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Lanthanide ions promote the hydrolysis of 2,3-Bisphosphoglycerate

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Abstract

The ^{31}P NMR studies showed that lanthanide ions promote the site-specific hydrolysis of 2,3-Bisphosphoglycerate (BPG) at pH 7.4 by cleaving the 2′ phosphomonoester bond. The effect of fourteen trivalent lanthanide ions and Sc^{3+}, and Y^{3+} were compared by the percentage of hydrolysis obtained by determining the inorganic phosphate produced. All the trivalent lanthanide ions promote the hydrolysis, but Sc^{3+} not. Among them, Ce^{3+} affects the reaction mostly. This was mainly attributed to the autooxidation of Ce^{3+} to Ce^{4+}, since the promoting effect of Ce^{3+} is related to the increasing Ce^{4+} amount in the solution and depressed by adding sulphite. Ce^{4+} promotes the hydrolysis more efficiently than Ce^{3+} do. The pseudo first-order rate constant for the hydrolysis of BPG by $Ce(SO_4)_2$ (18.7 mM) at pH 1 and pH 2, 37 °C is 3.1 h^{-1} and 0.65 h^{-1} respectively. A mechanism with a hydroxo species as reactive intermediate was proposed for the trivalent lanthanide ions. The site-specificity was explainable by this mechanism.

Introduction

2,3-Bisphosphoglycerate (2,3-BPG) plays an important role in human erythrocytes by regulating their blood oxygen transport and delivery. Because it is a heterotropic allosteric effector of oxygen binding by haemoglobin (Hb); by binding preferentially to the deoxygenated form of Hb, it decreases the apparent affinity of Hb for O_2. While the binding of 2,3-BPG to Hb has been known for over 30 years (Benesch & Benesch 1967; Chanutin & Curnish 1967). Over the last 30 years, several mathematical models of erythrocyte metabolism have been developed. Brief histories of the development of erythrocytes models are presented by Joshi and Palsson (Joshi & Palsson 1989), and Heinrich and Schuster (Heinrich & Schuster 1996). The most comprehensive model published to date is that of Palsson's group. This model includes glycolysis, the 2,3-BPG shunt. The PPP (pentose phosphate pathway). Adenine nucleotide metabolism. Various transmembrane processes. osmotic and electrostatic conditions, as well as pH effects on kinetic processes. In addition to, some enzymes also play an important role in regulating the concentration of 2,3-BPG in erythrocytes (Winn *et al.* 1981; Van Etten 1982; Bazan *et al.* 1989; Fothergill-Gilmore & Watson 1989). Synthase and phosphatase activities of erythrocyte bisphosphoglycerate mutase (BPGM) catalyze respectively the synthesis and degradation of 2,3-BPG, the main allosteric effector of hemoglobin. In spite of the presence of acid phosphatases in erythrocytes, the degradation of 2,3-BPG is very low because these acid phosphatases can not degrade 2,3-BPG and the phosphatase activity of BPGM is very slow (1000-fold lower than the synthase activity and lower than that of acid phosphatases). Consequently, 2,3-BPG is present in high concentration in erythrocytes. Although these models have identified the key features in the regulation and control of erythrocyte metabolism, many important aspects have remained unexplained. In particular. None of these models have satisfactorily accounted for 2,3-BPG metabolism. 2,3-BPG is an important modulator of haemoglobin oxygen affinity and hence an understanding of the regulation of

2,3-BPG concentration and turnover is important for understanding blood oxygen transport.

In recent years there has been growing interest in metal ion promoted hydrolysis of phosphate esters . It was studied as model systems for metallophosphatases and as potential catalysts for the hydrolysis of nucleic acids. The influence of different metal ions and complexes on the hydrolysis of various labile phosphate esters have been compared and some insight into the nature of the reactive species was proposed (Morrow 1994; Chin 1997; Chand *et al.* 2000; Sreedhara & Cowan 2001). Lanthanide ions and their complexes have recently been shown to be highly reactive in promoting the hydrolysis of phosphate diesters including RNA. It is noteworthy that lanthanides ions display catalytic activity also to phosphatidylinositol, in which a monoester P-O bond in the diacylglycerol side was hydrolyzed (Ciesiolka 1989; Zhu *et al.* 1998; Komiyama *et al.* 1999; Komiyama *et al.* 2001). We noted an increase in the oxygen affinity of hemoglobin (Hb) isolated from the erythrocytes after incubation with various lanthanides (Cheng *et al.* 2001). The effect was postulated to be the result of the acclerated BPG hydrolysis. BPG stabilizes deoxyhemoglobin (deoxyHb) and increases both the alkaline and acid Bohr effect (de Bruin *et al.* 1971; de Bruin *et al.* 1974; Brewer 1974; Benesch & Benesch 1974; Castilho *et al.* 2003). It plays important role in regulating the oxygen affinity of Hb. Stankiewicz *et al.* (Stankiewicz 1989) reported that oxyvanadium(IV) cation also stimulates the hydrolysis of BPG and thus induce the increase of oxygen affinity of hemoglobin. Up to now the mechanism of metal ion promoted phosphomonoester bond hydrolysis is not clear.

