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Sequence dependence of oxidation of some repeating pentapeptide sequences of elastin with electrolytically generated Mn(III): synthesis, kinetics and mechanistic study

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ABSTRACT: The repeating sequence of elastin, valylprolylglycylvalylglycine (VPGVG), its permutation pentamer glycylvalylglycylvalylproline (GVGVP), and its more hydrophobic pentamer glycylphenylalanylglycylvalylproline (GFGVP) were synthesized by classical solution-phase methods and characterized. The kinetics of the oxidation of these pentapeptides (PP) by Mn(III) was studied in the presence of sulphate ions in acidic medium at 25 °C. The reaction was followed spectrophotometrically at $\lambda_{max} = 500$ nm. A first-order dependence of rate on both [Mn(III)] and [PP] was observed. The rate is independent of concentration of the reduction product, Mn(II) and hydrogen ions. Effects of varying dielectric constant of the medium and addition of anions such as sulphate, chloride and perchlorate were studied. Activation parameters were evaluated using Arrhenius and Eyring plots. The oxidation products were isolated and characterized. A mechanism involving the reaction of PP 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 sequences where increased hydrophobicity results in an increased rate of oxidation. Further, it was observed that the pentamers with Pro as C-terminus are more susceptible to oxidation than the pentamer with Gly as C-terminus. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: elastin; pentapeptide sequences; oxidation; Mn(III)

INTRODUCTION

Oxidative reactions play an important role in a variety of biochemical events ranging from normal metabolism to ageing and disease processes.[1,2] Peptides and proteins represent major targets for modification in these reactions, and the identification of sites and structures of modifications may lead to a mechanistic understanding and approaches for prevention. In this context, Mn(III) oxidation is of special importance owing to its biological relevance.[3] Manganese(III) porphyrins have been studied as possible models for closely related biologically significant systems.[4] Several studies have been reported on the kinetics of manganese(III) oxidation of various substrates in different media.[5–7] Extensive work has been reported on the kinetics of oxidation of amino acids with various metal ions and several other oxidants.[8–12] However, similar studies on the kinetics of oxidation of peptides and proteins by Mn(III) have not been reported except for the oxidative behaviour of bromamine-B towards glycylglycine.[13]

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Elastic protein-based polymers have their origins in repeating sequences of the mammalian elastic protein elastin.[14,15] The most prominent repeating sequence occurs in bovine elastin; it can be written as $(Val^1\text{-}Pro^2\text{-}Gly^3\text{-}Val^4\text{-}Gly^5)_n$, where $n = 11$ without a single substitution. Another repeat first found in porcine elastin is $(Val^1\text{-}Pro^2\text{-}Gly^3\text{-}Gly^4)_n$, but this repeat has not been found to occur with $n > 2$ without substitution.[16] The monomers, oligomers and high polymers of these repeats have been synthesized and conformationally characterized.[17] These polymers have a number of medical and non-medical applications.[18,19] In this context, it was thought of interest to investigate the oxidative behaviour of Mn(III) towards the repeating elastin sequence Val-Pro-Gly-Val-Gly its permutation pentamer Gly-Val-Gly-Val-Pro and one of its more hydrophobic pentamers Gly-Phe-Gly-Val-Pro, and this is reported in the present paper.

The peptides were synthesized by classical solution phase methods. The classical methods for synthesis in solution are labour intensive, time consuming and skill intensive, in large part because the intermediates differ in solubility characteristics. With all these concerns, the solution method provides relatively pure materials that do not require much purification at the end of the synthesis.

On the other hand, the solid-phase synthesis always yields impure products that require extensive purification even at the component peptide stage. During the course of our physical studies, a large amount of material was required. For this purpose, an efficient, well-established and less expensive Boc chemistry was used instead of more expensive Fmoc chemistry.

The peptides Boc-Val-Pro-Gly-Val-Gly-OBzl, Boc-Gly-Val-Gly-Val-Pro-OBzl and Boc-Gly-Phe-Gly-Val-Pro-OBzl were synthesized as described previously.[20,21] The synthesis of Val-Pro-Gly-Val-Gly was carried out by a $2+3$ coupling strategy and Gly-Val-Gly-Val-Pro and Gly-Phe-Gly-Val-Pro were synthesized by a $3+2$ coupling strategy in solution phase methods. The Boc group was used for temporary N^{α}-protection and its removal was achieved with 4 M HCl in dioxane or trifluoroacetic acid. The C-terminal carboxyl group was protected by the benzyl ester and its removal was effected by hydrogenolysis using $HCOONH_4$–Pd C (10%).[22] All coupling reactions were achieved with isobutyl chloroformate. The protected peptides were purified by recrystallization and characterized by physical and analytical techniques. The purity of the free peptides was checked by paper chromatography and HPLC.

EXPERIMENTAL

All the amino acids used except glycine were of L-configuration unless specified otherwise. All *tert*-butyloxycarbonyl (Boc) amino acids, amino acid derivatives and trifluoroacetic acid (TFA) were purchased from Advanced Chem. Tech., (Louisville, KY, USA). Isobutyl chloroformate and *N*-methylmorpholine (NMM) were purchased from Sigma Chemicals (St. Louis, MO, USA). All solvents and reagents were of analytical grade or were purified according to the procedure recommended for peptide synthesis.[23] Thir-layer chromatography (TLC) was carried out on silica gel plates obtained from Whatman, with the following solvent systems: chloroform–methanol–acetic acid (90:10:3), R_f^1, and chloroform–methanol–acetic acid (85:15:3), R_f^2. The compounds on TLC plates were detected by UV irradiation after spraying with ninhydrin or by chlorine–tolidine. Paper chromatography was carried out on Whatman No. 1 chromatographic paper with the solvent system butanol–acetic acid–water (4:1:5, upper phase). The compounds on paper were detected by spraying with ninhydrin. Melting-points were determined with a Selaco Can. No. 103 melting-point apparatus and were uncorrected. Elemental analyses were carried out by Mic Anal (Tucson, AZ, USA). Optical rotation was measured using a Perkin-Elmer Model 243 digital polarimeter. Amino acid analysis was performed on a Waters HPLC Pico-Tag analyser. Hydrolysis of the sample was carried out using 6 M HCl containing 1% (v/v) phenol at 110 °C for 72 h in a sealed tube under vacuum from which the air had been removed using nitrogen. Product analysis was carried out by gas chromatography (GC 15A, Shimadzu, Kyoto, Japan).

