Asian Journal of Chemistry Vol. 19, No. 1 (2007), 510–516

Oxidation of Histidine by Pyridinium Bromochromate in Aquo-Acetic Acid Medium: A Kinetic Investigation

B.L. HIRAN*, R.K. MALKANI and SAPANA JAIN
Chemical Kinetics Lab., Department of Chemistry, College of Science,
M.L. Sukhadia University, Udaipur-313 001, India
Fax: (91)(294)2471150; E-mail: hiranbl@rediffmail.com

Amino acids do not react with CrO_3 and $K_2Cr_2O_7$ but react with pyridinium bromochromate. Oxidation of histidine with pyridinium bromochromate (PBC) has been studied in 30% acetic acid-70% water (v/v) mixture containing perchloric acid and leads to 2-imidazole aldehyde, carbon dioxide and ammonia as products. The reaction rate is first order with respect to each in [histidine] and [PBC] while inverse second order with respect to $[H^+]$. Michaelis-Menten type kinetics is observed with respect to histidine. The rate of reaction increases with decrease in polarity of solvent indicating ion-dipole interaction. Absence of primary kinetic isotope effect $(k_H/k_D) = 1.12$ at 303 K) rules out the α-C—H bond fission in the rate determining step. Activation parameters have been evaluated. A rate limiting C—C bond fission of the chromic-ester formed by zwitter ion of histidine and unprotonated PBC is consistent with the observed results. The rate law proposed and derived is in agreement with experimental results.

Key Words: Oxidation kinetics, Histidine, Pyridinium bromochromate, Acetic acid-water medium.

INTRODUCTION

Pyridinium bromochromate (PBC) has been reported as a mild and selective oxidizing reagent in synthetic chemistry[1]. Oxidation by PBC and other halochromates has been reviewed by Mahanti *et al.*[2] Oxidation of amino acids is of great importance both from a chemical point of view and its bearing on the mechanism of amino acid metabolism. Amino acids find a number of applications in biochemical research, metabolism, microbiology, nutrition, pharmaceuticals and fortification of foods and feeds. Histidine is a semi-essential amino acid and it has unique role in oxygen binding in haem (myoglobin). There is no report so far on oxidation of amino acids by CrO_3 and $K_2Cr_2O_7$. Experiments carried out in our lab confirmed no reaction with CrO_3 and $K_2Cr_2O_7$ at any acidity and temperature. Surprisingly, amino acids react with pyridinium bromochromate (PBC)[3–5] and quinolinium dichromate (QDC)[6–8]. Preliminary experiments in our laboratory on oxidation of histidine by PBC revealed contradictory results to the oxidation of methionine[3] and glycine[4] by PBC and oxidation of amino acids by quinolinium dichromate[6–8]. In view of this, we have taken up systematic kinetic study of the oxidation of histidine by PBC. The present investigation reports the results on the kinetics of the oxidation of histidine by PBC.

EXPERIMENTAL

BDH 'AnalaR' histidine was used. Purity was checked by m.p. Acetic acid was purified by refluxing over CrO_3, followed by fractional distillation in presence of acetic anhydride. Pyridinium bromochromate (PBC) was prepared by reported method[1]. The stock solution of PBC was prepared by dissolving known quantity of PBC in purified acetic acid and was standardized iodometrically. All other chemicals used were 'AnalaR' grade. All solutions for kinetic studies were prepared in double distilled water.

Kinetic measurements

The reactions were carried out under pseudo first order conditions by keeping large excess of histidine over PBC. Stock solution of PBC in acetic acid and reaction mixtures containing known volume of substrate, perchloric acid and acetic acid were brought to the thermostat temperature (± 0.02 K) separately. Perchloric acid was used as a source of hydrogen ion. Reactions were started by rapidly adding predetermined volume of thermostated PBC solution to the reaction mixtures. The rate of oxidation was followed iodometrically by taking aliquots (5.0 mL) at fixed intervals of time and taking all precautions to avoid air oxidation of iodide. The rate constants were computed from the linear plots of log [hypo] against time by linear least-square method. The results were reproducible within ± 4%. Order with respect to each reactant was determined by Ostwald isolation method.