In the present work, the BPG hydrolysis in presence of lanthanide ions was investigated by nuclear magnetic resonance (NMR) and the determination of the inorganic phosphates produced. The mechanism was deduced based on the results obtained under different conditions.

Experimental

Reagents

BPG was obtained from Sigma. The rare earth oxides (99.99%), cerium oxalate and ammonium ceric nitrate (99.99%) were obtained from Aldrich. All the other reagents are analytical pure. Water from Millipore purification system was used.

To prepare the lanthanide chloride solutions except cerium chloride, the corresponding oxides were dissolved in concentrated hydrochloric acid and then diluted with water, the pH was adjusted to ca. 5. The cerium(III) chloride solution was prepared by dissolving the completely calcinated cerium oxalate in concentrated hydrocloric acid and evaporating the solution to expel the excess HCl. After dilution with water, the pH was adjusted to ca. 5. The concentration of the lanthanides was determined by EDTA titration with xylenol orange as indicator. The solution of ceric sulphate was prepared as follows. Ceric oxide was heated to dissolve in concentrated sulphuric acid. Then excess H_2SO_4 was expelled by evaporating and diluting repeatedly until the solution became orange in color. The residue was allowed to stand with water for 24 h to dissolve out ceric sulfate. After filtering out the insoluble matter, the solution was diluted with water and the pH was adjusted to ca. 1. The stock solution of ammonium ceric nitrate was prepared by dissolving $Ce(NH_4)_2(NO_3)_6$ in water and the pH was adjusted to ca. 1.5. The concentration of cerium(IV) in the solution was determined by titration with ammonium ferric sulphate. The BPG stocking solution (50 mM) was prepared by dissolving BPG in 20 mM HEPES buffer of pH 7.4. The measured solutions were prepared by mixing the buffered BPG solution and lanthanide solution at pH 5. The buffered pH was not significantly changed (less than 0.4 pH unit).

Instruments

NMR spectrometer, UNITY 400, Varian; UV-Visible Recording Spectrophotometer, UV-260, Shimadzu.

Methods

^{31}P NMR studies on the mode of BPG hydrolysis in the presence of lanthanides

To study the BPG hydrolysis in presence of Lu^{3+}, Eu^{3+} and La^{3+}, a series of solutions containing 12 mM BPG and varied amount of lanthanides at pH 7.4 (HEPES buffer) were incubated at 37 °C, and the ^{31}P NMR spectra (161.9 MHz) were recorded at various time with phosphoric acid (85%) as external reference.

To study the effect of Ce(IV), the reaction mixtures of BPG (12 mM) and Ce^{4+} (30 mM) at pH 2 and 8 were incubated at 37 °C. The samples were taken at varied time and the ^{31}P NMR spectra were recorded under the conditions mentioned above.

BPG hydrolysis as monitored by determination of inorganic phosphate

All the experiments were carried out at 37 °C, the pH was controlled with HEPES buffer (pH 7.4) for the experiments with various trivalent lanthanide ions . For the experiments on ceric ion, pH was adjusted with NaOH or HCl. The concentration of inorganic phosphate $[PO_4]_t$ in the reaction mixtures at various reaction time was determined by using of molybdenum blue method. The degree of hydrolysis was calculated on the basis of two phosphate ester bonds in BPG. Lanthanides tend to hydrolyze and polymerize, especially in higher concentration, the percentage of hydrolysis and rate constants for hydrolysis in the present paper are the averages of the results of at least three times runs which coincide with each other within ± 5%.

(1) The comparative studies on the effect of various lanthanide ions. The reaction mixtures containing 0.1 mM BPG and 18.7 mM lanthanides chlorides with the pH adjusted to 7.4 (HEPES buffer) were kept at 37 °C in thermostat for 10 h and then the concentration of the inorganic phosphate was determined at various time by the method mentioned above. A blank was run in parallel without the addition of the lanthanide. All the experiments were repeated at least three times.

