Electrochemical oxidation of guanosine-5′-monophosphate at the pyrolytic graphite electrode

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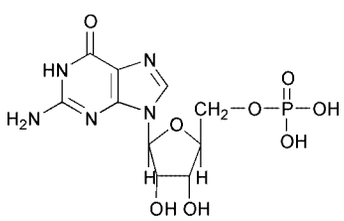
The electrochemical oxidation of guanosine-5′-monophosphate has been investigated in phosphate containing supporting electrolytes at pH 2.5–10.8 at a pyrolytic graphite electrode by cyclic sweep voltammetry, spectral studies, controlled potential electrolysis and related techniques. The initial course of the electrode reaction has been deduced to involve a pH dependent 4e, $4H^+$ oxidation to give a diimine species which undergoes a series of chemical reactions to give different products. The kinetics of the decay of the UV-absorbing intermediate generated during electrooxidation has been studied at different pH values and first-order rate constants for the disappearance of the UV-absorbing intermediate have been calculated. The electrooxidation of guanosine-5′-monophosphate has been found to be an EC reaction (electrode reaction followed by chemical reactions) in which charge transfer is followed by competitive chemical reactions. The ultimate products of oxidation, urea riboside phosphate, alloxan (pyrimidine-2,4,5,6(1*H*,3*H*)-tetraone) and a dimer were characterized by IR, ^{1}H NMR and mass spectrometry at pH 3.4. A detailed interpretation of the redox mechanism for the formation of these products is presented.

Introduction

Guanine nucleotides serve as energy sources[1] and play an important role in regulatory systems (*e.g.* cGMP) in a wide variety of tissues and organisms, facilitating maintenance of the internal milieu.[2] It has been reported that α-adrenergic receptor activation is associated with changes in the levels of guanine nucleotides in cells.[3] The purine nucleotide GTP is the key compound in biological energy metabolism. Guanosine-5′-monophosphate (GMP) is one of the four main ribosyl nucleotides present in RNA. However, the targets and mechanisms of action of GMP remain elusive. GMP is synthesized by a purine pathway from xanthine monophosphate and is the starting material for the synthesis of other guanosine phosphates. GMP is used as flavouring[4,5] and aroma substances and is extracted for this purpose from yeast nucleic acids or is produced on a large scale by mutants of certain microorganisms such as *Corynebacterium glutamicum*. Ando *et al.*[6] have studied the effect of GMP on blood pressure.

A literature survey revealed that a study on the electrochemical oxidation of GMP has attracted little attention and most of the papers reported deal with the protonation studies[7–9] and with the interaction of transition metal ions[10] and Pt complexes[11–13] with GMP. Over the last few years Goyal *et al.*[14,15] have been involved in investigating the oxidation properties of purines and their nucleosides at solid electrodes with the ultimate goal of elucidating the redox behaviour of nucleic acids. Thus, in the present study an attempt has been made to study the redox properties of a guanine nucleotide *viz*., guanosine-5′-monophosphate (GMP) using electrochemical and various analytical techniques. This article summarizes the results of the investigations related to the electron exchange between the GMP and electrode surface in the expectation that the information obtained will provide a basis for understanding some of the biochemical oxidation reactions of this important class of compounds.



Experimental

The disodium salt of guanosine-5′-monophosphate (GMP) was obtained from Sigma Chemical Co., USA and was used as received. The stock solution of GMP (1 mM) was prepared in double distilled water. The supporting electrolytes used throughout this study were phosphate buffers ($\mu = 1.0$ M) of different pH values, and these were prepared according to Christian and Purdy[16] from reagent grade chemicals. For voltammetric experiments 2.0 ml of the stock solution was mixed with 2.0 ml of the phosphate buffer of the desired pH so that the ionic strength of the solution was 0.5 M. The solutions were deoxygenated by means of a stream of nitrogen before the voltammograms were recorded. The working, counter and reference electrodes were pyrolytic graphite, platinum and saturated calomel electrodes, respectively.