Stability of reagent in solution

Solution of oxidant (PBC) in acetic acid-water-perchloric acid mixture obeys Beer-Lambert's law at λ_{max} 370 nm. It was found that there is no change in spectra and optical density on long standing and heating up to 60°C. This indicated PBC is stable in solution in acetic acid-water solution.

Detection of free radicals

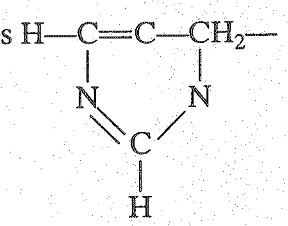
To the reaction mixture in kinetic conditions, acrylonitrile (free from stabilizer) was added in nitrogen atmosphere. No milky appearance was observed, hence any free radical formation in the reaction is ruled out.

Product analysis and stoichiometry

The oxidation of histidine by PBC gives 2-imidazole acetaldehyde. This was detected and estimated as its 2,4-dinitrophenylhydrazone (m.p. 491 K after recrystallization from alcohol) and confirmed by using authentic sample of 2-imidazole acetaldehyde. The yield of hydrazone obtained was 85 to 90%. Blank and parallel runs were performed and the correction applied in the result of estimation. The ratio of aldehyde and PBC consumed was found to be 3 : 2. Therefore, the reaction may be represented stoichiometrically as:

$$3RCH(NH_2)COOH + \underset{\text{(2 moles PBC)}}{2Cr(VI)} + 3H_2O \rightarrow 3RCHO + 2Cr(III) + 3NH_4^+ + 3CO_2 + 3H^+$$

where R represents



Ammonium ion by Nessler reagent and carbon dioxide by limewater test were also detected as product of oxidation.

RESULTS AND DISCUSSION

Under same kinetic conditions, experiments were carried out with CrO_3 and $K_2Cr_2O_7$ separately at different hydrogen ion concentrations. No reaction was observed. Further, addition of pyridine also could not initiate oxidation of histidine by CrO_3 and $K_2Cr_2O_7$.

Under psuedo first order conditions of [histidine] $\gg$ [PBC] at constant [histidine], [$HClO_4$], acetic acid % and constant temperature, plots of log [PBC] *i.e.*, log [hypo volume] *vs.* time were linear ($r > 0.9968$) indicating first order dependence of the rate on [PBC]. The variation of [PBC] has practically no effect on rate constant (Table-1), confirming that the observed first order rate constant is independent on initial concentration of PBC.

Keeping [PBC] constant, the rate of oxidation increases with increase in histidine concentration (Table-1). A plot of $1/k_{obs}$ against 1/[histidine] comes out to be a straight line ($r > 0.9868$) with an intercept on rate ordinate indicating that the oxidation proceeds through complex formation between histidine and PBC and follows Michaelis-Menten type kinetics.

The rate of oxidation of histidine can be expressed as:

$$-\frac{d[PBC]}{dt} = \frac{k[\text{Histidine}][PBC]}{K_m + [\text{Histidine}]}$$

From the slope and intercept ratio of plot of $1/k_{obs}$ against 1/[histidine], Michaelis-Menten constant $K_m = 1.37 \times 10^{-2}$ at 303 K has been obtained. Effect of substrate on reaction rate was studied at different temperatures and it was found that there was no appreciable change in K_m value.

At constant perchlorate ion concentration, first order rate constant decreases with increase in hydrogen ion concentration. The plot of log K_{obs} against log $[H^+]$ is a straight line ($r > 0.9928$) with negative slope equal to 1.8 indicating an inverse second order with respect to $[H^+]$. The plot of k_{obs} against $1/[H^+]^2$ is a straight line which reveals that the molecule of histidine is protonated. By increasing H^+ ion concentration, the protonation of protonated histidine will increase, which does not take part in the oxidation process. Since the protonated species of histidine cannot form coordinate bond with oxidant, *i.e.*, no complex formation, hence a decrease in rate. The results of the present investigation are contrary to Mahanti *et al.*[6–8], who proposed catalysis by H^+ and involvement of protonated amino acid as reactive species in oxidation by quinolinium dichromate.