*Boc-Gly-Xaa-Gly-Val-Pro-OH (Xaa-Val for **I**, Phe for **II**).* Boc-Gly-Val-Gly-Val-Pro-OBzl and Boc-Gly-Phe-Gly-Val-Pro-OBzl (0.015 mol) were separately hydrogenolysed in methanol (10 ml g^{-1} of peptide) using ammonium formate (2.0 equiv.) and 10% Pd/C (0.1 g g^{-1} peptide) for 30 min at room temperature. The catalyst was filtered and washed with methanol. The combined filtrate was evaporated *in vacuo* and the residue was dissolved in $CHCl_3$, washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure and triturated with diethyl ether, filtered, washed with diethyl ether and dried to obtain 7.2 g of **I** (yield 90.7%), m.p. 127 °C, $R_f^1$0.66, $R_f^2$0.75, $[\alpha]_D^{25}$(*c*, 1; MeOH) −64°, anal. calc. for $C_{24}H_{41}N_5O_8$ C 54.63, H 7.83, N 13.27%, found C 54.73, H 7.88, N 13.32%; and 7.4 g of **II** (yield 91.5%), m.p. 118 °C, $R_f^1$0.60, $R_f^2$0.50, $[\alpha]_D^{25}$(*c*, 1; MeOH) −67°, anal. calc. for $C_{28}H_{41}N_5O_8$ C 58.42, H 7.17, N 12.16%, found C 58.41, H 7.16, N 12.16%.

*Gly-Xaa-Gly-Val-Pro (Xaa - Val for **III**, Phe for **IV**).* **I** and **II** (0.01 mol) were deblocked with TFA (10 ml g^{-1} peptide) for 40 min. The solvent was removed under reduced pressure, triturated with diethyl ether, filtered and washed with diethyl ether to obtain the TFA salts of **III** and **IV** (yield, 100%).

*Boc-Val-Pro-Gly-Val-Gly-OH (**V**).* Boc-Val-Pro-Gly-Val-Gly-OBzl (4.9 g, 0.008 mol) in methanol (50 ml) was hydrogenolyzed using $HCOONH_4$—Pd/C (10%) and worked up as above to obtain 3.9 g of **V** (yield 92.8%), m.p. 80 °C, $R_f^1$0.60, $R_f^2$0.66, $[\alpha]_D^{25}$(*c*, 1; MeOH) −49°, anal. calc. for $C_{24}H_{41}N_5O_8$ C 54.63, H 7.83, N 13.27%, found C 54.66, H 7.85, N 13.25%.

*Val-Pro-Gly-Val-Gly (**VI**).* **V** (2.6 g, 0.005 mol) was deblocked with TFA (25 ml) for 40 min. The solvent was removed under reduced pressure, triturated with diethyl ether, filtered and washed with diethyl ether to obtain the TFA salt of **VI** (yield 100%).

Preparation of Mn(III) sulphate. A 0.05 M solution of manganese(III) sulphate was prepared by the electrolytic oxidation of Mn(II) sulphate in aqueous sulphuric acid by the procedure reported previously.[24] Even though the solution appeared to be stable for more than 1 month at $[H^+]$ >5.0 M, it was prepared fresh daily. All other reagents were prepared from analytical-reagent grade chemicals.

Preliminary studies. The maximum absorption (λ_{max}) of manganese(III) sulphate solution occurs at 500 nm. The standard redox potential E'_0 of Mn(III)/Mn(II), the oxidizing power of the oxidant, generally decreases on

complexation. The standard redox potential was measured under the specified experimental conditions. These details have been reported previously.[24] The formal redox potential E'_0 obtained at different $[H_2SO_4]$ and in presence of the complexing agents HSO_4^-, $P_2O_7^{4-}$ and Cl^- are 1.51, 1.48 and 1.42 V, respectively. Triply distilled water was used for preparing aqueous solutions.

Kinetic measurements. Mixtures of solutions containing the requisite amounts of PP, sulphuric acid (to maintain a known acid concentration), manganese(II) and water (to keep the total volume constant) were placed in stoppered boiling tubes. The mixture was thermally equilibrated in a water-bath at 25 °C. To the solution in this 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 appropriately. Plots of log(absorbance) vs time were linear. The rate constants k_{obs} calculated from these plots were reproducible to within ±3% error.

RESULTS

Dependence of rate on [Mn(III)] and [PP]

All kinetic runs were performed under pseudo-first-order conditions with [PP] ≫ [Mn(III)]. Plots of log[Mn(III)] vs time were linear even beyond 75% of the reaction, showing a first-order dependence of the rate on [Mn(III)] (Table 1); at constant $[Mn(III)]_0$, $[Mn(II)]_0$, $[H_2SO_4]$, $[Na_2SO_4]$ and temperature, the rate increased with increase in $[PP]_0$ (Table 1). Plots of $\log k_{obs}$ vs $\log[PP]_0$ (Fig. 1) were linear with slopes of 1.10, 0.98 and 1.09 for glycyl-valyl-glycyl-valyl-proline, glycyl-phenylalanyl-glycyl-valyl-proline and valyl-prolyl-glycyl-valyl-glycine, respectively.

