# Hydrophobicity Dependence of Oxidation of Tetrapeptides of Elastin Sequences with Mn(III): Synthesis, Characterization, Kinetics, and Mechanistic Study

B. K. KEMPE GOWDA, H. S. PRASAD, K. S. RANGAPPA, D. CHANNE GOWDA

*Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore-570006, India*

*Received 2 February 2001; accepted 9 July 2001*

ABSTRACT: The analogues of elastin sequences, glycyl-glycyl-alanyl-proline (GGAP), glycyl-glycyl-phenylalanyl-proline (GGFP), and glycyl-glycyl-isoleucyl-proline (GGIP) were synthesized by classical solution phase method and characterized. The kinetics of oxidation of these tetrapeptides (TETP) by Mn(III) has been studied in the presence of sulphate ions in acidic solution at 25°C. The reaction was followed spectrophotometrically at $\lambda_{max} = 500$ nm. A first-order dependence of rate on both [Mn(III)] and [TETP] was observed. The rate is independent of the concentration of the 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, or perchlorate were studied. Activation parameters have been evaluated using Arrhenius and Erying plots. The oxidation products were isolated and characterized. A mechanism involving the reaction of TETP 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 increased rate of oxidation. © 2001 John Wiley & Sons, Inc. Int J Chem Kinet 34: 39–48, 2002

## INTRODUCTION

Oxidative reactions play an important role in a variety of biochemical events ranging from normal metabolism to aging and disease processes [1,2]. Peptides and proteins represent major targets for modification in these reactions, and identification of sites and structures of modifications may lead to mechanistic understanding and approaches for prevention. In this account, oxidation by Mn(III) is of special importance due to its biological relevance [3]. Manganese(III) porphyrins have been studied as possible models for

*Correspondence to:* D. Channe Gowda; e-mail: dcgowda@yahoo.com.

ODI 10.1002/kin.10015

closely related biologically significant systems [4]. Several studies have been reported on the kinetics of oxidation of substrates with Mn(III) 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) oxidant have not been reported in the literature except the oxidative behavior of bromamine-B towards glycyl-glycine [13].

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 $(Val^1\text{-}Pro^2\text{-}Gly^3\text{-}Val^4\text{-}Gly^5)_n$ where $n$ is eleven 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 $n$ greater than 2 without substitution [16]. The monomers, oligomers, and high polymers of these repeats have been synthesized and confirmationally characterized [17]. These polymers have a number of medical and nonmedical applications [18,19]. The cross-linked polytetrapeptide matrices based on the repeating amino acid sequences, Gly-Gly-Ala-Pro, Gly-Gly-Ile-Pro, and Gly-Gly-Val-Pro were tested for cell adhesion promoting activity in both the absence and presence of fetal bovine serum [20]. The degree of cell attachment increased with increase of hydrophobicity. In this context, it was thought interesting to investigate the oxidative behavior of Mn(III) towards these tetrapeptides and this is reported in the present paper.

The peptides were synthesized by the classical solution phase methods. The classical methods for synthesis in solution are labor-intensive, time-consuming, and skill-intensive in large part because the intermediates differ in solubility characteristics. With all of 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 tert-butyloxycarbonyl (Boc) chemistry was used instead of more expensive 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry.

The synthesis was carried out by the stepwise solution phase method. The Boc group was used for temporary $N^{\alpha}$-protection and its removal was achieved with 4 N HCl in dioxane or trifluoroacetic acid. The C-terminus carboxyl group was protected by the benzyl ester and its removal was effected by hydrogenolysis using $HCOONH_4$/Pd–C (10%) [21]. All coupling reactions were achieved with isobutylchloroformate. The protected peptides were purified by recrystalization and characterized by physical and analytical techniques. The purity of the free peptides was checked by paper chromatography, HPLC and amino acid analysis.

## EXPERIMENTAL

All the amino acids used except glycine are of L-configuration unless otherwise specified. All tert-butyloxycarbonyl (Boc) amino acids, amino acid derivatives, 1-hydroxybenzotriazole (HOBt), and trifluoroacetic acid (TFA) were purchased from Advanced Chem. Tech., (Louisville, Kentucky, USA). Isobutylchloroformate and *N*-methylmorpholine (NMM) were purchased from Sigma Chemicals (St. Louis, USA). All solvents and reagents were of analytical grade or were purified according to procedure [22] recommended for peptide synthesis. Thinlayer chromatography (TLC) was carried out on silica gel plates obtained from Whatman Inc., with the following solvent systems: chloroform–methanol–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 the TLC plates were detected by UV light 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. The melting points were determined using Selaco Can. No-103 melting point apparatus and were uncorrected. Elemental analyses was carried out by Mic Anal (Tucson, Arizona, USA). The optical rotation was measured using a Perkin-Elmer 243 digital polarimeter. Amino acid analysis was performed on water HPLC Pico-Tag analyzer. Hydrolysis of the sample was carried out using 6 N HCl containing 1% phenol by volume at 110°C for 72 h in a sealed tube under vacuum from which the air has been removed using nitrogen. The product analysis was carried out with gas chromatography (GC 15A, Shimadzu, Japan).