The equipment used for the electrochemical studies and methods for the fabrication and preparation of the pyrolytic graphite electrode (PGE) have been described elsewhere.[17,18] The PGE used for the voltammetric studies had a geometric surface area of *ca.* ~9 mm^2. The renewal of the PGE surface after running each voltammogram was performed by polishing on a 600 grit metallographic disc and than washed with water and dried gently. The measurements were performed in triplicate. All potentials are referred to the SCE at 25±3 °C. Controlled potential electrolysis was carried out in a three compartment cell using a pyrolytic graphite plate (6 × 1 cm^2) as

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the working electrode and cylindrical Pt gauze as the auxiliary electrode. The number of electrons '*n*' involved in the electrooxidation was determined by connecting a coulometer in series. UV-Vis spectral changes associated with the electron transfer in GMP at PGE were monitored using a Shimadzu UV-2100/s spectrophotometer in a 1 cm cell. The progress of electrolysis was monitored by withdrawing a sample from the working electrode compartment of the H-cell at different time intervals and the spectra were recorded. In the second set of experiments, the potential was turned off by open circuited relaxation when the absorbance at λ_{max} reduced to 50%. Spectra at different times were then monitored to detect the wavelength region in which UV-Vis-absorbing intermediates were generated. The kinetics of decay, of the generated UV-Vis-absorbing intermediate, were monitored by turning off the potential when the absorbance at λ_{max} decreased to 50%. The solution was pipetted out from the electrolysis cell, transferred to a 1.0 cm quartz cell and placed in the spectrophotometer. The change in absorbance with time at selected wavelengths was then monitored. The values of rate constant k were calculated from the linear $\log(A - A_\infty)$ *versus* time plots.

The products of electrooxidation were obtained by exhaustively electrolysing about 10–12 mg of the compound in an H-cell at the peak Ia potential. A nitrogen stream was maintained throughout the course of electrolysis. The progress of electrooxidation was monitored by recording cyclic voltammograms at different time intervals. When the oxidation peak disappeared completely, the electrolysed solution was removed from the cell and made sufficiently acidic (pH = 1.0) to convert the disodium salt to free acid. The products obtained were lyophilised and separated by gel permeation chromatography in which a glass column packed with Sephadex G-10 (Sigma, bead size 40–120 μM) was used. For this purpose, the lyophilised product obtained was redissolved in 1–2 ml of distilled water and passed through the column using double distilled water as eluent and fractions of 5 ml each were collected. The flow rate of the eluent was fixed at 0.8 ml min^{-1}. The first peak P_1 (100–180 ml) that appeared in the gel-permeation chromatograph was due to phosphate as identified by qualitative testing of the yellow precipitate with $(NH_4)_2MoO_4$ and hence this eluent was discarded. The remaining phosphate free fractions P_2 (200–300 ml), P_3 (301–400 ml) and P_4(401–450 ml) were collected separately and again lyophilised for further analysis (mp, TLC, IR, ^{1}H NMR and FAB-MS).

Results and discussions

Linear sweep voltammetry of guanosine-5′-monophosphate at a sweep rate of 10 mV s^{-1} exhibited a single well-defined oxidation peak Ia in the entire pH range 2.5–10.8. The peak potential of this peak was dependent on pH and shifted towards a less positive potential with an increase in pH. The E_p *versus* pH plot (Fig. 1) was a straight line and the linear regression analysis indicated a correlation coefficient of 0.97. The dependence of E_p on pH can be expressed by the eqn. (1).

$$E_p \text{ (pH 2.5–10.8)} = [1345 - 47 \text{ pH}] \text{ mV } \textit{vs.} \text{ SCE} \quad (1)$$

In cyclic sweep voltammetry at a sweep rate of 100 mV s^{-1} one well-defined oxidation peak Ia was obtained when the sweep was initiated in the positive direction. In the reverse sweep three cathodic peaks IIc, IIIc and IVc were obtained. Peak IIc was obtained up to pH 5.8, whereas peaks IIIc and IVc were clearly observed in the entire pH range. In the second sweep towards positive potentials a new well-defined oxidation peak (IIa) was observed over the entire pH range at a potential less positive than that of peak Ia and it formed a quasi-reversible couple with peak IIc. In order to observe peak IIa, it is necessary to scan first peak Ia and then peak IIIc. The peak potential of peak IIa was also dependent on pH and shifted to less positive potential with an increase in pH. The plot of E_p *versus* pH was linear in nature (Fig. 1) and the E_p–pH relationship observed for peak IIa is expressed in eqn. (2).