Dependence of rate on [acid]

Kinetic measurements were performed in H_2SO_4–$NaHSO_4$ solution of different $[H^+]$. The effective $[H^+]$ used was evaluated with the aid of a calibration curve[25] of $[H_2SO_4]$ vs $[H^+]$. An increase in $[H^+]$ (0.1 to 2.0 M) had no effect on the rate.

Dependence of rate on Mn(II) and added salts

The effect on the rate of varying the concentration of Mn(II) (which is the reduction product of the oxidant) was investigated. An increase in [Mn(II)] (from 0.01 to 0.1 M) had no effect on the rate. Similarly, the effects of the anions Cl^- (from 0.01 to 0.5 M), SO_4^{2-} (from 0.01 to 0.5 M) and ClO_4^- (from 0.01 to 1.0 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.

Table 1. Effect of varying reactant concentration on the reaction rate, with $[H_2SO_4] = 0.2$ mol dm^{-3}, [Mn(II)] = 0.02 mol dm^{-3}, $T = 298$ K

$[Mn(III)] \times 10^3$ (mol dm^{-3})	$[PP] \times 10^3$ (mol dm^{-3})	$k_{obs} \times 10^5$ (s^{-1})		
		GFGVP	GVGVP	VPGVG
0.5	5.0	42.22	30.00	10.80
1.0	5.0	42.32	30.61	10.81
1.5	5.0	42.01	30.21	10.52
2.0	5.0	42.52	30.50	10.60
2.5	5.0	42.31	30.11	10.21
3.0	5.0	42.10	30.41	10.51
1.0	3.0	25.10	15.42	6.01
1.0	3.5	28.81	18.62	7.04
1.0	4.0	33.81	22.30	8.30
1.0	4.5	38.02	25.71	9.31
1.0	5.0	42.22	30.00	10.80
1.0	5.5	45.70	33.11	11.71
1.0	6.0	50.12	36.32	12.82
1.0	6.5	53.70	40.71	14.12

Effect of solvent composition

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

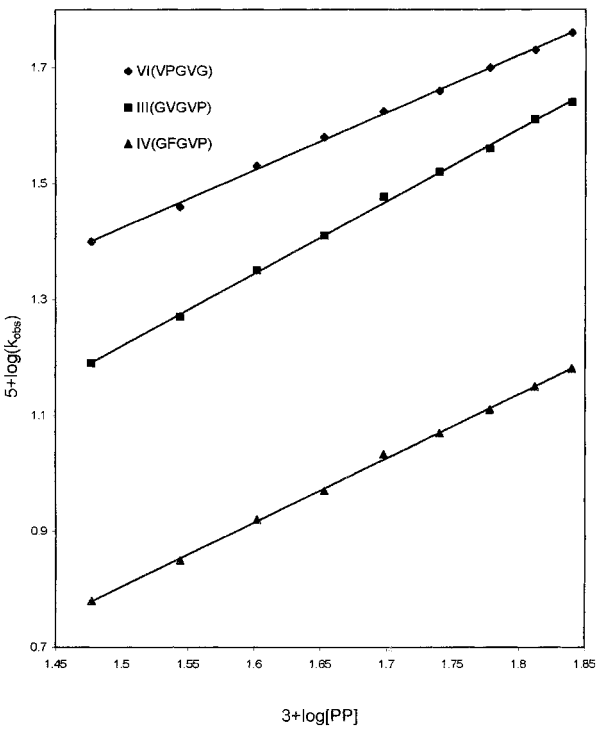


Figure 1. Effect of [PP] on the reaction rate

Table 2. Effect of varying dielectric constant on the reaction rate, with $[Mn(III)] = 1.0 \times 10^{-3}$ mol dm^{-3}, $[PP] = 5 \times 10^{-3}$ mol dm^{-3}, [Mn(II)] = 0.02 mol dm^{-3}, $[H^+]$ = 0.2 mol dm^{-3}, T = 298 K

MeOH (%, v/v)	Dielectric constant (D)	$k_{obs} \times 10^5$ (s^{-1})		
		GFGVP	GVGVP	VPGVG
0	76.73	42.22	30.00	10.80
10	72.37	53.01	39.81	12.81
20	67.48	71.92	58.82	15.80
30	62.71	97.93	87.02	21.32
40	58.06	144.61	143.92	29.50

Table 3. Temperature dependence of the oxidation of PP by Mn(III), with $[Mn(III)] = 1.0 \times 10^{-3}$ mol dm^{-3}, $[PP] = 5 \times 10^{-3}$ mol dm^{-3}, [Mn(II)] = 0.02 mol dm^{-3}, $[H^+]$ = 0.2 mol dm^{-3}

Temperature (K)	$(1/T) \times 10^{-3}$ (K^{-1})	$k_{obs} \times 10^5$ (s^{-1})		
		GFGVP	GVGVP	VPGVG
293	3.413	30.11	20.81	7.91
298	3.355	42.22	30.00	10.80
303	3.300	56.50	39.82	14.71
308	3.246	81.02	57.01	20.42
313	3.195	112.00	72.42	26.93

rate increased with increase in methanol content (Table 2). The plots of logk_{obs} vs $1/D$ (D = dielectric constant of the medium) were linear with positive slopes (Fig. 2). Measurements of rate constants were carried out in both the presence and absence of pentapeptides with Mn(III) and the rate constants were taken for the calculation of the effective k_{obs}, although the rate of oxidation of methanol in the absence of PP is negligible under the conditions employed.