**Boc-Phe-Pro-OBzl (I).** To Boc-Phe-OH (13.3 g, 0.05 mol) dissolved in acetonitrile (100 ml) and cooled to 0°C NMM (5.5 ml, 0.05 mol) was added. The solution was then cooled to $-15°C \pm 1°C$ and isobutylchloroformate (6.5 ml, 0.05 mol) was added under stirring while maintaining the temperature at $-15°C$. After stirring the reaction mixture for 10 min at this temperature, a precooled solution of HOBt (6.8 g, 0.05 mol)

was added. The reaction mixture was stirred for an additional 10 min and a precooled solution of HCl·H-Pro-OBzl (hydrochloride salt of Pro-OBzl) (12.1 g, 0.05 mol) and NMM (5.5 ml, 0.05 mol) in DMF (120 ml) was added slowly. After 20 min, the pH of the solution was adjusted to 8 by the addition of NMM and the reaction mixture stirred over night at room temperature. Acetonitrile was removed under reduced pressure and the residual DMF solution was poured into about 600 ml ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 min. The precipitated peptide was filtered, washed with water, 1 N HCl, and water and then dried. The crude peptide was recrystallised from ether and petroleum ether to obtain 19.5 g of I (yield, 86.5%).

**Boc-Gly-Phe-Pro-OBzl (II).** I (13.6 g, 0.03 mol) was deblocked with 4.1 N HCl/dioxane (10 ml/g of peptide) for 1.5 h. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether, and dried (yield, 100%). The HCl·H-Phe-Pro-OBzl (hydrochloride salt of Phe-Pro-OBzl) was neutralized with NMM (3.3 ml, 0.03 mol) and coupled to Boc-Gly (5.3 g, 0.03 mol) in acetonitrile (50 ml) and NMM (3.3 ml) using isobutylchloroformate (4.1 ml, 0.03 mol) and worked up the same as I to obtain 13.4 g of II (yield, 87.7%). The sample was recrystallized from ether/petroleum ether.

**Boc-Gly-Gly-Xaa-Pro-OBzl. (Xaa is Ala for III, Ile for IV, and Phe for V).** The peptides Boc-Gly-Ile-Pro-OBzl and Boc-Gly-Ala-Pro-OBzl were prepared as previously described [23]. Each peptide (Boc-Gly-Phe-Pro-OBzl, Boc-Gly-Ile-Pro-OBzl, and Boc-Gly-Ala-Pro-OBzl, 0.02 mol) was deblocked separately with 4.1 N HCl/dioxane (10 ml/g of peptide) for 1.5 h. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether, and dried (yield, 100%). The HCl·H-Gly-Xaa-Pro-OBzl was neutralized with NMM (2.2 ml, 0.02 mol) and coupled to Boc-Gly (3.5 g, 0.02 mol) in acetonitrile (35 ml) and NMM (2.2 ml, 0.02 mol) using isobutylchloroformate (2.6 ml, 0.02 mol) and worked up the same as I to obtain III–V. The samples were recrystallized from ether/petroleum ether.

**Boc-Gly-Gly-Xaa-Pro-OH. (Xaa is Ala for VI, Ile for VII, and Phe for VIII).** Each peptide (III–V, 0.01 mol) was hydrogenolyzed in methanol (10 ml/g of peptide) using ammonium formate (2.0 equiv.) and 10% Pd–C (0.1 g/g of 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 taken in to $CHCl_3$, washed with water, and dried over $Na_2SO_4$. The solvent was removed under reduced pressure and triturated with ether, filtered, washed with ether, and then dried to obtain VI–VIII.

**Gly-Gly-Xaa-Pro. (Xaa is Ala for IX, Ile for X, and Phe for XI).** Each peptide (VI–VIII, 0.009 mol) was deblocked with TFA (10 ml/g of peptide) for 40 min. The solvent was removed under reduced pressure, triturated with ether, filtered, washed with ether to obtain TFA salt of IX–XI (yield, 100%).

## Preparation of Mn(III) Sulphate

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

## Preliminary Studies

The maximum absorption ($\lambda_{max}$) of Mn (III) sulphate solution occurs at 500 nm. The standard reduction potential $E_0'$ of Mn(III)/Mn(II), the oxidizing power of the oxidant, generally decreases on complexation. The standard redox potential was measured at the specified experimental conditions. These details were 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 $Cl^-$ is 1.51, 1.48 and 1.42 V respectively. Triply distilled water was used for preparing aqueous solutions.

## Kinetic Measurements

Mixtures of solution containing requisite amounts of TETP, sulphuric acid (to maintain known acid concentration), Mn(II), and water (to keep the total volume constant) were prepared in stoppered boiling tubes. The mixture was thermally equilibrated in a water bath at 25°C. To the solution in this tube an aliquot of pre-equilibrated Mn(III) sulphate stock solution was added 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) versus time were linear. The rate constants $k_{obs}$ calculated from these plots were reproducible within $\pm$ 3% error.

## RESULTS

### Peptide Synthesis

The purity of the protected peptides (I–VIII) was checked by TLC and of the free peptides (IX–XI) by paper chromatography and HPLC. The yields, physical and analytical data of protected peptides are listed in Table I. The amino acid analysis of free peptides is shown in Table II.