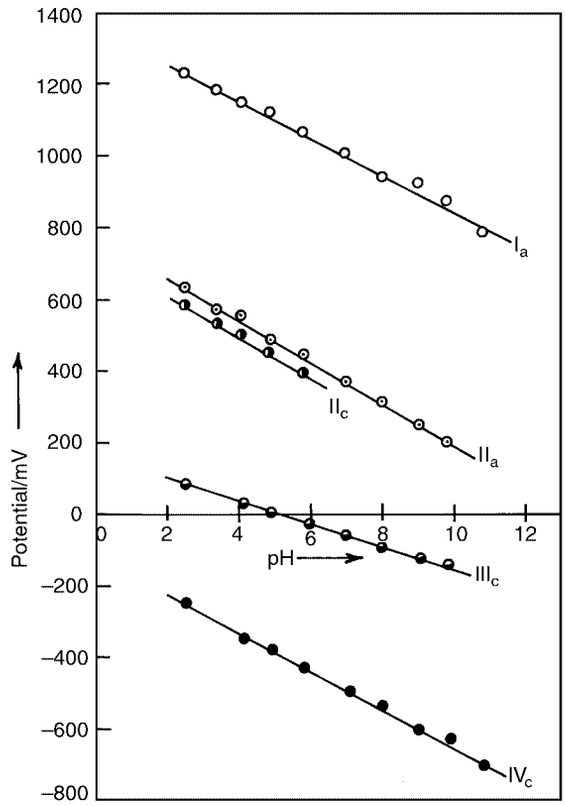


Fig. 1 Observed dependence of E_p on pH for the voltammetric oxidation and reduction peaks of 0.1 mM GMP at PGE.

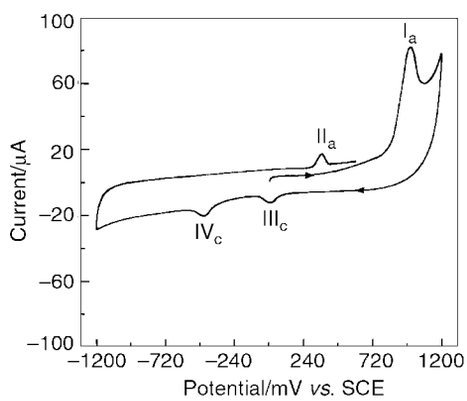


Fig. 2 A typical cyclic voltammogram (100 mV s^{-1}) of 0.5 mM solution of GMP in phosphate buffer of pH 7.0.

$$E_p \text{ (pH 2.5–9.8)} = [780 - 60 \text{ pH}] \text{ mV } \textit{vs.} \text{ SCE} \quad (2)$$

A typical cyclic voltammogram of GMP at PGE at pH 7.0 is shown in Fig. 2 and clearly depicts the oxidation and subsequent reduction of the species generated during voltammetric studies. The oxidation peak IIa formed a quasi-reversible couple with peak IIc as established from peak potential separation of anodic and cathodic peaks. The peak potential of the cathodic peaks IIc, IIIc and IVc were also dependent on pH and shifted towards more negative potentials with an increase in pH (Fig. 1). The dependence of E_p on pH for these reduction peaks can be represented as in eqn. (3)–(5).

$$E_p \text{ (pH 2.5–5.8)} = [725 - 60 \text{ pH}] \text{ mV } \textit{vs.} \text{ SCE} \quad (3)$$

$$E_p \text{ (pH 2.5–9.8)} = [165 - 33 \text{ pH}] \text{ mV } \textit{vs.} \text{ SCE} \quad (4)$$

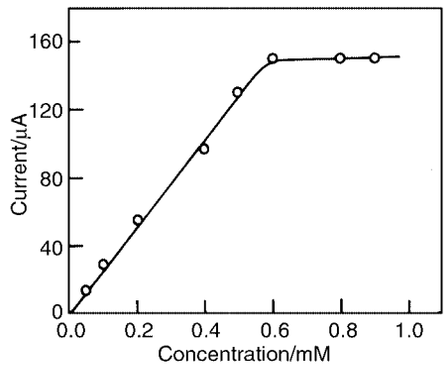


Fig. 3 Peak current (i_p) as a function of concentration of GMP at the PGE electrode for the oxidation peak Ia at pH = 7.0; $v = 100$ mV s^{-1}.

$$E_p \text{ (pH 2.5–10.8)} = [-120 - 55 \text{ pH}] \text{ mV } \textit{vs.} \text{ SCE} \quad (5)$$

The ratio of peaks IIa : IIc remained practically constant (~2.3) in the pH range 2.5–5.8. The peak currents for the reduction peaks IIIc and IVc were also found to be practically equal and their ratio was in the range 0.8 to 1.0. The peak current for peak Ia was found to increase with an increase in GMP concentration in the range 0.01 mM to 1.0 mM. A calibration graph of i_p *versus* GMP concentration was prepared from the data generated through CV studies. The plot of peak current i_p *versus* concentration for peak Ia (Fig. 3) was linear up to 0.6 mM of GMP and than became constant at higher concentrations. This behavior indicates that the electrode reaction associated with peak Ia involves an adsorption complication. The adsorption was further confirmed by the increase in peak current function $i_p/\sqrt{v}$ with increase in $\log v$.[19–21] Peak IIa and reduction peaks IIc–IVc were relatively small and presumably also influenced by adsorption phenomena.