(2) The effect of the autooxidation of cerium(III). The experiments with cerium(III) chloride was run by the same procedure with the reaction mixture degassing with purified nitrogen gas. The results were compared with those obtained from the solutions exposed to the air.

The amount of Ce(IV) in the reaction solution at various time was determined spectrophotometrically by measuring the absorbance at 340nm in H_2SO_4 (0.5 M). Solution of $Ce(NH_4)_2(NO_3)_6$ was used as the reference. The percentage of Ce(IV) was calculated.

(3) The promoting effect of cerium(IV) to BPG hydrolysis as the function of pH and background anions. The same experiments were conducted with ceric sulphate. The inorganic phosphate concentrations were determined after 0.5 h and the percentage of hydrolysis was calculated.

The effect of different anions and acidity on the Ce(IV) promoted hydrolysis was investigated by a series of similar experiments on the solutions containing 18.7 mM ceric sulphate, 0.1 mM BPG and varied concentration of $HClO_4$, HCl or H_2SO_4. The effect of sulphate ion was further studied comparatively by running parallel experiments with ammonium ceric nitrate and ceric sulphate.

Results and discussion

A series of preliminary experiments showed that BPG is stable in HEPES buffer (pH 7.4) and no hydrolytic product, inorganic phosphate could be detected (date not given). The P NMR spectra of BPG are featured by the chemical shifts of 3.98 and 3.30 ppm assigned to 2P and 3P in pH 7.4 solution (Lennon 1994) (Figure 1), but the chemical shifts are influenced by the pH value of the solutions, the downfield shift of phosphate of BPG and the hydrolytic products was attributed to the decrease of pH value (Russu 1990).

The spectra given in Figure 1 were scanned at 30 min after mixing up the solutions of BPG and lanthanide chlorides in various $[Lu^{3+}]/[BPG]$ mole ratios at 30 °C. The shift of all the signals is related to the binding of Lu^{3+}. The effect of pH is small but can not be excluded, since the changes in pH is less than 0.4 pH. the spectra are characterized by the appearance of 3′PGA signal (3.74 ppm) and phosphate signal (2.43 ppm) as well as the declination of the signals from 2P, these changes increase with the increasing ratio between $[Lu^{3+}]/[BPG]$ as shown in Figure 1. Meanwhile, the 3P signal remain unchanged, even up to 6 h no other change was observed (Figure 1E). Incubation at 37 °C for a longer time, up to 16 h, the hydrolysis of 2P approached completion and the 2P signal disappeared, but no effect was found for the 3P signal still (Figure 2). These results indicated that the Lu^{3+}-binding is not slow and the hydrolysis is specific for the 2′-phosphate group. The similar results were obtained with Eu^{3+} and La^{3+}.

The effects of different trivalent lanthanide ions were studied comparatively by determining the concentration of the inorganic phosphate produced. The results (Figure 3) showed that all the trivalent lanthanide cations (from La^{3+} to Lu^{3+}) can accelerate the BPG hydrolysis at pH 7.4 after incubation at 37 °C for 10 h. The variance in their promoting ability is not very significant, but the heavy lanthanides are more effective than the light ones. Y^{3+} is much less in efficiency and Sc^{3+} gave no effect practically. In these experiments, an excess of lanthanides and a low concentration of BPG were used to have the results determinable.