### Dependence of Rate on [Mn(III)] and [TETP]

All kinetic runs were performed under pseudo first order conditions of [TETP] $\gg$ [Mn(III)]. Plots of log [Mn(III)] vs. time were linear even beyond 75% of the reaction, showing first order dependence of the rate on [Mn(III)]. At constant $[Mn(III)]_0$, $[Mn(II)]_0$, $[H_2SO_4]$, $[Na_2SO_4]$, and temperature, the rate increased with increase in $[TETP]_0$. Plots of log $k_{obs}$ vs. log $[TETP]_0$ (Fig. 1) were linear with slopes of 1.01, 1.00, and 1.00 for Gly-Gly-Ala-Pro, Gly-Gly-Phe-Pro, and Gly-Gly-Ile-Pro, respectively.

### Dependence of Rate on [Acid]

Kinetic measurements were performed in $H_2SO_4$–$NaHSO_4$ solutions at different $[H^+]$. The effective $[H^+]$ used was evaluated with the aid of a standard curve [25] of $[H_2SO_4]$ vs. $[H^+]$. Increase in $[H^+]$ (0.1–2.0 M), had no effect on the rate.

### Dependence of Rate on [Mn(II)] and Added Salts

The effect on the rate of varying concentrations of Mn(II) (which is the reduction product of the oxidant) was investigated. Increase in [Mn(II)] (0.01–0.1 M) had no effect on the rate. Similarly the effect of anions $Cl^-$ (0.01–0.5 M), $SO_4^{2-}$ (0.01–0.5 M), and $ClO_4^-$ (0.01–1.0 M) on the rate was insignificant. The reaction product Mn(II) had no effect on the reaction, indicating that the product is not involved in a preequilibrium with the oxidant.

### Effect of Solvent Composition

The solvent composition of the medium was varied by adding methanol (0.0–40%) to the reaction mixture. The rate increased with increase in methanol content. The plots of log $k_{obs}$ vs. $1/D$ ($D$ = dielectric constant of the medium) were linear with positive slopes (Fig. 2). Measurement of rate constants were taken both in the presence and absence of TETP oxidized with Mn(III) and used for the calculation of effective $k_{obs}$, although the rate of oxidation of methanol in the absence of TETP is negligible under the present conditions employed.

### Activation Parameters

To determine the activation parameters, the reactions were carried out at different temperatures (20–40°C). The Arrhenius plots of log $k_{obs}$ vs. $1/T$ (Fig. 3)

**Table I** Physical and Analytical Data of Protected Peptides

| Peptides | M.P. (°C) | $R_f^1$ | $R_f^2$ | $R_f^3$ | Yield (%) | $[\alpha]_D^{25}$ (C,1; MeOH) | Molecular Formula | Elemental Analysis (%) C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|
| Boc-Phe-Pro-OBzl (I) | 100 | 0.73 | 0.82 | – | 86.5 | −0.52 | $C_{26}H_{32}N_2O_5$ | 68.90 (69.00) | 7.07 (7.10) | 6.12 (6.19) |
| Boc-Gly-Phe-Pro-OBzl (II) | 86 | 0.66 | 0.74 | – | 87.7 | −72 | $C_{28}H_{35}N_3O_6$ | 66.10 (65.99) | 6.94 (6.92) | 8.22 (8.24) |
| Boc-Gly-Gly-Ala-Pro-OBzl (III) | 56 | 0.63 | 0.70 | – | 89.6 | −79 | $C_{24}H_{34}N_4O_7$ | 57.90 (58.08) | 7.12 (7.10) | 11.61 (11.61) |
| Boc-Gly-Gly-Ile-Pro-OBzl (IV) | 77 | 0.61 | 0.69 | – | 89.6 | −75 | $C_{27}H_{40}N_4O_7$ | 60.66 (60.88) | 7.49 (7.56) | 10.69 (10.70) |
| Boc-Gly-Gly-Phe-Pro-OBzl (V) | 96 | 0.59 | 0.65 | – | 89.6 | −15.3 | $C_{30}H_{38}N_4O_7$ | 63.71 (63.58) | 6.69 (6.75) | 9.86 (9.88) |
| Boc-Gly-Gly-Ala-Pro-OH (VI) | 66 | – | 0.46 | 0.49 | 92.4 | −10.6 | $C_{17}H_{28}N_4O_7$ | 51.02 (50.99) | 7.02 (7.04) | 14.00 (13.99) |
| Boc-Gly-Gly-Ile-Pro-OH (VII) | 99 | – | 0.36 | 0.44 | 91.3 | −66 | $C_{20}H_{34}N_4O_7$ | 54.40 (54.28) | 7.73 (7.74) | 12.63 (12.66) |
| Boc-Gly-Gly-Phe-Pro-OH (VIII) | 105 | – | 0.39 | 0.48 | 93.4 | −33 | $C_{23}H_{32}N_4O_7$ | 57.77 (57.97) | 6.78 (6.76) | 11.74 (11.75) |

[a]Calculated elemental values are in parenthesis.