The effect of sweep rate (v) on peak potential (E_p) and peak current (i_p) of peaks Ia and IIa was studied in the sweep range 5 mV s^{-1} to 1 V s^{-1} at pH 7.0. The peak potentials of peak Ia and IIa were found to shift towards more positive potentials with an increase in sweep rate. The ratio of peak currents of IIc : IIa did not reach 1.0 even at a sweep rate of 1 V s^{-1}. The nature of the plot of $[(\Delta E_{p/2})/\delta \log v]$ *vs.* $\log v$ for peak Ia was S-shaped suggesting that the nature of the electrode reaction is EC in which charge transfer is followed by irreversible chemical reactions.[22,23]

The controlled potential electrolysis of GMP at different pH values and concentrations was performed in phosphate buffers at a potential 100 mV more positive than peak Ia. The number of electrons n involved in the electrooxidation was determined by graphical integration of the current–time curve.[24] The plot of i_p *versus* time was exponential, however the $\log i_p$ *versus* t plot was linear only for the first 25 min of electrolysis and thereafter a large deviation from a straight line was noticed. This deviation clearly indicated that for the first 25 min electrode reaction followed a simple path and thereafter subsequent chemical reactions play an important role as suggested by Meites[25] and Cauquis *et al.*[26] The average experimental n values obtained under different conditions were close to 4.1 ± 0.2.

Spectral studies

UV-Vis spectra of GMP exhibited two well-defined λ_{max} values at 201 and 252 nm and a broad bump centered around 280 nm in the entire pH range studied (2.5–10.8). The progress of electrolysis was monitored by recording UV-spectral changes during electrooxidation of GMP at different pH values to detect the formation of UV-Vis absorbing intermediates. Curve 1 in Fig.4A is the initial spectrum of GMP at pH 7.0 just before electrooxidation. Upon application of a potential of 1.1 V corresponding to peak Ia the absorbance in the wavelength region 245–285 nm decreased while the absorbance in the shorter wavelength region 205–240 nm and 285–320 nm increased as shown by curves 2 to 9 in Fig. 4A. The exhaustively electrolysed solution (Curve 10) recorded after 210 min of electrolysis exhibited an absorption maxima *ca.* 208 nm due to the final product. Two clear isosbestic points at 242 and 290 nm were also obtained.

In the second set of experiments after electrolyzing the solution of GMP for 45 min (corresponding to curve 5 in Fig. 4A) the potentiostat was open circuited and spectra were recorded at different time intervals. It was observed that the absorbance in the region 200–300 nm decreased systematically due to the decay of the intermediate. The spectral changes observed during electrooxidation of GMP at pH 3.4 are illustrated in Fig. 4B and the changes were essentially similar to that observed at pH 7.0.

Thus, these spectral studies indicate that an intermediate is generated on electrolysing the solution of GMP in a buffer of the desired pH which decays due to competitive chemical reactions. The decay of the intermediate was monitored by recording absorbance at selected wavelengths of 225, 254 and 280 nm. These λs were selected on the basis of UV-Vis spectral changes observed during electrolysis as described above. In each case, the time *versus* absorbance profile showed an exponential decay in absorbance with an increase in time, which then ultimately became constant (Fig. 5) and hence, the absorbance at this point was considered as A_∞. The first order plots of $\log(A - A_\infty)$ *versus* time (A_∞ and A are final absorbance and the absorbance at the reaction time, respectively) were linear. The values of the rate constant (k) were calculated from the slope value of linear best fit equation of $\log(A - A_\infty)$ *versus* time plots and are presented in Table 1. On examining Table 1 it can be seen that the value of k at pH 7.0 is almost thrice that observed at pH 3.4. Also, the value of k at pH 9.8 was almost twice that at pH 3.4. The rate of decay of the intermediate was thus found as pH dependent and suggests that the rate of decay of the UV-absorbing species is an acid–base catalyzed reaction.

Table 1 Rate constant values observed for the first order decay of the UV-absorbing intermediate generated during electrooxidation of GMP at different pH values at PGE

pH	λ_{max}/nm	$k^a/10^{-3}$ s^{-1}
3.4	225	0.8
	254	1.1
	280	1.3
7.0	225	3.1
	254	3.1
	280	3.4
9.8	225	2.5
	254	2.9
	280	2.8

[a] Average of at least two replicate determinations.

