KINETICS AND MECHANISM OF OXIDATIVE DECARBOXYLATION AND DEAMINATION OF GLYCINE AND DL-α-PHENYLGLYCINE BY PHENYL IODOSOACETATE

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KINETICS of phenyl iodosoacetate (PIA) oxidation of several organic substrates[1-3] and the reaction of amino acids using different oxidants[4-9] other than PIA are well documented. The present investigation was undertaken to study the oxidation of amino acid using PIA.

Glycine (V.P. Chest Institute, New Delhi) and DL-α-phenylglycine (Fluka, AR) were used as such. Perchloric acid, sodium perchlorate and sodium acetate were of AR grade and used as such without further prufication. Acetic acid was purified by refluxing with chromium trioxide. Doubly-distilled water was used for all kinetic measurements. PIA was prepared by the modified method of Boeseken and Schneider[10].

The course of the reaction was followed iodometrically. The reaction was carried out under pseudo-unimolecular condition taking the amino acid always in excess. The rate constants were calculated from the log (titre) *vs* time plot by least-square method.

Reaction mixtures containing varying ratios of PIA and amino acid were allowed to equilibrate at 40° for 24 hr. Estimation of unreacted PIA showed that one mole of amino acid consumed one mole of PIA. The reaction could be written as $RCHNH_2COOH + PhI(OAc)_2 + H_2O \rightarrow RCHO + NH_3 + PhI + 2HOAc$, where R=H for glycine and Ph for phenylglycine. In the case of phenylglycine, the product benzaldehyde was estimated as its 2,4-dinitrophenylhydrazone (92%).

The reaction was investigated with varying concentrations of amino acid at constant [PIA] (table 1). The reaction was first order in amino acid as evidenced by the unit slope of the plot of log [amino acid] *vs* log k_1. The second-order rate constants obtained by dividing the pseudo-first order rate constants by the respective amino acid concentration is constant indicating first-order dependence on amino acid.

The reaction was first order in PIA as evidenced by the linearity of log (titre) *vs* time plots. The pseudo-first order rate constants calculated in PIA at different initial concentrations are independent of oxidant concentration (table 2).

Table 1 *Effect of varying [amino acid] in PIA-glycine and PIA-phenylglycine reactions*

(A) Glycine			(B) Phenylglycine		
[Amino acid] M	$k_1 \times 10^4$ sec^{-1}	$k_2 \times 10^3$ lit mol^{-1} sec^{-1}	[Amino acid] $\times 10^2$ M	$k_1 \times 10^4$ sec^{-1}	$k_2 \times 10^2$ lit mol^{-1} sec^{-1}
0.2	2.43	1.21	1.0	7.28	7.28
0.4	4.95	1.24	1.5	11.13	7.42
0.6	7.80	1.30	2.0	14.21	7.10
0.8	9.76	1.22	2.5	15.06	7.23

(A) [PIA] = 0.002 M: Solvent = 20% HOAc (v/v); Temp. 50°
(B) [PIA] = 0.001 M: Solvent = 50% HOAc (v/v); Temp. 35°

Table 2 *Effect of varying [PIA] on the reaction rate*

(A) Glycine		(B) Phenylglycine	
[PIA] $\times 10^3$ M	$k_1 \times 10^4$ sec^{-1}	[PIA] $\times 10^3$ M	$k_1 \times 10^4$ sec^{-1}
1.0	6.12	0.5	7.18
2.0	6.47	1.0	7.28
3.0	6.66	1.5	6.92
4.0	6.68	2.0	7.03

(A) [Glycine] = 0.5 M: Solvent = 20% HOAc (v/v): Temp. – 50°
(B) [Phenylglycine] = 0.01 M: Solvent = 50% HOAc (v/v): Temp = 35°

It was observed that addition of perchloric acid to the reaction mixture decreases the rate (table 3). Increase in the concentration of pyridine increases the reaction rate (table 4).

The reaction was conducted at different ionic strengths using sodium perchlorate keeping the other variables constant. Ionic strength had practically no effect. The reaction rates were measured at different acetic acid-water mixtures. It was observed that an

Table 3 *Effect of varying [$HClO_4$] on the reaction rate*

(A) Glycine		(B) Phenylglycine	
[$HClO_4$] M	$k_1 \times 10^4$ sec^{-1}	[$HClO_4$] M	$k_1 \times 10^4$ sec^{-1}
Nil	6.47	Nil	7.28
2.05	4.97	0.01	4.63
0.10	4.30	0.02	3.19
0.15	3.94	0.03	1.99
0.20	3.54	0.04	1.65

(A) [Glycine] = 0.5 M: [PIA] = 0.002 M: Temp. 50°
(B) [Phenylglycine] = 0.01 M: [PIA] = 0.001 M: Temp. 35°