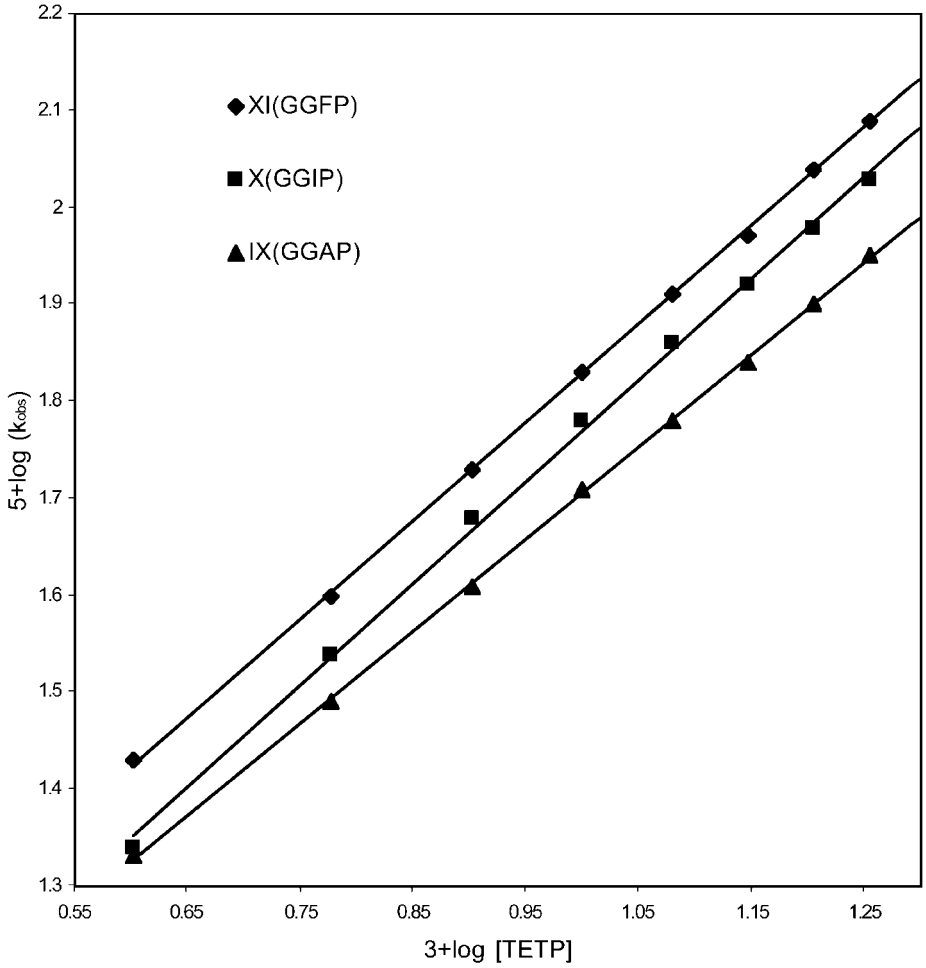



**Figure 1** Effect of varying reactant concentration on the rate. [Mn(II)] = 0.02 mol $dm^{-3}$, and [$H_2SO_4$] = 0.2 mol $dm^{-3}$ at temperature 25°C.

were found to be linear. The activation energies ($E_a$) were calculated from the slope of the plots. From this value, the thermodynamic parameters $\Delta H^{\#}$, $\Delta S^{\#}$, $\Delta G^{\#}$ and the frequency factor (log $A$) were evaluated (Table III).

## Test for Free Radicals

Addition of the reaction mixture to aqueous acrylamide monomer solution initiates polymerization, indicating the in situ formation of free radical species during the reaction sequence.

**Table II** Amino Acid Composition of Free Peptides

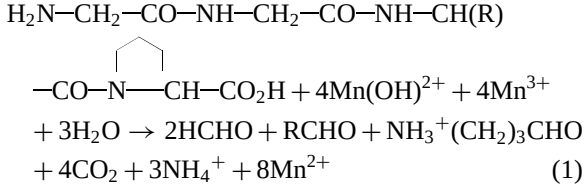
| Peptides | Gly | Pro[a] | Ala | Ile | Phe |
|---|---|---|---|---|---|
| GGAP (IX) | 2.07 (2.00) | 1.00 | 0.98 (1.00) | – | – |
| GGIP (X) | 2.10 (2.00) | 1.00 | – | 0.97 (1.00) | – |
| GGFP (XI) | 1.98 (2.00) | 1.00 | – | – | 1.02 (1.00) |

Calculated values are in parenthesis.
[a]Pro taken as 1.0.

## Reaction Stoichiometry

The mixtures containing TETP (0.001 M), acid (0.1 M), and excess Mn(III) (0.01 M) were kept for 24 h at 25°C. The unconsumed Mn(III) was then determined, 8 moles of oxidant were sufficient to oxidise 1 mole of TETP leading to aldehydes, carbon dioxide, ammonia, Mn(II), and hydrogen ion.

Based on these results the following stoichiometric equations are suggested.



