alcohols by PHPB[5]. However, the rate constant of decomposition of the complex or the rate constant of the reaction, as the case may be, showed a wide variation. These values were, therefore, subjected to correlation analysis.

Initially the rate constants were analysed in terms of Taft's polar and steric effects separately[11]. The results are given in Eqs 8 and 9.

$$\log k_2 = \underset{(\pm 0.34)}{-2.03\ \sigma^* - 1.63} \quad \ldots(8)$$

$$r^2 = 0.8358;\ n = 9;\ sd = 0.22$$

$$\log k_2 = \underset{(\pm 0.05)}{-0.74\ E_s - 1.79} \quad \ldots(9)$$

$$r^2 = 0.9681;\ n = 9;\ sd = 0.097$$

In view of the unsatisfactory correlations obtained with single substituent-parameter equations, the rate constants were analysed in terms of Pavelich-Taft's[12] dual substituent-parameter equation (10)

$$\log k_2 = \rho^* \sigma^* + \delta E_s + \log k_o \quad \ldots 10$$

The correlations are excellent; the reaction constants being negative (Table 4). The correlations showed that the reaction is susceptible to both polar and steric effects of the substituents. The values of reaction constants support the proposed mechanism. The negative polar reaction constant is in accordance with the net flow of electrons towards the oxidant. An increase in the electron density at the reaction centre facilitates the flow of electron from the substrate towards the oxidant. The negative steric reaction constant points to a steric acceleration. This may be due to the high ground state energy of the amino acids with bulky groups. As the steric crowding is relieved in the product $(R\text{-}CH{=}NH_2)^+$ and as well as in the transition state leading to it, there is not much difference in the transition state energy of the crowded and uncroweded molecules. The steric acceleration, therefore, results.

Table 4—Temperature dependence of the reaction constants of the oxidation of the amino acids by PHPB

Temp.(K)	ρ^*	δ	r^2	sd
308	-0.71 ± 0.01	-0.54 ± 0.01	0.9999	0.004
318	-0.58 ± 0.01	-0.58 ± 0.01	0.9998	0.003
328	-0.44 ± 0.01	-0.39 ± 0.01	0.9998	0.005

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