

Anodically generated manganese(III) sulphate for the oxidation of dipeptides in aqueous sulphuric acid medium: A kinetic study

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Abstract. The kinetic of oxidation of dipeptides (DP) namely valyl-glycine (Val-Gly), alanyl-glycine (Ala-Gly) and glycyl-glycine (Gly-Gly), by Mn(III) have been studied in the presence of sulphate ions in acid medium at 26°C. The reaction was followed spectrophotometrically at $\lambda_{\text{max}} = 500$ nm. A first-order dependence of the rate on both $[\text{Mn(III)}]_0$ and $[\text{DP}]_0$ was observed. The rate is independent of the concentration of reduction product, Mn(II) and hydrogen ions. The effects of varying the dielectric constant of the medium and addition of anions such as sulphate, chloride and perchlorate were studied. The activation parameters have been evaluated using Arrhenius and Eyring plots. The oxidation products were isolated and characterized. A mechanism involving the reaction of DP with Mn(III) in the rate-limiting step is suggested. An apparent correlation was noted between the rate of oxidation and the hydrophobicity of these dimers, where increased hydrophobicity results in increased rate of oxidation.

Keywords. Oxidation; dipeptides; manganese(III).

1. Introduction

There has been a great deal of attention focussed on the oxidation of organic substrates by high-valent metal ions. Of these, manganese(III) oxidation is of special importance due to its biological relevance.¹ Manganese(III) porphyrins have been studied as possible models for closely related biologically significant systems.² Some studies have been reported on the kinetics of manganese(III) oxidation of various substrates in perchlorate, sulphate, acetate and pyrophosphate medium.^{3,4} Peptides and proteins are the most characteristic chemical compounds found in living cells. Peptides, such as enkephalins, oxytocin, vasopressin, leutinizing hormone releasing hormone (LHRH), opioid peptides and elastic sequence play a very important role in biology. These peptides are susceptible to enzymes. Extensive work has been reported on the enzymatic degradation of these peptides. Although the kinetics of oxidation of amino acids with various metal ions and halogens^{5,6} in acid and alkaline media has been studied, oxida-

tion of these biologically active peptides have not been reported in literature.

We have synthesised three dipeptides viz. valyl-glycine (Val-Gly), alanyl-glycine (Ala-Gly) and glycyl-glycine (Gly-Gly) which are fragments of elastic sequences⁷ to study the kinetics of oxidation with Mn(III) in acid medium at 26°C to elucidate the mechanism of these redox reactions.

2. Peptide synthesis

All of the amino acids used except glycine are of L-configuration unless otherwise specified. All *t*-butyloxycarbonyl (Boc) amino acids, amino acid derivatives, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), 1-hydroxybenzotriazole (HOBt), trifluoroacetic acid (TFA) and N-methylmorpholine (NMM) were purchased from Advanced Chem. Tech. (Louisville, KY, USA). All solvents and reagents were of analytical grade or were purified according to procedures recommended for peptide synthesis. Thin layer chromatography (TLC) was carried out on silica gel plates obtained from Whatman Inc., with the following solvent systems: chloroform-methanol-

*For correspondence

acetic acid (95:5:3), R_f^1 ; chloroform–methanol–acetic acid (90:10:3), R_f^2 ; and chloroform–methanol–acetic acid (85:15:3), R_f^3 . The compounds on TLC plates were detected by UV light after spraying with ninhydrin or by chlorine/toluidine. The melting points were determined by using Thomas–Hoover melting point apparatus and are uncorrected.

2.1 Boc- X_{aa} -Gly-OBzl [X_{aa} = Val or Ala or Gly]

Boc- X_{aa} (0.02 mol) and HOBt (3.37 g, 0.022 mol) in DMF (40 ml) was cooled to $-15 \pm 1^\circ\text{C}$ and EDCI (4.21 g, 0.022 mol) was added. After stirring for 20 min, a pre-cooled solution of Gly-OBzl.Tos (6.78 g, 0.02 mol) and NMM (2.4 ml, 0.022 mol) in DMF (50 ml) was added and stirred overnight at room temperature. After evaporating DMF under reduced pressure, the residue was taken up by chloroform and extracted with 10% citric acid, water, 5% sodium bicarbonate, water and dried over sodium sulphate. The solvent was removed under reduced pressure and recrystallized from ether/ethyl acetate to obtain 6.34 g (87%) of Boc- X_{aa} -Gly-OBzl. R_f^1 0.58, R_f^2 0.72 and R_f^3 0.66, m.p. 80°C (lit⁸ $80\text{--}82^\circ\text{C}$); 6.0 g (93%) of Boc- X_{aa} -Gly-OBzl. R_f^1 0.51 and R_f^2 0.63, m.p. $89\text{--}90^\circ\text{C}$ (lit⁸ $90\text{--}92^\circ\text{C}$) and 5.7 g (89%) of Boc- X_{aa} -Gly-OBzl. R_f^1 0.49 and R_f^2 0.58, m.p. $81\text{--}82^\circ\text{C}$ (lit⁹ $82\text{--}83^\circ\text{C}$).