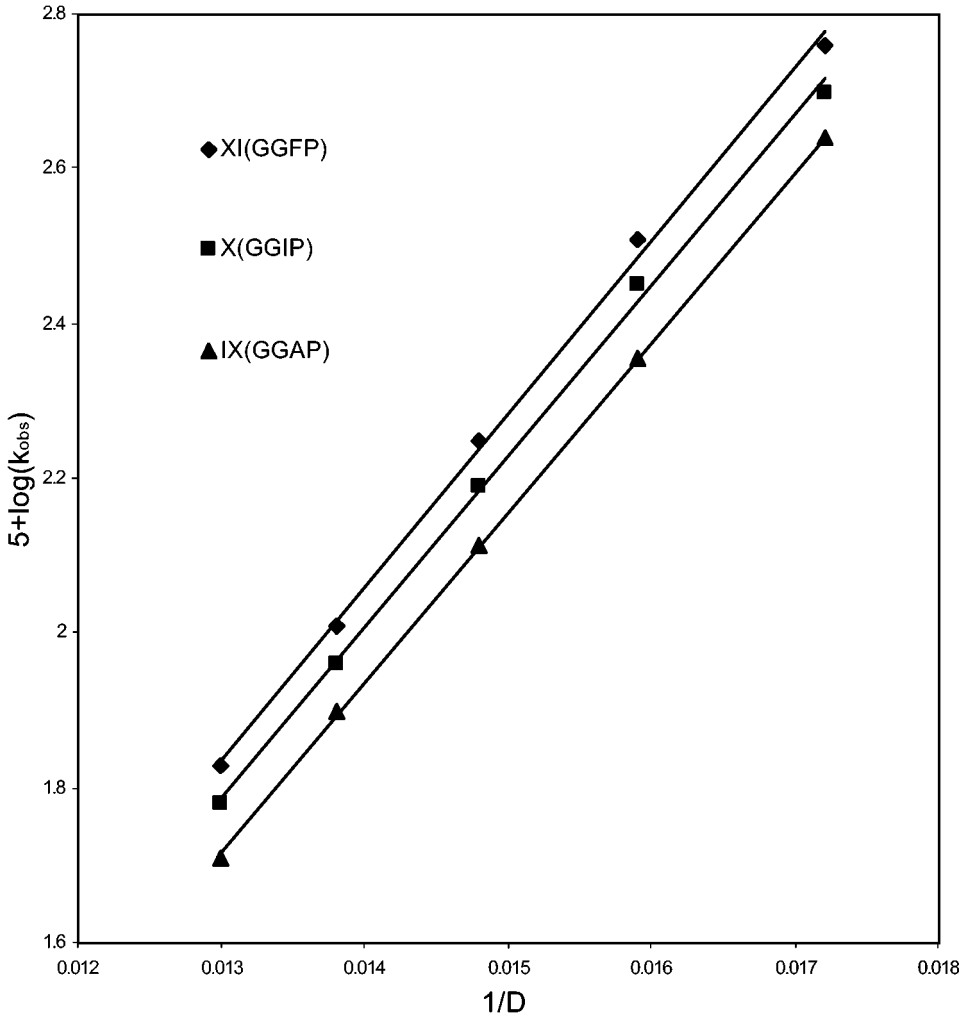



**Figure 2** Effect of varying dielectric constant on the rate. $[Mn(III)] = 2 \times 10^{-3}$ mol dm$^3$, $[TETP] = 1.0 \times 10^{-2}$ mol dm$^3$, $[Mn(II)] = 0.02$ mol dm$^3$, and $[H_2SO_4] = 0.2$ mol dm$^3$ at temperature 25°C.

R:$CH_3$—for Gly-Gly-Ala-Pro; $CH_3$—$CH_2$—$CH(CH_3)$—for Gly-Gly-Ile-Pro and $C_6H_5$—$CH_2$—for Gly-Gly-Phe-Pro.

## 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). Aldehydes were analyzed qualitatively by gas chromatography. The retention values of formaldehyde, acetaldehyde, isobutyraldehyde, phenylacetaldehyde, and 4-aminobutyaraldehyde are 6.0, 5.14, 27.4, 31.09, and 32.1 respectively, which are identical with authentic samples. Ammonia and $CO_2$ were detected by the conventional tests.

**Table III** Activation Parameters for the Oxidation of TETP by Mn(III)

| Substrate | $E_a$ (kJ mol$^{-1}$) | $\Delta H^{\#}$ (kJ mol$^{-1}$) | $\Delta S^{\#}$ (J K$^{-1}$ mol$^{-1}$) | $\Delta G^{\#}$ (kJ mol$^{-1}$) | Log $A$ |
|---|---|---|---|---|---|
| GGAP | 59.93 | 57.42 | −110.05 | 90.73 | 7.33 |
| GGFP | 54.70 | 52.18 | −132.49 | 92.32 | 6.31 |
| GGIP | 58.44 | 55.92 | −119.10 | 92.00 | 7.02 |

$[Mn(III)] = 2 \times 10^{-3}$ mol dm$^{-3}$, $[TETP] = 1.0 \times 10^{-2}$ mol dm$^3$, $[Mn(II)] = 0.02$ mol dm$^3$, and $[H^+] = 0.2$ mol dm$^3$.