Activation parameters

To determine the activation parameters, the reactions were carried out at different temperatures (20–40 °C, Table 3). The Arrhenius plots of logk_{obs} vs $1/T$ (Fig. 3) were linear. The activation energies (E_a) were calculated from the slope of the plots. From these values, the thermodynamic parameters $\Delta H^{\neq}$, $\Delta S^{\neq}$ and $\Delta G^{\neq}$ and the frequency factor (log$A$) were evaluated (Table 4).

Test for free radicals

Addition of reaction mixture to aqueous acrylamide monomer solutions did not initiate polymerization, indicating the absence of *in situ* formation of free radical species in the reaction sequence.

Reaction stoichiometry

Mixtures containing PP (0.001 M), acid (0.1 M) and

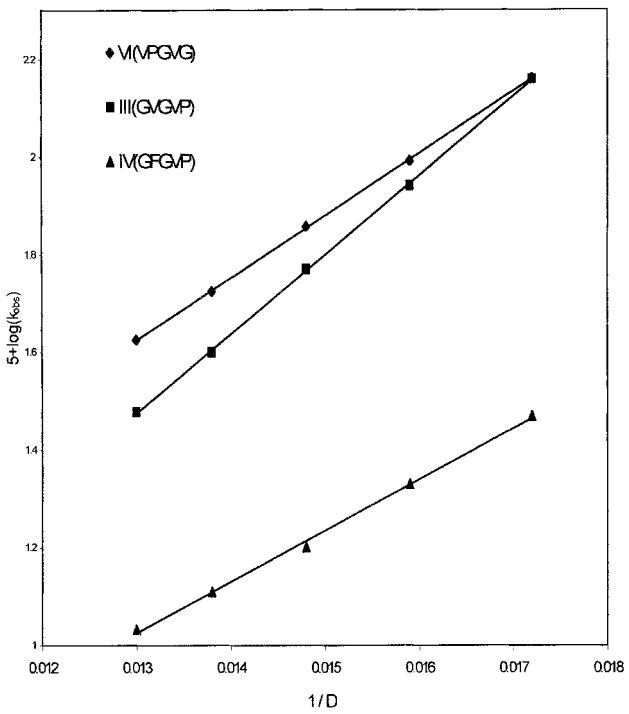


Figure 2. Effect of dielectric constant on the reaction rate

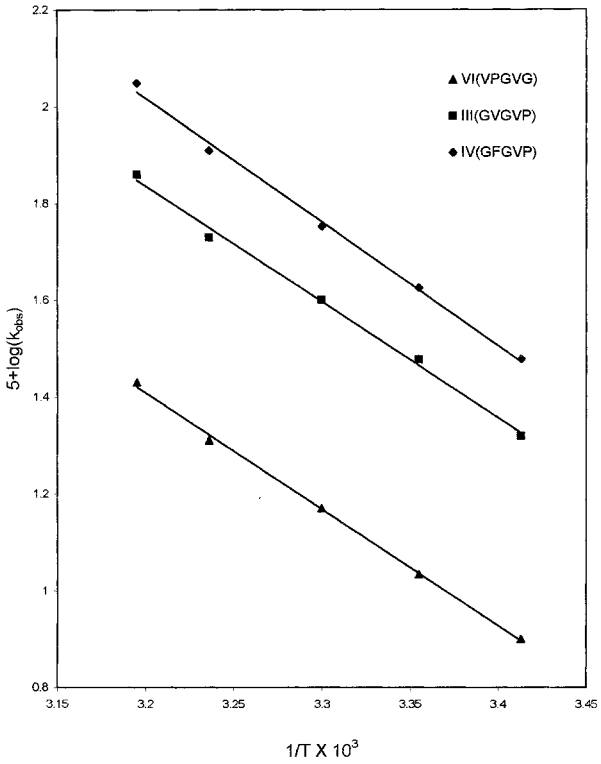


Figure 3. Effect of temperature on the reaction rate

Table 4. Activation parameters for the oxidation of PP by Mn(III), with [Mn(III)] = 1.0×10^{-3} mol dm^{-3}, [PP] = 5×10^{-3} mol dm^{-3}, [Mn(II)] = 0.02 mol dm^{-3}, [H^+] = 0.2 mol dm^{-3}

Substrate	E_a (kJ mol^{-1})	$\Delta H^{\neq}$ (kJ mol^{-1})	$\Delta S^{\neq}$ (J K^{-1} mol^{-1})	$\Delta G^{\neq}$ (kJ mol^{-1})	Log$A$
GFGVP	47.291	44.771	−170.59	96.46	4.323
GVGVP	50.928	48.408	−153.08	93.94	5.385
VPGVG	54.183	51.66	−136.43	93.00	6.118

excess Mn(III) (0.03 M) were kept for 24 h at 25 °C. The unconsumed Mn(III) was then determined; 10 mol of oxidant were sufficient to oxidize 1 mol of PP, leading to aldehydes, carbon dioxide, ammonia, Mn(II) and hydrogen ion. Based on these results, the following stoichiometric equations are suggested.