2.2 X_{aa} -Gly

Boc- X_{aa} -Gly-OBzl (0.015 mol) was saponified in methanol (50 ml) using 1 M NaOH (2.0 equiv.) for 2 h at room temperature. After evaporating the solvent under reduced pressure, the residue was taken up by water and washed with chloroform (3 \times 25 ml). The aqueous layer was cooled and neutralized with cold 1 M HCl and extracted with chloroform (40 ml). The organic phase was washed with cold 0.1 M HCl, 50% saturated NaCl and dried over Na_2SO_4 . The solvent was removed *in vacuo* and triturated with ether, filtered, washed with ether and dried to obtain 3.78 g (92%) of Boc-Val-Gly-OH. R_f^2 0.22 and R_f^3 0.34; 3.4 g (92.1%) of Boc- X_{aa} -Gly-OH. R_f^2 0.24 and R_f^3 0.35 and 3.15 g (90.5%) of Boc- X_{aa} -Gly-OH. R_f^2 0.26 and R_f^3 0.37.

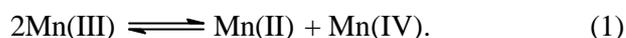
Boc- X_{aa} -Gly-OH (0.01 mol) was deblocked with TFA (10 ml/g of peptide) by stirring for 40 min. The solvent was removed under reduced pressure, the

residue was triturated with ether, filtered, washed with ether to obtain TFA. X_{aa} -Gly-OH (100%).

3. Kinetic measurements

3.1 Preparation of Mn(III) sulphate

A 0.05 M solution of manganese(III) sulphate was prepared¹⁰ using the standard anodic oxidation of 0.2 M solution of manganese(II) sulphate in 5 M sulphuric acid performed in an undivided cell with a platinum foil anode (generation area 4.0 cm^2) and a thin platinum spiral cathode (effective area 0.2 cm^2). The manganese(III) sulphate solution contained an excess but known concentration of manganese(II) sulphate to suppress the disproportionation reaction,



Though the solution appeared to be stable for more than a month at $[\text{H}^+] > 5.0\text{ M}$, solution of manganese(III) sulphate prepared afresh daily was used in the experiments. All other reagents were prepared from AR grade chemicals. Triply distilled water was used for preparing aqueous solutions.

3.2 Experimental

Solutions containing the requisite amounts of DP, sulphuric acid (to maintain known acid concentration), manganese(II) sulphate and water (to keep the total volume constant) were placed in stoppered boiling tube. The mixtures were thermally equilibrated in a water bath at 26°C . To the solutions in each tube, was added an aliquot of pre-equilibrated manganese(III) sulphate stock solution to give a known overall concentration. The progress of the reaction was monitored for two half-lives by measuring the absorbance of unreacted Mn(III) at 500 nm using a Spectrochem MK II spectrophotometer. The reaction mixture was quenched iodometrically. Plots of $\log(\text{absorbance})$ vs. time were linear. The rate constants, k_{obs} , calculated from these plots were reproducible within $\pm 3\%$ error.

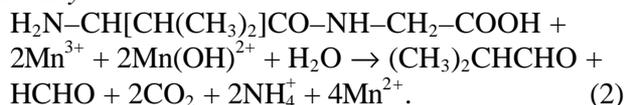
4. Stoichiometry

The mixtures containing DP (0.001 M), acid (0.1 M) and excess Mn(III) (0.005 M) were kept for 24 h at

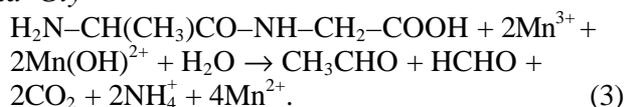
26°C. The unconsumed Mn(III) was then determined iodometrically. Four moles of oxidant were sufficient to oxidise one mole of DP leading to aldehydes, carbon dioxide, ammonia and Mn(II).