Product characterization

The ultimate products of electrooxidation of GMP were isolated and characterized at pH 3.4 at PGE. The exhaustively electrolysed solution was acidified, lyophilised and the freeze dried material obtained exhibited three spots in the TLC ($R_f = 0.31$, 0.56 and 0.72) indicating the formation of three products. The products were separated by gel permeation chromatography (see Experimental) and the plot of absorbance *versus* volume exhibited four peaks. The first peak P_1 (100–180 ml) was found to contain phosphate and was discarded.

The lyophilised material obtained from the volume under the second peak P_2 between 200–300 ml gave a clear molecular ion peak at $m/z = 273$ (20%) in the mass spectrum suggesting the product to be urea riboside phosphate. The other high mass peaks observed in the mass spectrum were at 254 (8.0%), 213 (9.9%), 175 (18.4%), 154 (45.1%), 149 (40.2%).

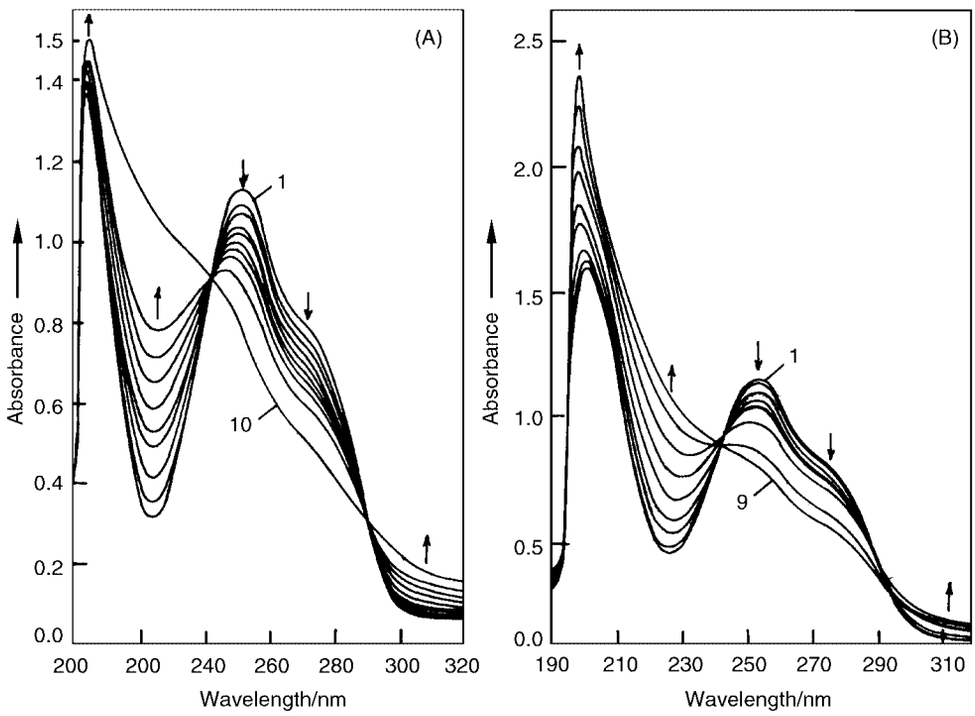


Fig. 4 UV-Vis spectral changes observed for the electrooxidation of 0.1 mM GMP in phosphate buffers at PGE. Arrows show direction of change upon application of potential. (A) At pH = 7.0; E_{appl} = 1.1 V *vs.* SCE. Curves were recorded at (1) 0; (2) 5; (3) 15; (4) 30; (5) 45; (6) 60; (7) 80; (8) 100; (9) 140 and (10) 210 min of electrolysis. (B) At pH = 3.4; E_{appl} = 1.28 V *vs.* SCE. Curves were recorded at (1) 0; (2) 5; (3) 10; (4) 20; (5) 30; (6) 45; (7) 90; (8) 150 and (9) 210 min of electrolysis.