Since we have observed retardation by H^+, therefore involvement of protonated PBC and protonated amino acid has been ruled out.

$$2H^+ + \text{Histidine} \rightleftharpoons \text{Histidine } H_2^{2+} \qquad (1)$$

TABLE-1
EFFECT OF OXIDANT CONCENTRATION, PERCHLORIC ACID AND ACETIC ACID ON RATE

(Temperature = 303 K)

10^3 [PBC] (mol dm^{-3})	Acetic acid (%)	$[H^+]$ (mol dm^{-3})	$k_{obs} \times 10^5$ (sec^{-1})
0.50	30	1.00	6.28
0.80	30	1.00	6.25
1.20	30	1.00	6.15
1.60	30	1.00	6.24
2.00	30	1.00	6.28
3.00	30	1.00	6.21
2.00	30	0.20	112.20
2.00	30	0.30	49.20
2.00	30	0.50	19.95
2.00	30	0.80	10.77
2.00	30	1.50	2.90
2.00	20	1.00	5.01
2.00	40	1.00	8.94
2.00	50	1.00	10.65
2.00	60	1.00	15.60

There is no change in rate by increase in concentration of sodium sulphate, sodium nitrate and sodium perchlorate. Hence, ion-ion interaction in rate determining step is ruled out[9].

At fixed ionic strength and $[H^+]$, the rate of oxidation of histidine with PBC increases with increase in acetic acid (Table-2).

TABLE-2
EFFECT OF HISTIDINE CONCENTRATION AT DIFFERENT TEMPERATURES

$[HClO_4] = 1.0$ mol dm^{-3}; $[PBC] = 2.0 \times 10^{-3}$ mol dm^{-3}; acetic acid = 30% (v/v)

[Histidine] × 10^2 (mol dm^{-3})	10^5 k_{obs} (s^{-1})					
	303 K	308 K	313 K	318 K	323 K	$K_m \times 10^2$
1.25	5.01	5.88	6.80	8.47	11.11	1.37
1.66	5.62	7.24	8.93	12.05	15.38	1.38
2.00	6.28	8.41	9.97	14.76	19.95	1.34
3.30	7.98	11.11	14.70	20.22	38.46	1.34
5.00	9.59	13.34	20.01	35.71	125.01	1.35

$\Delta E_a^{\neq} = 43.76 \pm 1.80$ kJ mol^{-1}; $\Delta S^{\neq} = -178.64 \pm 7.15$ J mol^{-1} K^{-1}; $\Delta F^{\neq} = 97.88 \pm 3.92$ kJ mol^{-1}

In other words, a decrease in rate with increase in dielectric constant is observed. This is due to the polar character of the transition state as compared to the reactants. The plot of log k_{obs} against 1/D (dielectric constant) is linear ($r > 0.9948$) with positive slope ($= 40.81$) indicating ion-dipole type of interaction[10].

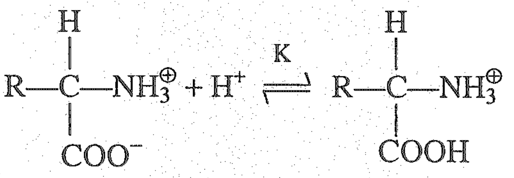
The addition of pyridine has no effect on the rate of reaction. This proves the stability of PBC in acetic acid-water containing perchloric acid medium in the concentration range studied.

The rate constants for oxidation of histidine $RCH(NH_2)COOH$ and α-deuteriohistidine $RCD(NH_2)COOH$, at 0.020 mol dm^{-3} [histidine], 1.0 mol dm^{-3} [$HClO_4$], 0.0020 mol dm^{-3} [PBC], HOAc : H_2O = 30 : 70 (v/v) and temperature 303 K are 6.28×10^{-5} and 5.58×10^{-5} sec^{-1} respectively. Thus the absence of primary kinetic isotope effect (k_H/k_D) = 1.12 (at 303 K) rules out the α-C—H bond fission in the rate determining step.