Gly-Xaa-Gly-Val-Pro (Xaa = Val or Phe):

$$H_2N\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH(R)\text{-}CO\text{-}NH\text{-}CH_2\text{-}CO\text{-}NH\text{-}$$

$$CH[CH\text{—}(CH_3)_2]\text{-}CO\text{-}N\text{—}CH\text{-}COOH + 5Mn^{3+} + 5Mn(OH)^{+2} + 4H_2O \longrightarrow 2HCHO + RCHO + (CH_3)_2CHCHO + {}^{+}NH_3(CH_2)_3CHO + 5CO_2 + 4NH_4^+ + 10Mn^{2+} \quad (1)$$

R = $(CH_3)_2CH$- for Gly-Val-Gly-Val-Pro and
R = $C_6H_5CH_2$- for Gly-Phe-Gly-Val-Pro

Val-Pro-Gly-Val-Gly:

$$H_2N\text{-}CH[CH\text{—}(CH_3)_2]\text{-}CO\text{-}N\text{—}CH\text{-}CO\text{-}NH\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH[CH(CH_3)_2]\text{-}CO\text{-}NH\text{-}CH_2\text{-}COOH + 5Mn^{+3} + 5Mn(OH)^{+2} + 4H_2O \longrightarrow 2HCHO + 2(CH_3)_2CHCHO + {}^{+}NH_3(CH_2)_3CHO + 5CO_2 + 4NH_4^+ + 10Mn^{2+} \quad (2)$$

Product analysis

After the reaction was completed, the reaction products were extracted with diethyl ether and subjected to column chromatography on silica gel (60–200 mesh) using gradient elution (dichloromethane to chloroform). Aldehydes were determined qualitatively by gas chromatography. The retention values of formaldehyde, isobutyraldehyde, phenylacetaldehyde and 4-aminobutyaraldehyde are 6.0, 27.4, 31.09 and 32.1, respectively, which are identical with those for authentic samples. Ammonia and CO_2 were detected by conventional tests.

DISCUSSION

Data published by Diebler and Sutin[26], Packler and Chawla[27] and Wells and Davies[28] have shown that in the presence of F^- ion, an aqueous solution of Mn(III) consists of hexaaquamanganese(III) {$[Mn(H_2O)_6]^{+3}$}, $Mn(III)_{(aq)}$, hydroxopentaaquamanganese(II) {$[Mn(OH)(H_2O)_5]^{+2}$}, $Mn(OH)^{2+}{}_{(aq)}$ and $MnF^{2+}{}_{(aq)}$. Hence it can be assumed with justification that the Mn(III) species present in sulphuric acid medium are $Mn(III)_{(aq)}$, $Mn(OH)^{2+}{}_{(aq)}$ and $MnSO_4{}^+$. Therefore, it was shown[29] that manganese(III) sulphate in aqueous sulphuric acid contains $Mn^{3+}{}_{(aq)}$ and $Mn(OH)^{2+}{}_{(aq)}$ as reaction species:

$$Mn^{3+}{}_{(aq)} + H_2O \rightleftharpoons Mn\ (OH)^{2+}{}_{(aq)} + H^+ \quad (3)$$

The hydrolysis constant calculated was $K_h = 0.93 \pm 0.03$. The absorption spectra of both $Mn^{3+}{}_{(aq)}$ and $Mn(OH)^{2+}{}_{(aq)}$ have been reported to be similar in both the visible and UV regions. Our observation of the electronic absorption spectra is consistent with the values reported.

The electrochemical studies of Biedermann and Palombari[30] also indicated a significant amount of $Mn(OH)_2^+$:

$$Mn(OH)^{2+} + H_2O \rightarrow Mn(OH)_2^+ + H^+ \quad (4)$$

However, a fresh solution of mangenese(III)sulphate was always prepared and used immediately after cessation of the electrolysis, thereby eliminating any reaction due to $Mn(OH)_2^+$. The absence of a sulphate ion effect on the reaction rate indicates that $MnSO_4^+{}_{(aq)}$ is not the active species under the present conditions. The fact that there is no hydrogen ion dependence on the rate suggests that $Mn^{3+}{}_{(aq)}$ and $Mn(OH)^{2+}$ are the reactive species.[31] The molar absorptivity ε ranges between 131 and 110 dm^3 mol^{-1} cm^{-1} at [H^+] = 1.20–2.50 mol dm^{-3}. The high value of ε has been attributed to the presence of hydrolysed $Mn(OH)^{2+}$ species. Therefore, it is probable that $Mn(OH)^{2+}$ is the likely reactive species. Scheme 1 accounts for the observed experimental results.

$$Mn(OH)^{2+} + PP \xrightarrow[(i)]{k_1} X \qquad \text{slow and rate-determining step}$$

$$X \xrightarrow[(ii)]{k_2} \text{products} \qquad \text{fast}$$

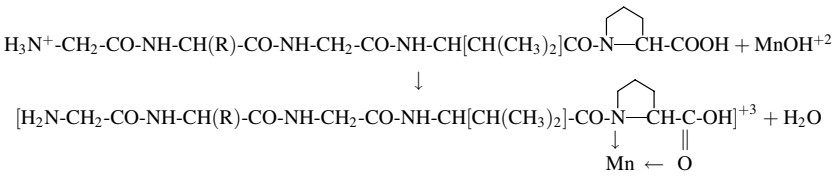
$$\text{rate} = k'[Mn(OH)^{2+}][PP]$$

Scheme 1

Amis[32] has shown that plots of log k_{obs} vs 1/D gives a

MECHANISM:

For Gly-Xaa-Gly-Val-Pro



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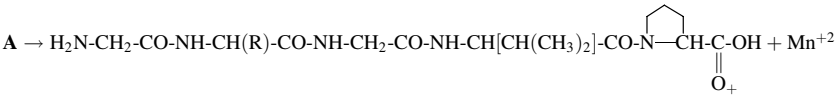

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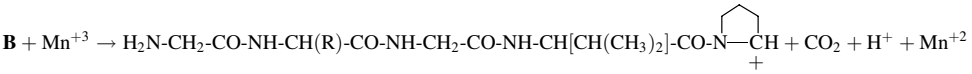

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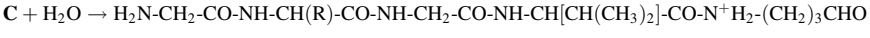