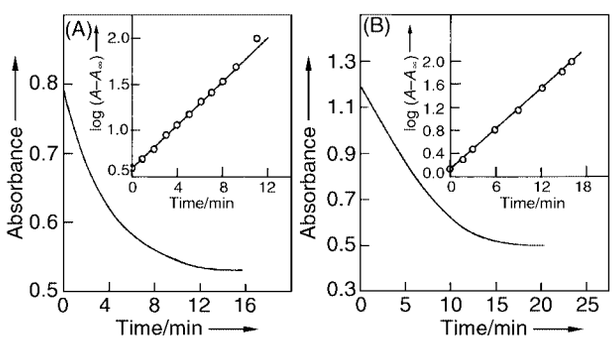


Fig. 5 Time course of the absorption changes at (A) pH = 7.0, λ_{max} = 280 nm; (B) pH = 9.8, λ_{max} = 254 nm. **Inset**: First-order kinetic plot of decay. The solid line represents the best fit of the data by linear regression, with (A) Correlation coefficient = 0.9951. (B) Correlation coefficient = 0.9625.

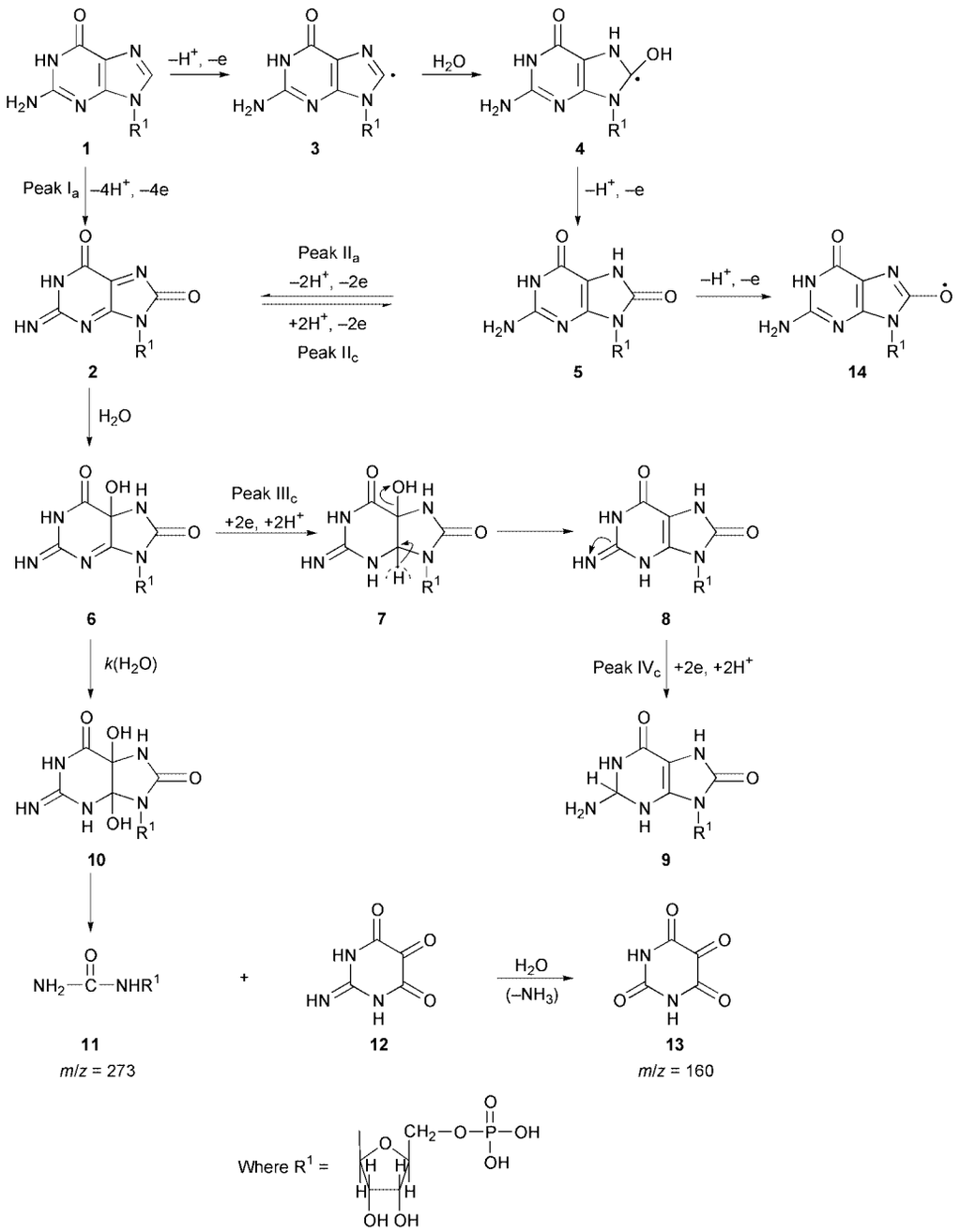
The volume 301–400 ml under peak P_3 was lyophilized and the colourless material obtained exhibited strong IR bands at 1720, 1640, 1410, 1260, 1170, 1040, 1020, 808 and 708 cm^{-1} and was identical to that of an authentic sample of alloxan. The ^1H NMR spectrum of this product exhibited sharp peaks at δ = 11.24 (s, N–H), 11.46 (s, N–H) and 8.24 (s, O–H) further supporting the structure as alloxan. The mass spectrum of the material also exhibited a molecular ion peak at *m*/*z* = 160 (35%) confirming the product as alloxan.