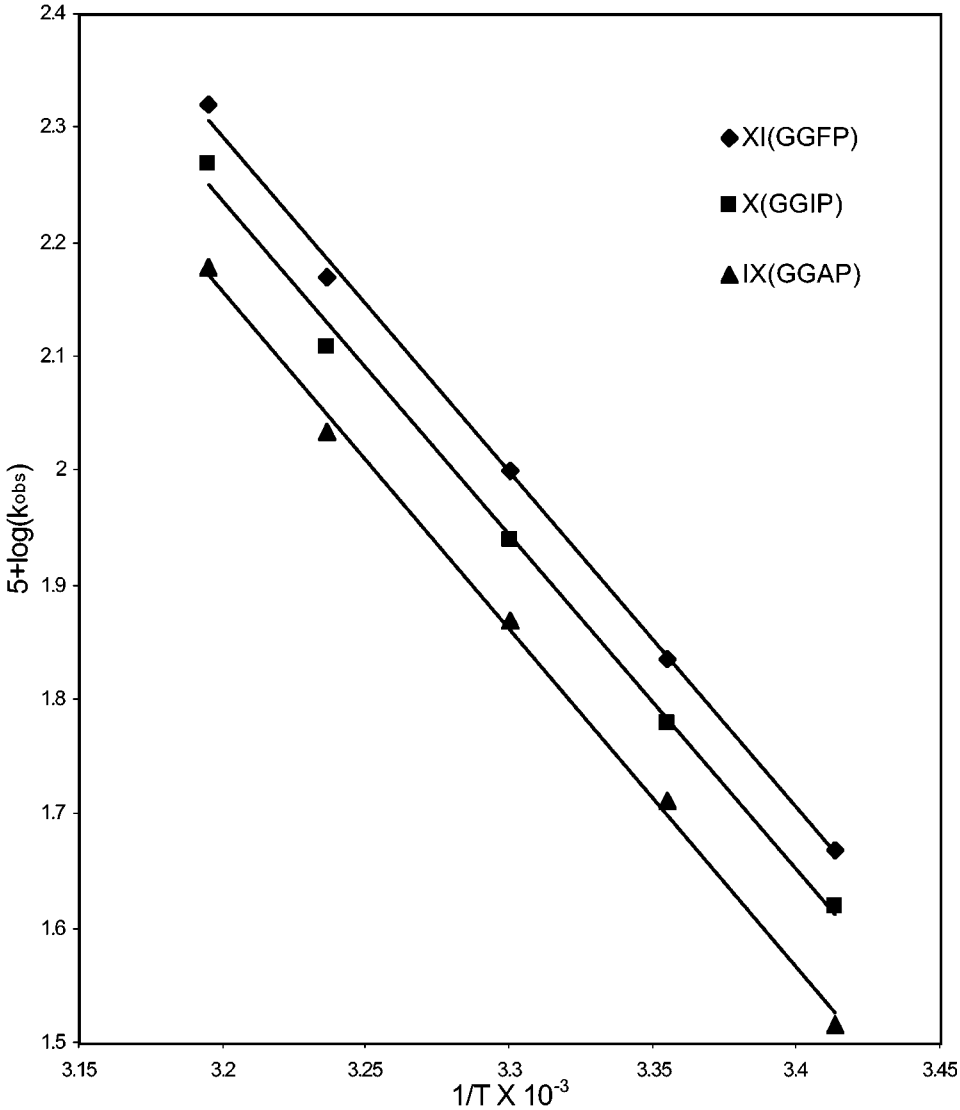



**Figure 3** Temperature dependence of the oxidation of TETP by Mn(III). $[Mn(III)] = 2 \times 10^{-3}$ mol dm$^3$, $[TETP] = 1.0 \times 10^{-2}$ mol dm$^3$, $[Mn(II)] = 0.02$ mol dm$^3$, and $[H_2SO_4] = 0.2$ mol dm$^3$ at different temperatures.

## DISCUSSION

Data published by Diebler and Sutin [26], Packler and Chawla [27], and Davies [28], have shown that in the presence of $F^-$ ion, aqueous solutions of Mn(III) consist of hexaaquomanganese (III) $\{[Mn(H_2O)_6]^{3+}\}$, $Mn(III)_{(aq)}$, hydroxopentaaquomanganese (II) $\{[Mn(OH)(H_2O)_5]^{2+}\}$, $Mn(OH)^{2+}{}_{(aq)}$, and $MnF^{2+}{}_{(aq)}$. Along the same line it may be assumed with justification that the Mn(III) species present in sulphuric acid solution 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.

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

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 Biederman and Polombari [30] also indicated a significant amount of $Mn(OH)_2{}^+$, as shown in Eq. (3).

$$Mn(OH)^{2+} + H_2O \rightarrow Mn(OH)_2{}^+ + H^+ \qquad (3)$$

But a fresh solution of Mn(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 effect on the reaction rate indicates that $MnSO_4{}^+{}_{(aq)}$ is not the active species under the present condition. Since there is no hydrogen ion dependence on the rate, this suggests that $Mn^{3+}{}_{(aq)}$ and $Mn(OH)^{2+}$ are the reactive species [31]. The molar absorption coefficient $\varepsilon$ ranges between 131 and 110 $dm^3\ mol^{-1}\ cm^{-1}$ at $[H^+] = 1.20$–$2.50$ (mol $dm^{-3}$). The high value of $\varepsilon$ has been attributed to the presence of the hydrolyzed species $Mn(OH)^{2+}$. Therefore, it is probable that $Mn(OH)^{2+}$ is the likely reactive species.