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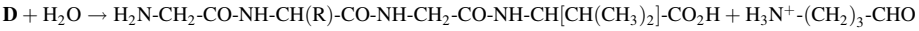

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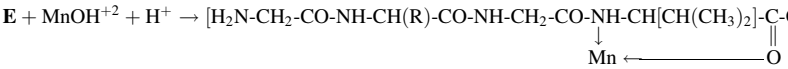

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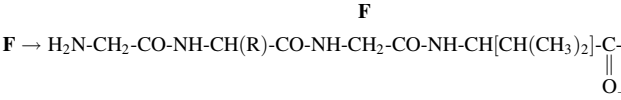

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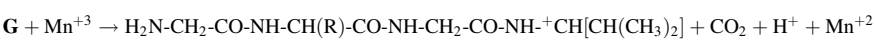

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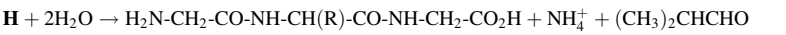

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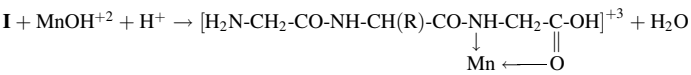

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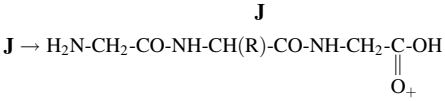

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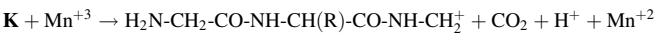

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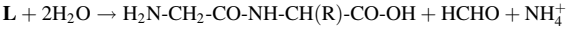

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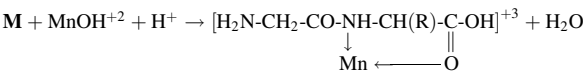

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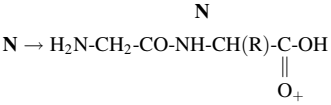

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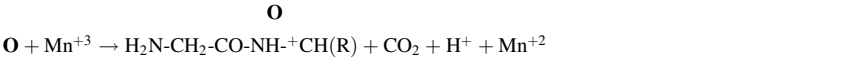

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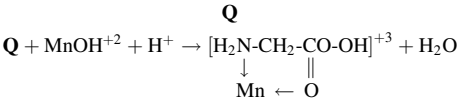


R

$$\mathbf{R} \rightarrow \mathrm{H_2N\text{-}CH_2\text{-}\underset{\overset{\|}{O_+}}{C}\text{-}OH} + \mathrm{Mn^{+2}}$$

S

$$\mathbf{S} + \mathrm{Mn^{+3}} \rightarrow \mathrm{H_2N\text{-}^{+}CH_2} + \mathrm{CO_2} + \mathrm{H^+} + \mathrm{Mn^{+2}}$$

T

$$\mathbf{T} + \mathrm{H_2O} \rightarrow \mathrm{CH_2O} + \mathrm{NH_4^+}$$

R = $CH(CH_3)_2$ for GVGVP

R = $C_6H_5CH_2$ for GFGVP

For Val-Pro-Gly-Val-Gly

$$\mathrm{H_3N^+\text{-}CH\{CH(CH_3)_2\}\text{-}CO\text{-}N\text{—}CH\text{-}CO\text{-}NH\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH\{CH(CH_3)_2\}\text{-}CO\text{-}NH\text{-}CH_2\text{-}COOH} + \mathrm{MnOH^{+2}}$$

$$\downarrow$$

$$\mathrm{[H_2N\text{-}CH\{CH(CH_3)_2\}\text{-}CO\text{-}N\text{—}CH\text{-}CO\text{-}NH\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH\{CH(CH_3)_2\}\text{-}CO\text{-}\underset{\downarrow}{NH}\text{-}CH_2\text{-}\underset{\overset{\|}{O}}{C}\text{-}OH]^{+3}} + \mathrm{H_2O}$$

$$\mathrm{Mn \leftarrow O}$$

A

$$\mathbf{A} \rightarrow \mathrm{H_2N\text{-}CH\{CH(CH_3)_2\}CO\text{-}N\text{—}CH\text{-}CO\text{-}NH\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH\{CH(CH_3)_2\}\text{-}CO\text{-}NH\text{-}CH_2\text{-}\underset{\overset{\|}{O_+}}{C}\text{-}OH} + \mathrm{Mn^{+2}}$$

B

$$\mathbf{B} + \mathrm{Mn^{+3}} \rightarrow \mathrm{H_2N\text{-}CH\{CH(CH_3)_2\}CO\text{-}N\text{—}CH\text{-}CO\text{-}NH\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH\{CH(CH_3)_2\}\text{-}CO\text{-}NH\text{-}CH_2^+} + \mathrm{CO_2} + \mathrm{H^+} + \mathrm{Mn^{+2}}$$

C

$$\mathbf{C} + \mathrm{H_2O} \rightarrow \mathrm{H_2N\text{-}CH\{CH(CH_3)_2\}CO\text{-}N\text{—}CH\text{-}CO\text{-}NH\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH\{CH(CH_3)_2\}\text{-}CO\text{-}NH_2} + \mathrm{CH_2O}$$

D

$$\mathbf{D} + \mathrm{H_2O} \rightarrow \mathrm{H_2N\text{-}CH\{CH(CH_3)_2\}CO\text{-}N\text{—}CH\text{-}CO\text{-}NH\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH\{CH(CH_3)_2\}\text{-}COOH} + \mathrm{NH_4^+}$$