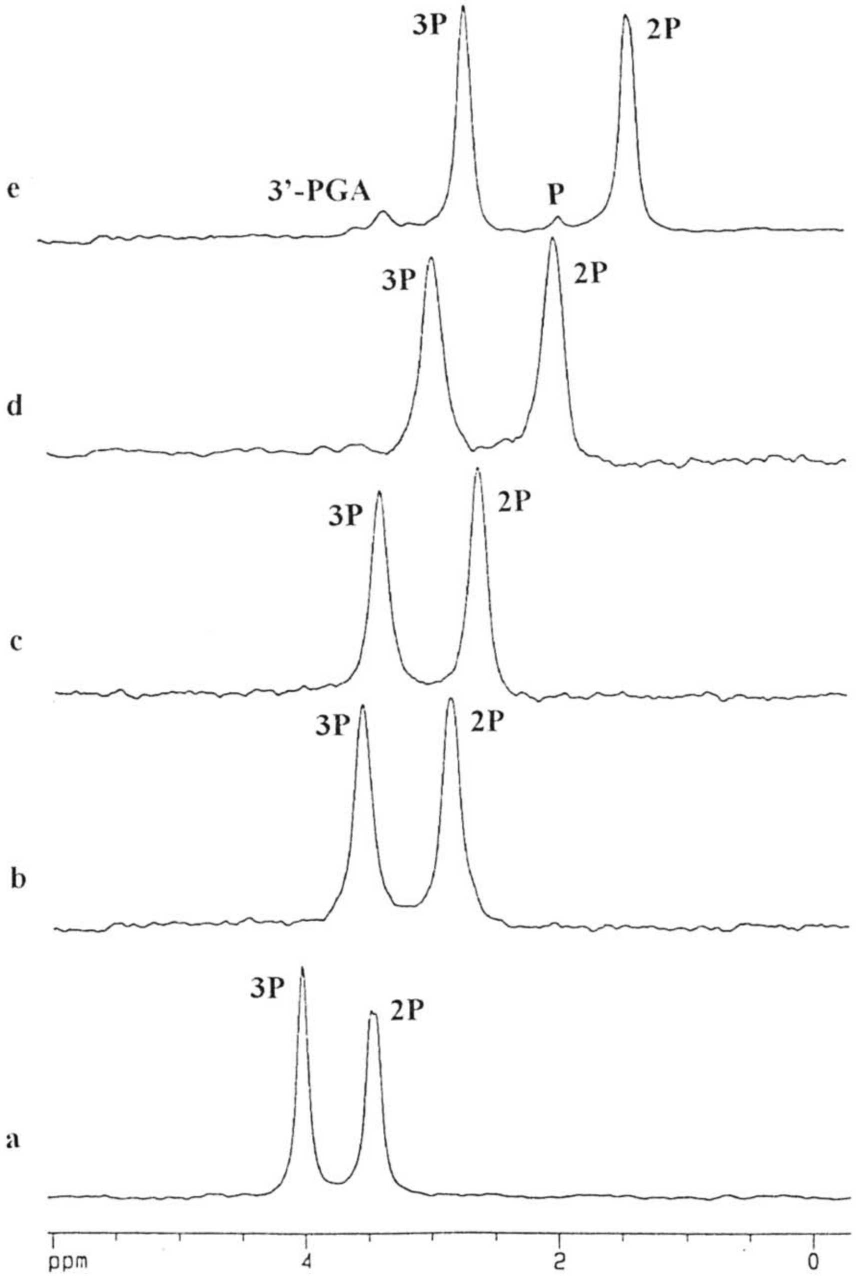

Fig. 1. ^{31}P NMR(161.9 MHz) spectra of hydrolyzing BPG in presence of Lu^{3+} at 30 °C and pH 7.4. $[BPG]_0 = 12$ mM. $[\ ]_0$-initial concentration (a) pure sample of BPG at pH 7.4(b) $[Lu^{3+}]_0/[BPG]_0 = 0.3$, 0.5 h; (c) $[Lu^{3+}]_0/[BPG]_0 = 0.45$, 0.5 h; (d) $[Lu^{3+}]_0/[BPG]_0 = 0.75$, 0.5 h; (e) $[Lu^{3+}]_0/[BPG]_0 = 1$, 6 h.

It is noteworthy that Ce^{3+} displayed the highest activity among the trivalent lanthanide ions, though it is a light lanthanide and stands in between La^{3+} and Pr^{3+} which are both very weak in the promoting action. We noted that BPG hydrolysis proceeds rapidly in the presence of Ce^{3+} chloride at pH 7.4, but it became much slower when the oxygen was replaced by bubbling in nitrogen. The results suggested that the effect of Ce^{3+} is probably related to the dissolved oxygen. A similar experiment given in Figure 3 indicated the activity of Ce^{4+} is even higher. Since Ce^{3+} is the only lanthanide ion which is oxidizable spontaneously