The lyophilised material obtained from volume 401–450 under peak P_4 exhibited a molecular ion peak at *m*/*z* = 661 (M + H^+ 90%) in the mass spectrum, indicating the formation of a dimer as product. The accurate mass measurement in a 3-nitrobenzyl matrix gave an intense pseudomolecular ion peak at 661.4511 (86%; $C_{20}H_{25}N_{10}O_{14}P$, calculated *m*/*z* = 661.4517). Some other important peaks and their relative abundance noticed in mass spectrum are as follows: 649 (17%), 633 (20%), 589 (18%), 566 (22%), 549 (20%), 491 (25%), 391 (30%), 295 (25%), 175 (82%), 154 (65%), 137 (47%), 136 (60%). The ^1H NMR spectrum of the product exhibited sharp signals at δ = 10.8 (s, 1H, N^1–H), 10.5 (s, 1H, N^1–H), 6.9 (s, 2H, C^2–NH_2) and 6.6 (s, 2H, C^2–NH_2). However, at higher field regions (δ = 6–3) a complex overlapping series of multiplets were observed in the spectrum owing to the various C^1–H and O–H resonances of ribose residues. The NMR spectra indicate that both C^8–H groups are missing but both C–NH_2 groups are intact and are in the same environments on both the guanosyl residues. Thus, it appears that the dimer consists of two guanosyl residues linked by oxygen atom at the C^8 position.

Hence, in view of these results it is concluded that the product obtained under peak P_4 is an oxygen bridged dimer linked by the C^8 position of the two guanosyl residues. Cyclic voltammograms of a dilute solution of dimer (<0.01 mM) were recorded at pH 7.0 and a bump at around 1 V was noticed. Thus, the oxidation products of GMP at pH 3.4 are urea riboside phosphate, alloxan and an oxygen linked dimer.

Redox mechanism

Linear and cyclic sweep voltammetry, coulometry and product identification studies of GMP indicated that oxidation peak Ia is a 4e, $4H^+$ reaction. Thus, compound **1** on 4e, $4H^+$ oxidation will give a diimine species (**2**). This oxidation is basically similar to that of guanine[27] or guanosine.[14] in which 4e, $4H^+$ oxidation is reported to occur *via* the formation of 8-hydroxy derivatives The diimine obtained in the case of oxidation of various purine bases.[28,29] has been reported to be highly unstable due to two C=N bonds. Thus, it is expected that the diimine (**2**) would be rapidly attacked by the water molecule in a chemical follow up step to give imine alcohol species **6** (Scheme 1). Further hydration of imine alcohol **6** will lead to diol species **10**. The rate of attack of water on imine alcohol **6** is found to follow pseudo-first order kinetics and the *k* values indicate it to be an acid–base catalyzed reaction. Thus, the intermediate **6**, formed upon electrolysis, is the UV-absorbing intermediate species observed during spectral changes. The reduction peak IIIc involving 2e, $2H^+$ also supports its formation. At pH 3.4, the imidazole ring of the diol (**10**) opens giving urea riboside phosphate (**11**) and alloxan (**13**) as the final products. Such hydrolysis reactions of

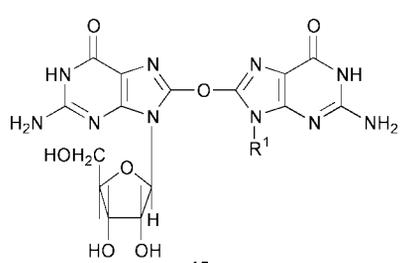


Scheme 1 Proposed mechanism for the production of urea riboside phosphate and alloxan at pH 3.4.

purine nucleosides in acidic medium causing the opening of imidazole ring to give the corresponding products is well documented in the literature[14,30,31] The ultimate products, species **11** and **13**, have been characterized on the basis of ^{1}H NMR, mass and related studies.