Table 4 *Effect of varying [Pyridine] on the reaction rate*

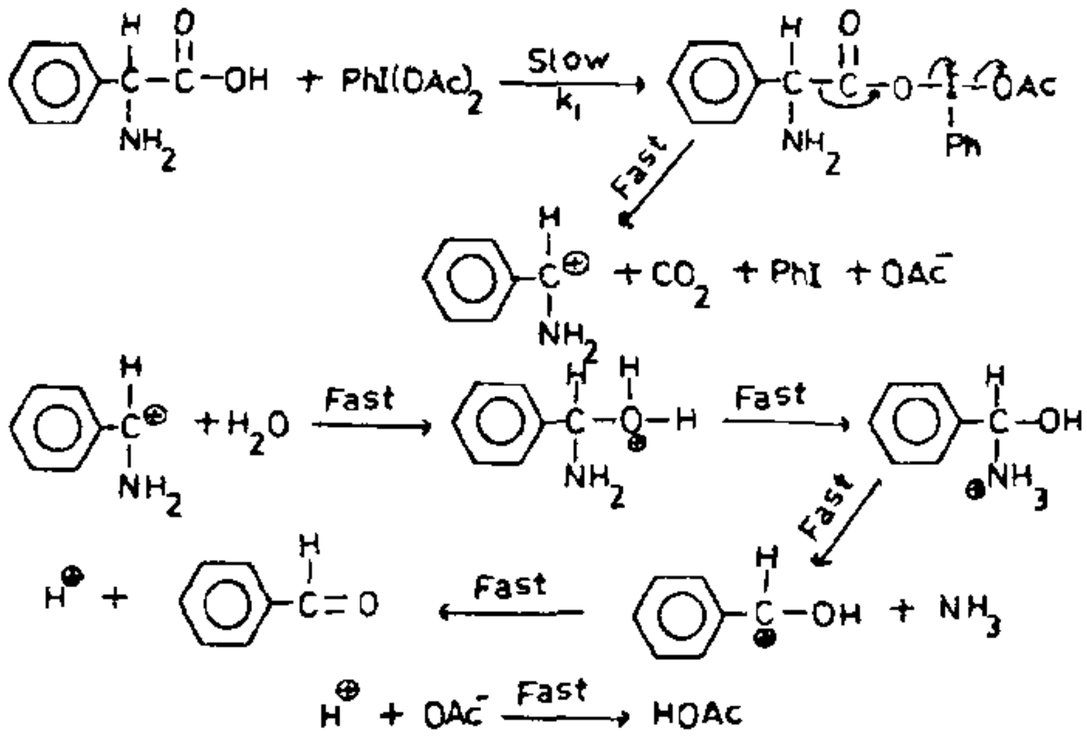
(A) Glycine		(B) Phenylglycine	
[Pyridine] M	$k_1 \times 10^4$ sec^{-1}	[Pyridine] M	$k_1 \times 10^4$ sec^{-1}
Nil	6.47	Nil	7.28
0.125	7.72	0.125	13.09
0.250	9.97	0.250	16 62
0.375	11.57	0.375	20.00
0 500	12 36	0 500	22.02

(A) [Glycine] = 0.5 M: [PIA] = 0.002 M: HOAc = 20% (v/v: Temp. 50°
(B) [Phenylglycine] = 0.01 M: [PIA] = 0.001 M: HOAc = 50% (v/v) Temp. 35°

increase in the acetic acid content of the medium considerably decreased the reaction rate (table 5). Polymerisation test[11] showed the absence of free radical formation.

The rate measurements were made at three different temperatures, *viz* 45, 50 and 55° for glycine and at 30, 35 and 40° for phenylglycine. The rate constants and the activation parameters are given in table 6.

The first-order dependence on the [substrate] and [oxidant] reveal that the overall rate may involve the interaction of PIA and amino acid in the rate-determining step to give a mono ester. The mono ester could be of the type involving O–I linkage with the elimination of acetic acid. This mono ester in a fast step probably forms a carbonium ion intermediate. Immediately the water molecule could attack the carbonium ion, to form an oxonium ion which in turn gives ammonia and an aldehyde. A typical reaction sequence with phenylglycine is shown in the following scheme.



Based on the above scheme, the rate law for the reaction could be represented by eq (1).

$$-\frac{d[PIA]}{dt} = k_2[PIA][\text{amino acid}] \qquad (1)$$

The negative effect of the $[H^+]$ could be explained by considering the following equilibrium

$$S + H^+ \rightleftharpoons SH^+$$

amino acid substrate cation

which shows the removal of reactive species (S), thus accounting for the decrease in the reaction rate. This effect coupled with the negative acetic acid effect does point out that the reaction with unprotonated amino acid should be more predominant.

Addition of pyridine was found to catalyse the reaction but the order is not unity. This catalysing effect of pyridine may be due to the decrease in the hydrogen ion concentration of the medium.