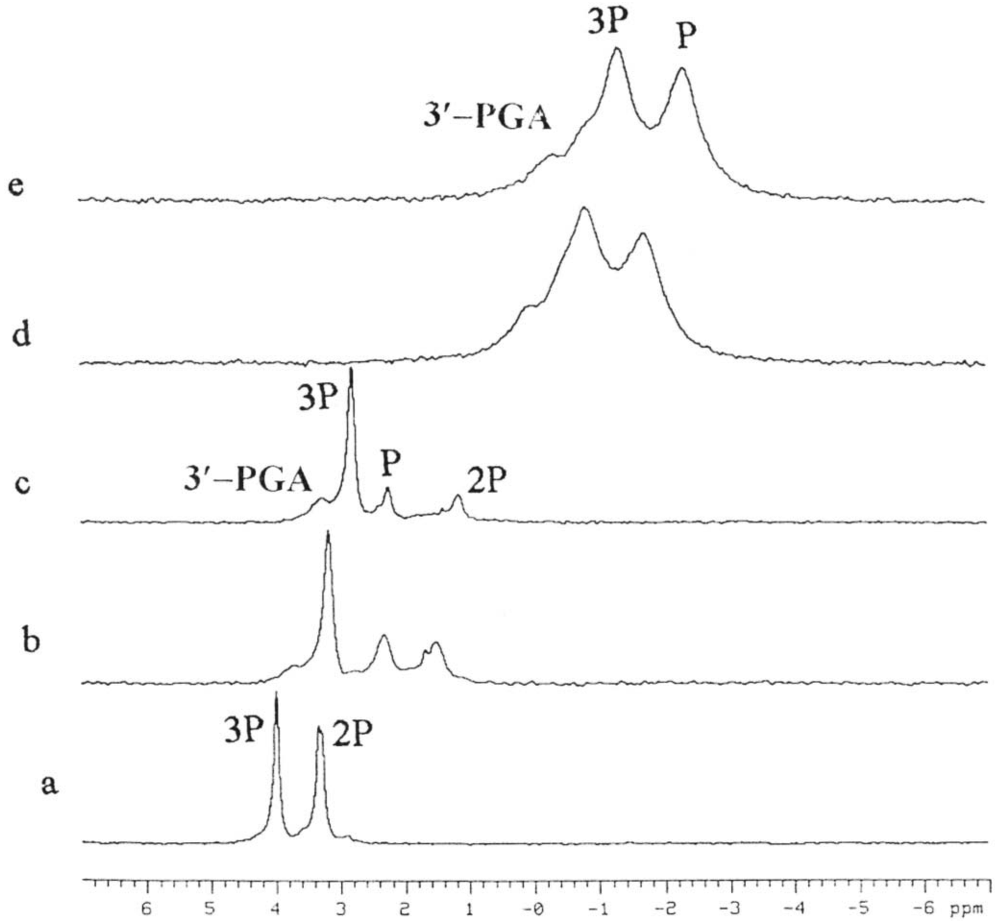

Fig. 2. ^{31}P NMR(161.9 MHz) spectra of hydrolyzing BPG by Lu^{3+} at 37 °C and pH 7.4. $[BPG]_0 = 12$ mM. $[\ ]_0$-initial concentration (a) pure sample of BPG at pH 7.4; (b) $[Lu^{3+}]_0/[BPG]_0 = 1$ incubated for 6 h; (c) $[Lu^{3+}]_0/[BPG]_0 = 1$ incubated for 16 h; (d) $[Lu^{3+}]_0/[BPG]_0 = 4$ incubated for 6 h; (e) $[Lu^{3+}]_0/[BPG]_0 = 4$ incubated for 16 h.

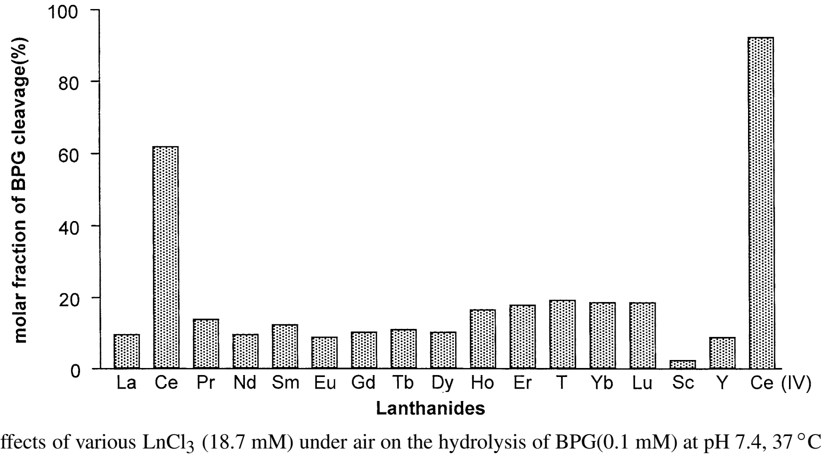

Fig. 3. Effects of various $LnCl_3$ (18.7 mM) under air on the hydrolysis of BPG(0.1 mM) at pH 7.4, 37 °C for 10 h.