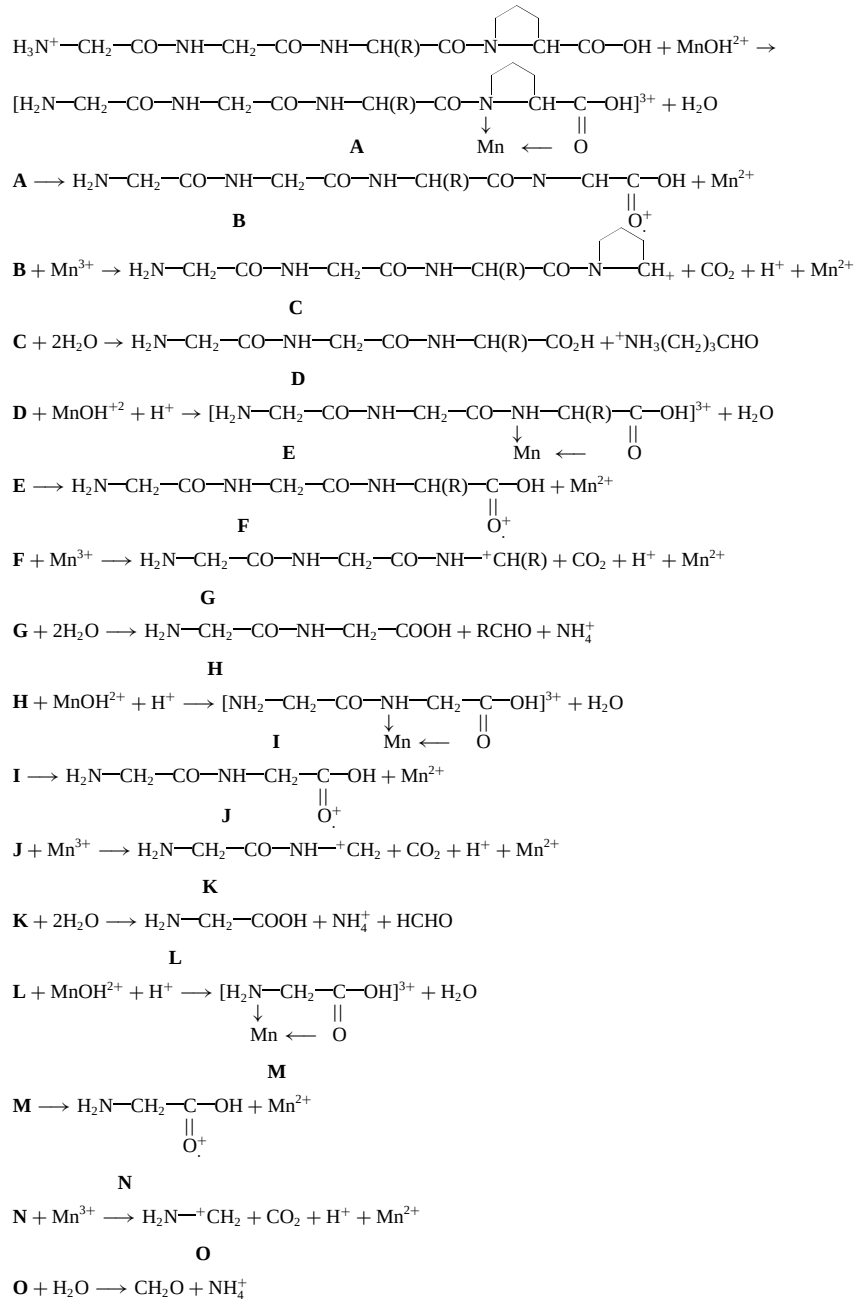
The following Scheme I accounts for the observed experimental results:

$$Mn(OH)^{2+} + TETP \xrightarrow{k_1} X \qquad \text{(i) slow and rds}$$

$$X \xrightarrow{k_2} \text{Products} \qquad \text{(ii) fast}$$

**SCHEME 1** Hence, Rate $= k'\,[Mn(OH)^{2+}]\,[TETP]$

**MECHANISM:**



**SCHEME 2** R: $CH_3$— for GGAP, $CH_3CH_2CH(CH_3)$— for GGIP, $C_6H_5CH_2$— for GGFP

Amis [32] has shown that plots of log $k_{obs}$ vs. 1/$D$ give 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 Scheme II.

The rate of oxidation of TETP by Mn(III) was compared with that of oxidation of tripeptides, Gly-Ala-Pro, Gly-Ile-Pro and Gly-Phe-Pro, dipeptides, Ala-Pro, Ile-Pro and Phe-Pro, and free amino acids by Mn(III) under identical experimental conditions, and it was found that the rate of oxidation of tetrapeptide was slower than 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 tetrapeptides is expected to be slower than the monomers, dipeptides, and tripeptides. 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 rate of oxidation of tetrapeptides was found to be GGFP > GGIP > GGAP which is well in agreement with their hydrophobicity [33].