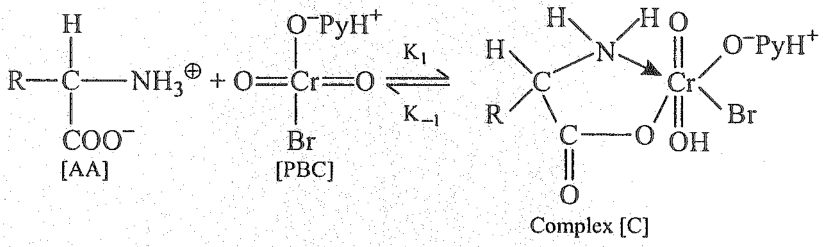
Rate of reaction increased with increase in temperature. The reaction was studied at different temperatures (303–323 K) keeping all other experimental conditions constant. A plot of log k_{obs} against 1/T is a straight line ($r > 0.995$). This confirms the validity of Arrhenius equation in the case of oxidation of histidine by PBC. Thermodynamic parameters have been calculated (Table-2). The value of activation energy ($\Delta E_a^{\neq}$) is 43.76 kJ mol^{-1} suggesting that C—C bond cleavage is involved in the rate determining step, which is further confirmed by primary kinetic isotope effect (k_H/k_D = 1.12 at 303 K). The entropy of activation (–178.2 J K^{-1} mol^{-1}) is negative and high suggesting that the transition state is more rigid and extensively solvated than the reactants. Negative entropy also suggests formation of cyclic intermediate from acyclic species.

Amino acids are known to exist as neutral, zwitter ions, anions and cations in aqueous solutions and dissociation depends on the pH of the solution. Karim and Mahanti[5–7] in oxidation of amino acids by quinolinium dichromate have suggested protonated amino acid as active species. We conclude here that amino acid in the form of zwitter ion is taking part as active species in these oxidations since increase in amino acid decreases rate of reaction.

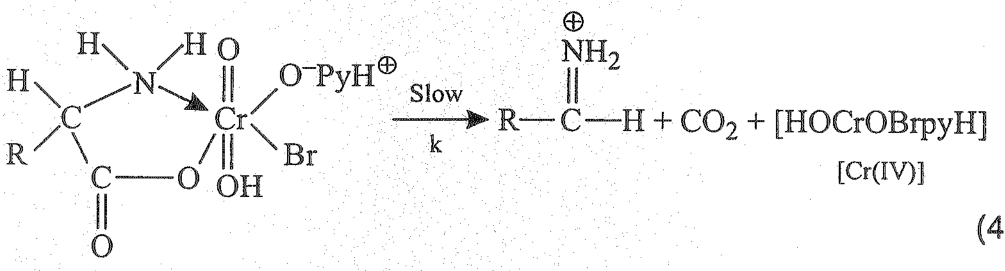
The following reaction scheme can explain all the observed experimental results:



$$R-\underset{COO^-}{\overset{H}{C}}-NH_3^{\oplus} + H^+ \overset{K}{\rightleftharpoons} R-\underset{COOH}{\overset{H}{C}}-NH_3^{\oplus} \qquad (2)$$

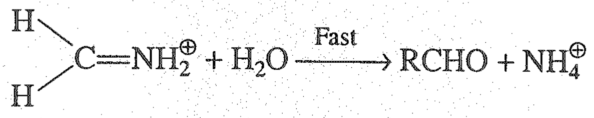


(3)



$$\xrightarrow[k]{\text{Slow}} R-\overset{\overset{\oplus}{NH_2}}{\overset{\|}{C}}-H + CO_2 + \underset{[Cr(IV)]}{[HOCrOBrpyH]} \qquad (4)$$