The authors thank Dr K. Ganapathy for providing facilities and CSIR for the award of a fellowship to KL.

10 August 1984; Revised 1 October 1984

Table 5 *Effect of dielectric constant of the medium on the reaction rate*

% Acetic acid	$k_1 \times 10^4$ sec^{-1} at		
	45°	50°	55°
20	3.179	6.286	13.580
40	1.455	2.941	6.458
60	0.745	1.499	2.959
80	0.723	1.320	2.000

[Glycine] = 0.5 M: [PIA] = 0.002 M:

Table 6 *Activation parameters for the reaction of glycine and phenylglycine by PIA*

Parameters	(A) Glycine	(B) Phenyl-glycine
$k_2 \times 10^2$ (lit mol^{-1} sec^{-1})	0.1257 (at 50°)	7.282 (at 35°)
E_a (kJ mol^{-1})	125.3	105.4
$\Delta H\ddagger$ (kJ mol^{-1})	122.6	102.8
$\Delta G\ddagger$ (kJ mol^{-1})	97.3	82.2
$\Delta S\ddagger$ (JK^{-1} mol^{-1})	78.5	66.8

(A) [Glycine] = 0.5 M: [PIA] = 0.002 M: HOAc = 20% v/v
(B) [Phenylglycine] = 0.01 M: [PIA] = 0.001 M: HOAc = 50% v/v

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SOLUBILISATION OF ROCK PHOSPHATE BY *THIOBACILLUS FERROOXIDANS*

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MICROBIAL solubilisation of rock phosphate, especially of the low grade variety, is receiving greater attention in recent years. This process can compensate for the higher cost of preparing phosphate fertilizers in the industries. India possesses substantial deposits of rock phosphate which till now remains unutilized due to the presence of several impurities and a low phosphorus content[1]. Laboratory studies have been carried out to utilize rock phosphates, rendering them soluble by microbial means in presence of a suitable carbon source[2-4]. We report here, for the first time, the involvement of iron-oxidizing chemoautotroph *Thiobacillus ferrooxidans* for such a solubilisation process.

The cells of *T. ferrooxidans* were grown in the modified 9K medium and harvested accordingly[5]. Rock phosphate samples were collected from Indian origin. For our experiment the samples were ground to < 63 microns particle size and analysed[6]. The slurry of rock phosphate, mixed with or without pyrite *viz* Pyriteferous shales of Amjhore (India), was made in acidified water (pH 2.4 adjusted with 0.01N H_2SO_4) containing 0.1% ammonium sulphate. The suspensions in 250 ml Erlenmeyer flasks were inoculated with 5 ml cell suspension (2×10^9 cells/ml) and incubated at 28°C on a rotary shaker (150 rev/min). The amount of phosphate released from rock phosphate was estimated[7] periodically. The uninoculated samples were treated in the same manner served as controls.

The results (figures. 1a & b) show the efficiency of the bacterial strain *T. ferrooxidans* in solubilizing phosphate from a sterilized pyrite mixed low grade rock phosphate (P_3-MRP). It could be concluded that the maximum phosphate leach rate was achieved at 6% (w/v) rock phosphate concentration (16.4 mg phosphate/l/hr), whereas the maximum solubilisation (98%) was obtained at 2% (w/v). Considering the release of phosphate with time (figure 1a) it was evident that the extent of solubilisation increased steadily up to 15 days, although it continued upto 25 days. The increase in solubility of phosphate between 20 and 25 days was very small (figure 1b).

The effective role of pyrite during this process is reflected from another low grade rock phosphate sample P_1-MRP (compare B with C, figure 2). The deleterious effect of the presence of calcite to restrict phosphate release was observed. This can be due to the secondary reactions in which Ca^{2+} ions released from calcite at pH 2.4 may effect the solubility product of calcium phosphate. The sample in association with pyrite showed 38.0% phosphate solubilisation, whereas 56% solubilisation was possible when calcite free sample, obtained by the treatment with triammonium citrate solution[8] was used (compare C with D, figure 2). Maximum solubilisation (72.5%) was achieved when the medium with calcite free sample was supplemented with essential nutrients (0.05% magnesium sulphate and 0.01% potassium chloride), other than nitrogen source (compare D with E, figure 2). The calcite free unsterilized, uninoculated sample also showed significant phosphate solubilisation (compare A with F, figure 2). However, the bacterium showed nearly similar phosphate release from calcite free sterilized and unsterilized samples (compare D with H, figure 2). The strain was found equally effective in solubilizing phosphate from high grade rock phosphates (unpublished data).

It seems evident from these results that *T. ferrooxidans* together with acidiphilic microbes present in the samples is efficient in solubilizing phosphate from rock phosphate, irrespective of their total phosphate content and other chemical constituents (table 1). The