## Spectral Evidence for the Formation of TETP–Mn (III) Complex

The study of UV–Visible spectra separately of pure Mn(III), TETP (glycyl-glycyl-alanyl-proline, glycyl-glycyl-phenylalanyl-proline, and glycyl-glycyl-isoleucyl-proline), and a mixture of Mn(III) and TETP show deviation in peak wave length($\lambda_{max}$) and absorbance(Abs) as follows.

| Substrate | $\lambda_{max}$ in nm | Abs | Complex | $\lambda_{max}$ in nm | Abs |
|---|---|---|---|---|---|
| Mn(III) | 500 | 0.970 | | | |
| GGAP | 215.0 | 2.789 | Mn(III) + GGAP | 241.5 | 2.987 |
| GGIP | 223.0 | 3.000 | Mn(III) + GGIP | 238.5 | 3.063 |
| GGFP | 222.0 | 2.913 | Mn(III) + GGFP | 239.5 | 3.023 |

## BIBLIOGRAPHY


1. Stadtman, E. R. Science 1992, 257, 1220.
2. Berlett, B. S.; Stadtman, E. R. 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, K. S.; Chandraju, S.; Made Gowda, N. M. Synth React Inorg Met-Org Chem 1998, 28, 275.
7. Rangappa, K. S.; Chandraju, S.; Made Gowda, N. M. Int J Chem Kinet 1998, 30, 7.
8. Rangappa, K. S.; Manjunathaswamy, H.; Ragavendra, M. P.; Channe Gowda, D. Carbohydr Res 1998, 307, 253.
9. Rangappa, K. S.; Manjunathaswamy, H.; Ragavendra, M. P.; Channe Gowda, D. J Org Chem 1998, 63, 531.
10. Rangappa, K. S.; Ragavendra, M. P.; Mahadevappa, D. S. Carbohydr Chem 1997, 16, 359.
11. Mahadevappa, D. S.; Ananda, S.; Made Gowda, N. M.; Rangappa, K. S. J Chem Soc Perkin Trans II, 1985, 11, 39.
12. Rangappa, K. S.; Chandaraju, S.; Mahadevappa, D. S. Transition Met Chem 1996, 21, 519.
13. Asha Iyengar, T.; Mahadevappa, D. S. Indian J Chem 1992, 31A, 752.
14. Sandberg, L. B.; Leslie, J. G.; Leach, C. T.; Torres, V. L.; Smith, A. R.; Smith, D. W. Pathol Biol 1985, 33, 266.
15. Yeh, H.; Ornstein-Goldstein, N.; Indik, Z.; Sheppard, P.; Anderson, N.; Rosenbloom, J. C.; Cicila, G.; Yoon, K.; Rosenbloom, J. Collagen Relat Res 1987, 7, 235.
16. Sandberg, L. B.; Soskel, N. T.; Leslie, J. B. Engl J Med 1981, 304, 566.
17. Urry, D. W.; Long, M. M. CRC Crit Rev Biochem 1976, 4, 1.
18. Urry, D. W.; McKee, L. D.; Williams, T.; Olsen, D. B.; Cox, B. A. Medical Application of Bioelastic Materials, in Biotechnological Polymers: Medical, Pharmaceutical and Industrial Applications, Technomic Publishing: Atlanta, Georgia, 1993; pp. 82.
19. Urry, D. W.; McPherson, D. T.; Xu, J.; Daniell, H.; Guda, C.; Channe Gowda, D.; Jing, N.; Parker, T. M. Polymeric Materials Encyclopedia, Salamone, J. C. (Ed.); CRC Press: Boca Raton, 1996; pp. 7263.
20. Nicol, A.; Channe Gowda, D.; Parker, T. M.; Urry, D. W. J Biomed Mater Res 1993, Vol. 27, pp. 801.
21. Anwer, M. K.; Spatola, A. F. Synthesis 1980, 929.
22. Andreu, D.; Merrifield, R. B.; Steiner, H.; Boman, H. G. Proc Natl Acad Sci USA 1983, 80, 6475.
23. Channe Gowda, D.; Kempe Gowda, B. K.; Rangappa, K. S. Synth React Inorg Met-Org Chem (in press).
24. Kamaluddin, Indian J Chem 1980, 19A, 431.
25. Pinto, I.; Sherigara, B. S.; Udupa, H. V. K. J Chem Soc Jpn 1983, 63, 3625.
26. Diebler, M.; Sutin, N. J Phys Chem 1964, 68, 174.
27. Pacjker, P.; Chawla, I. D. Inorg Chem Soc 1964, 38, 1130.

28. Wells, C. F.; Davies, G. O. J Chem Soc 1967, A 1858.
29. Sherigara, B. S.; Bhat, K. I.; Pinto, I.; Made Gowda, N. M. Int J Chem Kinet 1995, 27, 675.
30. Biedermann, G.; Palombari, R. Acta Scand Ser A 1978, A32, 381.
31. Siskos, P. A.; Peterson, N. C.; Huie, R. E. Inorg Chem 1984, 23, 1134.
32. Amis, E. S. J Chem Educ 1953, 30, 351.
33. Urry, D. W.; Channe Gowda, D.; Parker, T. M.; Luan, C. H.; Reid, M. C.; Harris, C. M.; Pattanaik, A.; Harris, D. R. Biopolymers 1992, 32, 1243.