E

$$\mathbf{E} + \mathrm{MnOH^{+2}} + \mathrm{H^+} \rightarrow \mathrm{[H_2N\text{-}CH\{CH(CH_3)_2\}CO\text{-}N\text{—}CH\text{-}CO\text{-}\underset{\downarrow}{NH}\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH\{CH(CH_3)_2\}\text{-}\underset{\overset{\|}{O}}{C}\text{-}OH]^{+3}} + \mathrm{H_2O}$$

$$\mathrm{Mn \longleftarrow O}$$

F

$$\mathbf{F} \rightarrow \mathrm{H_2N\text{-}CH\{CH(CH_3)_2\}CO\text{-}N\text{—}CH\text{-}CO\text{-}NH\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH\{CH(CH_3)_2\}\text{-}\underset{\overset{\|}{O_+}}{C}\text{-}OH} + \mathrm{Mn^{+2}}$$

G

$$\mathbf{G} + \mathrm{Mn^{+3}} \rightarrow \mathrm{H_2N\text{-}CH\{CH(CH_3)_2\}CO\text{-}N\text{—}CH\text{-}CO\text{-}NH\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH\{CH(CH_3)_2\}} + \mathrm{CO_2} + \mathrm{H^+} + \mathrm{Mn^{+2}}$$

H

$$\mathbf{H} + 2\mathrm{H_2O} \rightarrow \mathrm{H_2N\text{-}CH\{CH(CH_3)_2\}CO\text{-}N\text{—}CH\text{-}CO\text{-}NH\text{-}CH_2\text{-}CO\text{-}OH} + \mathrm{(CH_3)_2CHCHO} + \mathrm{NH_4^+}$$

I

$$\mathbf{I} + \mathrm{MnOH^{+2}} + \mathrm{H^+} \rightarrow \mathrm{[H_2N\text{-}CH\{CH(CH_3)_2\}CO\text{-}N\text{—}CH\text{-}CO\text{-}\underset{\downarrow}{NH}\text{-}CH_2\text{-}\underset{\overset{\|}{O}}{C}\text{-}OH]^{+3}}$$

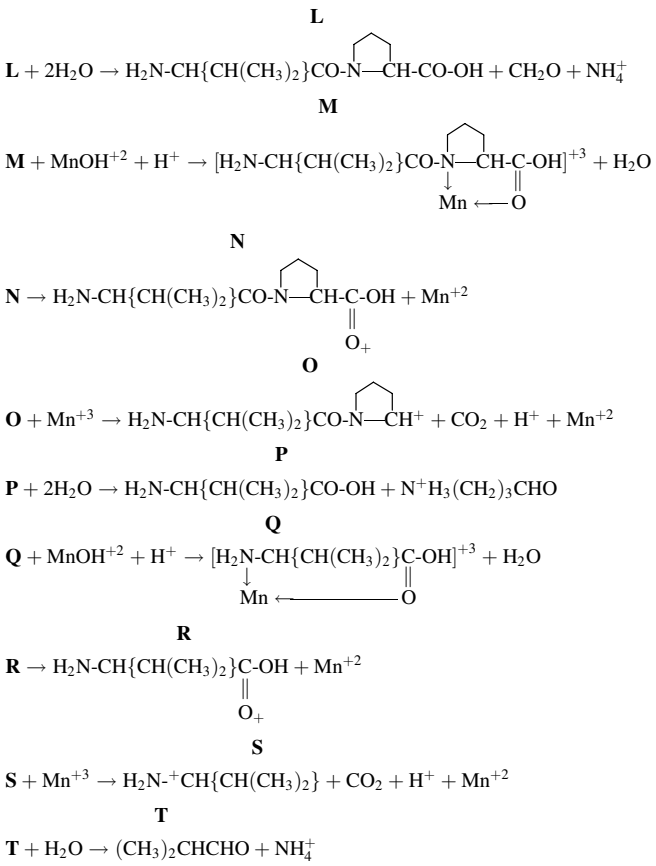
$$\mathrm{Mn \longleftarrow O}$$

J

$$\mathbf{J} \rightarrow \mathrm{H_2N\text{-}CH\{CH(CH_3)_2\}CO\text{-}N\text{—}CH\text{-}CO\text{-}NH\text{-}CH_2\text{-}\underset{\overset{\|}{O_+}}{C}\text{-}OH} + \mathrm{Mn^{+2}}$$

K

$$\mathbf{K} + \mathrm{Mn^{+3}} \rightarrow \mathrm{H_2N\text{-}CH\{CH(CH_3)_2\}CO\text{-}N\text{—}CH\text{-}CO\text{-}NH\text{-}CH_2^+} + \mathrm{CO_2} + \mathrm{H^+} + \mathrm{Mn^{+2}}$$



Scheme 2

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 Scheme 2.

The rates of oxidation of pentapeptides by Mn(III) were compared with those of oxidation of constituent tripeptides, dipeptides and free amino acids by Mn(III) under identical experimental conditions, and it was found that the rates of oxidation of pentapeptides were slower than those of either tripeptides, dipeptides and free amino acids. The change in each case is due to the increased distance between the functional groups and consequently weaker electrostatic effects. Hence the oxidation of the pentapeptides is expected to be slower than that of the monomers, dipeptides and tripeptides. Further, an apparent correlation was noted between the rate of oxidation and the hydrophobicity of those sequences where increased hydrophobicity results in an increased rate of oxidation. The most hydrophobic pentamer, Gly-Phe-Gly-Val-Pro, oxidized at a faster rate than the less hydrophobic pentamers, Gly-Val-Gly-Val-Pro and Val-Pro-Gly-Val-Gly. The probable reason for the increased oxidation rate for the more hydrophobic peptides is that the carboxylic groups are more destabilized, which enhances the rate of formation of a transition-state complex with Mn(III), and the oxidation rate may be high. Further, it was observed that the pentamers with Pro as C-terminus, Gly-Val-Gly-Val-Pro and Gly-Phe-Gly-Val-Pro, are more susceptible to oxidation than the pentamer with Gly as C-terminus, Val-Pro-Gly-Val-Gly. This is in good agreement with our earlier findings.[33,34]

Spectral evidence for the formation of PP–Mn(III) complex

The study of the UV–visible spectra separately of pure Mn(III), PP (Gly-Phe-Gly-Val-Pro, Gly-Val-Gly-Val-Pro and Val-Pro-Gly-Val-Gly) and a mixture of Mn(III) and PP showed deviations in the peak wavelength (λ_{max}) and absorbance (Abs.) as shown in Table 5.