Based on the experimental results, the following stoichiometric equations are shown below.

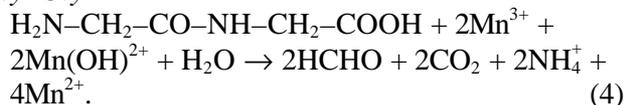
Val-Gly



Ala-Gly



Gly-Gly



5. Product analysis

After the reaction was completed, the reaction products were extracted with ether and subjected to column chromatography on silica gel (60–200 mesh) using gradient elution (dichloromethane to chloroform). After initial separation, the products were further purified by recrystallization. Aldehydes were quantified in the ether extract by the formation of 2,4-dinitrophenylhydrazone (DNP) derivatives isolable up to 95% yield. Ammonia and CO₂ were detected by the conventional tests.

6. Results and discussion

All kinetic runs were performed under pseudo first-order conditions of $[\text{DP}] \gg [\text{Mn}(\text{III})]$. Plots of $\log [\text{Mn}(\text{III})]$ vs time were linear ($r > 0.998$) even beyond 75% of the reaction, showing first-order dependence of the rate on $[\text{Mn}(\text{III})]$ (table 1). At constant $[\text{Mn}(\text{III})]_0$, $[\text{Mn}(\text{II})]_0$, $[\text{H}_2\text{SO}_4]$, $[\text{Na}_2\text{SO}_4]$ and temperature, the rate increased with increase in $[\text{DP}]_0$ (table 1). Plots of $\log k_{\text{obs}}$ vs $\log [\text{DP}]_0$ were linear with slopes of 0.99, 1.00 and 1.06 for Val-Gly, Ala-Gly and Gly-Gly respectively. Increase in $[\text{H}^+]$ (0.6 to 1.8 M), had no effect on the rate, $[\text{Mn}(\text{II})]$ (0.006 to 0.016 M), Cl^- (0.001 to 0.01 M), SO_4^{2-} (0.001 to 0.01 M) and ClO_4^- (0.001 to 0.01 M) on the rate were insignificant. The reaction product

Mn(II) had no effect on the reaction, indicating that the product is not involved in a pre-equilibrium with the oxidant.

The solvent composition of the medium was varied by adding methanol (0.0 to 40%) to the reaction

Table 1. Effect of varying reactants concentration on the rate^a.

[Mn(III)] (10 ³ M)	[DP] (10 ² M)	$k_{\text{obs}} \times 10^5 \text{ (s}^{-1}\text{)}$		
		Val-Gly	Ala-Gly	Gly-Gly
0.6	1.0	4.73	3.59	2.63
0.8	1.0	4.80	3.50	2.61
1.0	1.0	4.75	3.59	2.63
1.2	1.0	4.60	3.40	2.55
1.4	1.0	4.55	3.60	2.70
1.6	1.0	4.49	3.72	2.69
1.8	1.0	4.90	3.85	2.61
1.0	0.6	2.82	1.91	1.54
1.0	0.8	3.76	2.55	2.07
1.0	1.0	4.75	3.59	2.63
1.0	1.2	5.63	3.86	3.20
1.0	1.4	6.54	4.48	3.72
1.0	1.6	7.50	5.08	4.32
1.0	1.8	8.42	5.76	4.90

^a $[\text{Mn}(\text{II})]_0 = 0.01 \text{ M}$, $[\text{H}_2\text{SO}_4] = 0.1 \text{ M}$, at 26°C

Table 2. Effect of varying dielectric constant (D) on the rate^a.

MeOH (% v/v)	Dielectric constant		$k_{\text{obs}} \times 10^5 \text{ (s}^{-1}\text{)}$		
	(D)	10 ³ /D	Val-Gly	Ala-Gly	Gly-Gly
0	76.73	13.00	4.75	3.49	2.63
10	72.37	13.80	8.94	5.50	3.64
20	67.48	14.80	11.52	9.36	5.76
30	62.71	14.40	15.90	16.64	9.00
40	58.06	17.80	17.20	32.50	16.00

^a $[\text{Mn}(\text{III})]_0 = 0.001 \text{ M}$, $[\text{DP}]_0 = 0.01 \text{ M}$, $[\text{Mn}(\text{II})]_0 = 0.01 \text{ M}$, $[\text{H}_2\text{SO}_4] = 0.1 \text{ M}$, at 26°C

Table 3. Temperature dependence of the oxidation of DP by Mn(III)^a.