Complex [C]



$$H_2C{=}NH_2^{\oplus} + H_2O \xrightarrow{\text{Fast}} RCHO + NH_4^{\oplus} \qquad (5)$$

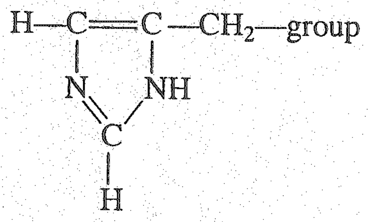
$$Cr(IV) + \underset{[PBC]}{Cr(VI)} \xrightarrow{\text{Fast}} 2Cr(V) \qquad (6)$$

$$2Cr(V) + 2RCH(NH_2)COOH + 2H_2O \xrightarrow{\text{Fast}} 2RCHO + 2NH_4^{\oplus} + 2CO_2 + 2Cr(III) \qquad (7)$$

The overall reaction may be represented as:

$$3RCH(NH_2)COOH + 2Cr(VI) + 3H_2O \longrightarrow 3RCHO + 2Cr(III) + 3NH_4^+ + 3CO_2 + 3H^+ \qquad (8)$$

where R represents



The rate law derived, based on the above mechanism, is as follows:

$$\text{Rate of reaction} = -\frac{d[C]}{dt} \alpha[C] \qquad (9)$$

$$= k[C] \qquad (10)$$

Concentration of complex [C] can be calculated by applying the steady state concept.

This gives

$$[C] = \frac{k_1'[AA][PBC]_t}{k_{-1}' + k + k_1'[AA]}$$

$$= \frac{[AA][PBC]_t}{\frac{k_{-1}' + k}{k_1'} + [AA]}$$

$$= \frac{[AA][PBC]_t}{K_m + [AA]}$$

where $$K_m = \frac{k'_{-1} + k}{k'_1}$$

Hence $$\begin{aligned} \text{Rate} &= k[C] \\ &= k\frac{[AA][PBC]_t}{K_m + [AA]} \\ &= k_{obs}[PBC] \end{aligned}$$

The observed pseudo first order rate constants, k_1, will then be:

$$k_{obs} = \frac{k[AA]}{K_m + [AA]}$$

or $$\frac{1}{k_{obs}} = K_m \frac{1}{k} \frac{1}{[AA]} + \frac{1}{k}$$

Therefore, K_m can be calculated by slope/intercept of plot $1/k_{obs}$ *vs.* $1/[AA]$.

This rate law is consistent with the results. The decrease in rate with H^+ can be explained by eqn. (1).

ACKNOWLEDGEMENT

Authors are grateful to Dr. Rama Shanker (Retired Professor) for his valuable suggestions and for providing deuterated histidine.

REFERENCES

1. N. Narayanan and T.R. Balasubramanian, *J. Chem. Res.*, 336 (1986).
2. M.K. Mahanti and K.K. Banerji, *J. Indian Chem. Soc.*, **79**, 31 (2002).
3. V. Sharma, P.K. Sharma and K.K. Banerji, *Indian J. Chem.*, **36**, 418 (1997).
4. N. Nalwaya, A. Jain and B.L. Hiran, *J. Indian Chem. Soc.*, **79**, 587 (2002).
5. N. Nalwaya, A. Jain and B.L. Hiran, *Kinetics and Catalysis.*, **45**, 345 (2004).
6. E. Karim and M.K. Mahanti, *Oxid. Commun.*, **21**, 559 (1988).
7. E. Karim and M.K. Mahanti, *Oxid. Commun.*, **19**, 582 (1996).
8. E. Karim and M.K. Mahanti, *Oxid. Commun.*, **15**, 211 (1992).
9. K.J. Laidler, Chemical Kinetics, Harper and Row, 3rd Edn., p. 183 (1987).
10. E.S. Amiss, Solvent Effect on Reaction Rates and Mechanism, Academic Press, New York (1967).

(*Received*: 10 December 2005; *Accepted*: 23 June 2006) AJC-4970