Peak IIIc appeared in the CV of GMP and is probably due to the reduction of imine alcohol species **6** in a 2e, 2H$^+$ reaction to give dihydro species **7** which rapidly loses a molecule of water to give 8-hydroxyguanosine monophosphate (**5**). Peak IVc appears to be due to the reduction of species **8** in a 2e, 2H$^+$ reaction to give amino derivative of 8-hydroxyguanosine monophosphate (**9**). It appears that 8-hydroxyguanosine monophosphate (**5**) is formed from the reduction of species **6** and hence peak IIa is noticed only in the second cycle of CV. It must be realized that the proposed mechanism explains one of the several possible pathways and hence the presence of intermediates **3**, **4**, **5**, **7**, **8** and **9** is assumed for the formation of the final products.

The characterization of product at $m/z = 661$ (M + H$^+$) clearly indicates that the electrooxidation of **1** lead to the formation of a dimeric nucleoside. Based on ^{1}H NMR and



15

$m/z = 660$

FAB-MS evidence it has been concluded that dimer **15** consists of two guanosyl residues linked through an oxygen bridge between C^8 positions of both rings. One route for the formation of such a dimer involves electrochemical oxidation of **1** to a free radical species **3** with an unpaired electron at the C^8 position. The free radical **3** can undergo dimerization or other chemical and electrochemical reactions. The occurrence of the oxidation reaction at the C^8 position has been reported for the oxidation

of several purines and their nucleosides[14,27,32] Thus, the oxidation of GMP also occurs in a 1e, $1H^+$ reaction giving a free radical species **3**. The radical **3** hydrolyses rapidly giving the hydroxylated radical **4** which on further 1e, $1H^+$ oxidation gives hydroxyguanosine monophosphate (**5**). Further oxidation of species **5** in a 1e, $1H^+$ reaction can give a free radical **14**, which on reaction with radical **3** can give dimer **15**. The reaction of two different free radicals (**3** and **14**) to give dimer **15** seems a little difficult because one would expect that two molecules of **14** or two molecules of **3** should combine to give dimers. However, these dimers were not observed in the final products of oxidation and the proposed dimer **15**, a major product clearly satisfies the analytical data obtained. It is expected that the dimer should possess two ribosyl phosphate units. However, the characterized product has only one such unit. It seems that the phosphate molecule of one of the ribosyl unit undergoes hydrolysis during the formation of dimer **15** due to steric hindrance. It must be realized that the free radical formed at various stages can also resonate to give different free radicals in Scheme 1 and thus several dimers involving radical–radical coupling are possible. The combination of two different free radicals to give a dimer or oligomers has also been reported in the literature.[33]

Thus, these studies clearly reveal that guanosine monophosphate oxidizes by a pathway essentially similar to that of purine nucleosides in phosphate buffers of different pH values. The oxidation behaviour of GMP was also compared with its parent base guanine to monitor the effect of the ribosyl phosphate substituent. The results for GMP clearly indicate that in the presence of a ribose phosphate group in guanine the behaviour of the nucleotide is much more complicated then that observed for the corresponding base guanine. The 4e, $4H^+$ oxidation of guanine has been found to occur in two 2e, $2H^+$ steps and the formation of 8-hydroxyguanine was observed. The ultimate products of oxidation were found to be 25-diiminoimidazol-4-one and 5-guanidinohydantoin.[27] On the other hand, oxidation of GMP gave alloxan, urea ribosyl phosphate and an oxygen linked dimer through C^8–C^8 positions. The formation of the first two compounds essentially occurs *via* a mechanism similar to that proposed for guanine including several additional hydrolysis steps whereas the formation of the dimer occurred through a free radical mechanism. The rate of disappearance of the UV-absorbing intermediate (k) also indicated a significant difference at pH 7.0. A comparison of k values clearly indicated that the decay of UV-absorbing intermediates generated in the case of GMP occurred at almost half the rate of that observed for guanine. Thus the ribosyl phosphate significantly affects the mechanism of electrode reaction and probably causes the reaction to proceed *via* the free radical mechanism.

Thus, it can be concluded on the basis of these investigations that guanine nucleotides are also oxidized in biosystems leading to the formation of several products. The present studies on GMP not only help in fully understanding the mechanism of electron transfer reactions of this compound, but also provide information about the nature of the intermediates and products that can possibly form during the metabolic activities. It has already been reported[14] that the dimer formed as the oxidation product of guanosine at lower pH cause nephritis with edema in albino mice and hence is toxic in nature. This has also prompted us to evaluate the toxicity of dimer **15** and the studies are in progress and will be reported later.

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