Substrate	$k_{\text{obs}} \times 10^5 \text{ (s}^{-1}\text{) at temp (K)}$				
	294	299	304	309	314
Val-Gly	3.50	4.75	6.10	8.00	12.02
Ala-Gly	2.76	3.59	4.92	6.46	8.72
Gly-Gly	2.01	2.63	3.39	4.27	5.63

^a $[\text{Mn}(\text{III})]_0 = 0.001 \text{ M}$, $[\text{DP}]_0 = 0.01 \text{ M}$, $[\text{Mn}(\text{II})]_0 = 0.01 \text{ M}$, $[\text{H}_2\text{SO}_4] = 0.1 \text{ M}$

Table 4. Activation parameters for the oxidation of DP by Mn(III)^a.

Substrate	E_a	ΔH^\ddagger	ΔS^\ddagger	ΔG^\ddagger	Log A
	(kJ mol ⁻¹)	(kJ mol ⁻¹)	(J K ⁻¹ mol ⁻¹)	(kJ mol ⁻¹)	
Val-Gly	42.75	40.22	-192.82	97.86	3.1
Ala-Gly	44.72	41.94	-189.29	99.47	3.3
Gly-Gly	40.26	37.74	-206.19	99.99	2.4

^a[Mn(III)]₀ = 0.001 M, [DP]₀ = 0.01 M, [Mn(II)]₀ = 0.01 M, [H₂SO₄] = 0.1 M

mixture. The rate increases with increase in methanol content (table 2). The plots of log k_{obs} vs $1/D$ (D = dielectric constant of the medium) were linear ($r = 0.999$) with positive slopes. Measurements of rate constants were done both in the presence and absence of dipeptides with Mn(III) and the rate constants were taken for the calculation of effective k_{obs} , although the rate of oxidation of methanol in the absence of DP is negligible under the present conditions employed.

To determine the activation parameters, the reactions were carried out at different temperatures (21–41°C). The Arrhenius plots of log k_{obs} vs $1/T$ (table 3), were found to be linear ($r = 0.999$). The activation energies (E_a) were calculated from the slope of the plots. From this value, the activation parameters, ΔH^\ddagger , ΔS^\ddagger , ΔG^\ddagger and the frequency factor (log A) (table 4), were evaluated.

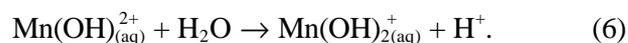
Addition of acrylamide to the reaction mixture did not cause polymerization suggesting the absence of free-radical involvement during the oxidation.

Data published by Diebler, Sutin¹¹, Fackler and Chawla¹² and Wells¹³, have shown that in the presence of F^- ion, aqueous solution of Mn(III) sulphate consists of hexaquomanganese(III), Mn(III)_(aq), Mn(OH)_(aq)²⁺ and MnF_(aq)²⁺. Along the same line it can be assumed with justification that Mn(III) species present in sulphuric acid solution are Mn(III)_(aq), Mn(OH)_(aq)²⁺ and MnSO_(4aq)⁺. Therefore, it was shown¹⁴ that manganese(III) sulphate in aqueous sulphuric acid solution contains Mn_(aq)³⁺ and Mn(OH)_(aq)²⁺ as reactive species.



The hydrolysis constant of manganese(III) sulphate calculated was $k_h = 0.93 \pm 0.03$ at 25°C. The absorption spectra of both Mn_(aq)³⁺ and Mn(OH)_(aq)²⁺ have been reported to be similar in both the visible and UV-region. Our observation of the electronic absorption

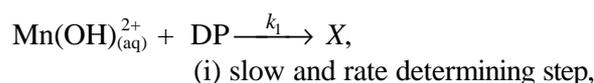
spectra is consistent with the values reported. Kinetic studies have shown that Mn_(aq)³⁺ is more reactive. Formation of dihydroxo species Mn(OH)_{2(aq)}⁺, produced by further hydrolysis of Mn(OH)_(aq)²⁺ is another possibility.¹⁵



However, a fresh solution of manganese(III) sulphate is always prepared and used immediately after cessation of the electrolysis, thereby eliminating any reaction due to Mn(OH)_{2(aq)}⁺. The molar absorption coefficient, ' ϵ ' ranges between 131–110 M⁻¹ cm⁻¹ at [H⁺] = 1.20–2.50 (M). The high value of ' ϵ ' has been attributed to the presence of hydrolyzed species Mn(OH)_(aq)²⁺. Since there is no hydrogen ion dependence on the rate, this suggests that Mn(OH)_(aq)²⁺ is not the reactive species. The absence of a sulphate effect on the reaction rate indicates that MnSO_{4(aq)}⁺ is not the active species in the present condition. Therefore, Mn(OH)_(aq)²⁺ and Mn_(aq)³⁺ are the reactive species as shown in the mechanism.