Table 5. UV–visible spectral data

Substrate	λ_{max} (nm)	Abs.	Complex	λ_{max} (nm)	Abs.
Mn(III)	500	0.970			
GVGVP	222.4	2.892	Mn(III) + GVGVP	242.5	3.082
GFGVP	220.5	2.829	Mn(III) + GFGVP	240.5	3.063
VPGVG	221.5	2.842	Mn(III) + VPGVG	235.5	3.200

REFERENCES

1. Stadtman ER. *Science* 1992; **257**: 1220.
2. Berlett BS, Stadtman ER. *J. Biol. Chem.* 1997; **272**: 20313.
3. Boucher J. *Coord. Chem. Rev.* 1972; **7**: 289.
4. Carlvin M. *Rev. Pure Appl. Chem.* 1965; **15**: 1.
5. Davies G. *Coord. Chem. Rev.* 1969; **4**: 199.
6. Rangappa KS, Chandraju S, Made Gowda NM. *Synth. React. Inorg. Met.–Org. Chem.* 1998; **28**: 275.
7. Rangappa KS, Chandraju S, Made Gowda NM. *Int. J. Chem. Kinet.* 1998; **30**: 7.
8. Rangappa KS, Manjunathaswamy H, Ragavendra MP, Channe Gowda D. *Carbohydr. Res.* 1998; **307**: 253.
9. Rangappa KS, Manjunathaswamy H, Ragavendra MP, Channe Gowda D. *J. Org. Chem.* 1998; **63**: 531.
10. Rangappa KS, Ragavendra MP, Mahadevappa DS. *Carbohydr. Chem.* 1997; **16**: 359.
11. Mahadevappa DS, Ananda S, Made Gowda NM, Rangappa KS. *J. Chem. Soc., Perkin Trans. 2* 1985; **11**: 39.
12. Rangappa KS, Chandaraju S, Mahadevappa DS. *Transition Met. Chem.* 1996; **21**: 519.
13. Asha Iyengar T, Mahadevappa DS. *Indian J. Chem.* 1992; **31A**: 752.
14. Sandberg LB, Leslie JG, Leach CT, Torres VL, Smith AR, Smith DW. *Pathol. Biol.* 1985; **33**: 266.
15. Yeh H, Ornstein-Goldstein N, Indik Z, Sheppard P, Anderson N, Rosenbloom JC, Cicila G, Yoon K, Rosenbloom J. *Collagen Relat. Res.* 1987; **7**: 235.
16. Sandberg LB, Soskel NT, Leslie JB. *N. Engl. J. Med.* 1981; **304**: 566.
17. Urry DW, Long MM. *CRC Crit. Rev. Biochem.* 1976; **4**: 1.
18. Urry DW, Nicol A, Gowda DC, McKee LD, Williams T, Olsen DB, Cox BA. In *Biotechnological Polymers: Medical, Pharmaceutical and Industrial Applications, a Conference in Print*, Gebelein CG (ed). Technomic Publishing Co. Inc., Atlanta Georgia. 1993; 82.
19. Urry DW, McPherson DT, Xu J, Daniell H, Guda C, Channe Gowda D, Jing N, Parker TM. Polymeric Materials Encyclopaedia New York, Boca Raton, FL: CRC Press, 1996; **9**: 7263.
20. Prasad KU, Iqbal MA, Urry DW. *Int. J. Pept. Protein Res.* 1985; **24**: 408.
21. Luan CH, Parker TM, Channe Gowda D, Urry DW. *Biopolymers* 1992; **32**: 1251.
22. Anwer MK, Spatola AF. *Synthesis* 1980; 929.
23. Andreu D, Merrifield RB, Steiner H, Boman HG. *Proc. Natl. Acad. Sci. USA* 1983; **80**: 6475.
24. Kamaluddin. *Indian J. Chem.* 1980; **19A**: 431.
25. Pinto I, Sherigara BS, Udupa HVK. *J. Chem. Soc. Jpn.* 1983; **63**: 3625.
26. Diebler M, Sutin N. *J. Phys. Chem.* 1964; **68**: 174.
27. Packler P, Chawla ID. *Inorg. Chem. Soc.* 1964; **38**: 1130.
28. Wells CF, Davies GO. *J. Chem. Soc. A* 1967; 1858.
29. Sherigara BS, Bhat KI, Pinto I, Made Gowda NM. *Int. J. Chem. Kinet.* 1995; **27**: 675.
30. Biedermann G, Palombari R. *Acta Chem. Scand. Ser. A* 1978; **32**: 381.
31. Siskos PA, Peterson NC, Huie RE. *Inorg. Chem.* 1984; **23**: 1134.
32. Amis ES. *J. Chem. Educ.* 1953; **30**: 351.
33. Kumar MN, Channe Gowda D, Rangappa KS. *Int. J. Chem. Kinet.* 2001; in press.
34. Channe Gowda D, Kempe Gowda BK, Rangappa KS. *Synth. React. Inorg. Met.–Org. Chem.* 2001; in press.