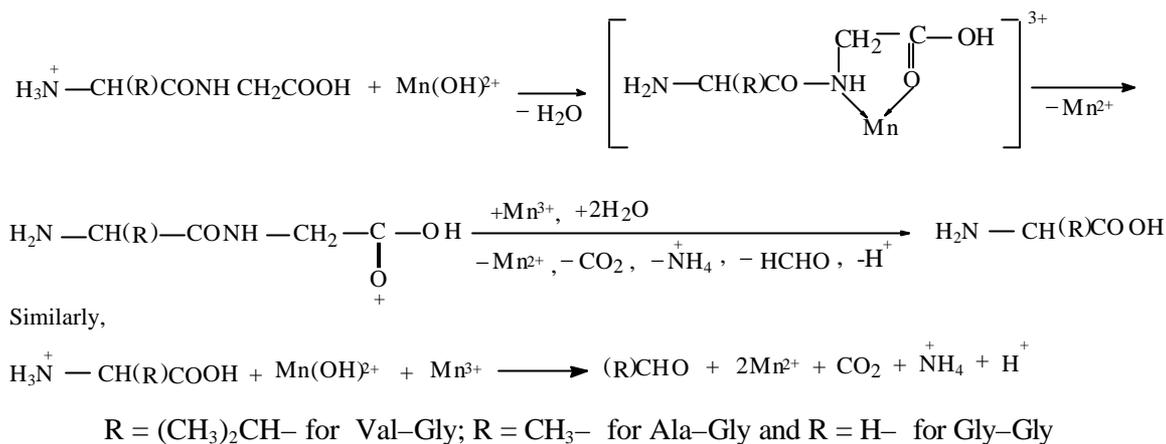
Scheme 1 accounts for the observed experimental results.

Amis¹⁶ has shown that plots of log k_{obs} vs $1/D$ gives a straight line with a positive slope for positive ion-dipole interaction. The positive dielectric effect in the present investigation shows charge dispersal in the transition state, pointing towards a positive ion-dipole reaction and hence supports the scheme in scheme 2.



$$\text{Hence, rate} = k_1 [\text{Mn}(\text{OH})_{(\text{aq})}^{2+}] [\text{DP}]. \quad (7)$$

Scheme 1.



Scheme 2.

Table 5. Deviations in peak wavelengths and absorbance.

Substrate	I_{max}		Complex	I_{max}	
	(nm)	Abs		(nm)	Abs
Mn(III)	500	0.97			
Val-Gly	232	2.48	Mn(III) + Val-Gly	437	2.60
Ala-Gly	229	2.46	Mn(III) + Ala-Gly	430	2.63
Gly-Gly	224	2.45	Mn(III) + Gly-Gly	445	2.70

The rate of oxidation of dipeptides by Mn(III) was compared with that of oxidation of amino-acids, Val, Ala, and Gly and it was found that the rate of oxidation of dipeptide was slower than free amino-acids. The change is due to the increased difference between the functional groups and consequently weaker electrostatic effects. Hence, the oxidation of dipeptides is expected to be slower than the monomers. Further, an apparent correlation was noted between the rate of oxidation and the hydrophobicity of these sequences, where increased hydrophobicity results in increased rate of oxidation. The order of oxidation of dipeptides was found to Val-Gly > Ala-Gly > Gly-Gly, which is in well agreement with their hydrophobicity.¹⁷

5.1 Spectral evidence for the formation of DP-Mn(III) complexes

The study of UV-Visible spectra separately, of pure Mn(III), DP (Val-Gly, Ala-Gly and Gly-Gly) and mixtures of Mn(III) and DP show deviations in peak wave length and absorbance as in table 5. It is noted that the values of the complexes are different from each of the substrate and Mn(III) in its pure form.

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