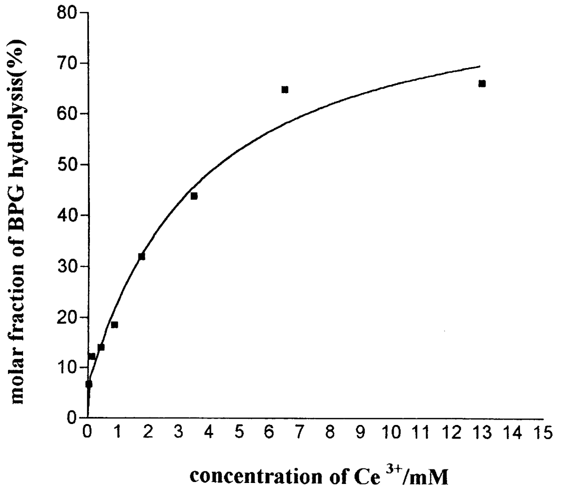

Fig. 4. Influence of concentration of Ce^{3+} on the hydrolysis of BPG at 37 °C, pH 7.4 and 10 h. $[BPG]_0 = 0.1$ mM. $[\ ]_0$-initial concentration.

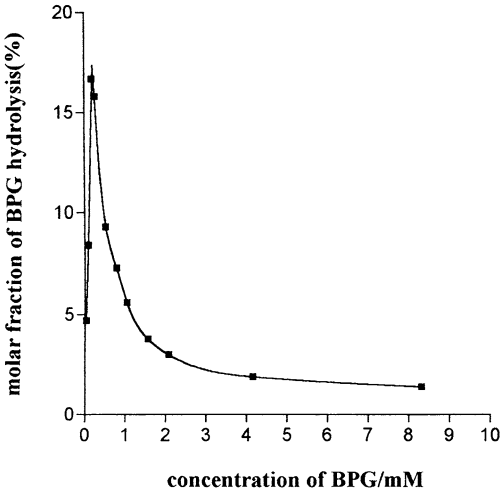

Fig. 5. Influence of concentration of BPG on the hydrolysis of BPG at 37 °C, pH 7.4 and 10 h. $[Ce^{3+}]_0 = 0.26$ mM. $[\ ]_0$-initial concentration.

under aerobic condition to a significant extent, it is reasonable to speculate that the autooxidation of Ce^{3+} plays important role in Ce^{3+} promoted BPG hydrolysis. In order to clarify the mediator is the cerium(IV) ion or the reactive oxygen species formed during the autooxidation of Ce^{3+}, we determined simultaneously the degree of hydrolysis of BPG and the concentration of cerium(IV) in Ce^{3+}-BPG solutions, which were exposed to the air. The results listed in Table 1 revealed that the percentage of hydrolysis increased in parallel with the increasing percentage of Ce^{4+} at various reaction time. The possibility of oxidative cleavage was excluded, since additional experiments showed that hydrogen peroxide did not promote the hydrolysis. On the other hand, a solution of K_2SO_3 was added to inhibit the autooxidation of Ce^{3+} and the hydrolysis was determined at various time. The results show that the hydrolysis was retarded significantly. Thus we conclude at that Ce^{4+} contributes much to the extraordinary rapidness of the Ce^{3+} in promoting BPG hydrolysis.

As shown in Figures 4–5, a positive dependence on Ce^{3+} concentration was observed up to 10 mM of Ce^{3+} with mole ratio Ce:BPG∼100. The degree of hydrolysis increased due to the large amount of Ce^{4+} -bond hydroxide gels formed. The hydrolysis depends on Ce^{4+}-bond hydroxide gels and BPG concentrations significantly. Furthermore, with 0.26 mM Ce^{3+}, the degree of hydrolysis increased with the increasing BPG concentration at low Ce:BPG ratio (∼1:1) and then a strong inhibitory effect was apparent at high BPG concentration. This indicates that the promoting effect on BPG hydrolysis is likely different from their effect on phosphodiester hydrolysis (Komiyama *et al.* 1994; Nakasaki & Chin 1994) which is a catalytic process, in which the efficiency is high and turnover is efficient.

To clarify the mechanism and site-specificity of ceric ion promoted BPG hydrolysis, the reaction was studied by scanning the ^{31}P NMR spectra of BPG in presence of Ce^{4+} at pH 2 and pH 8. As shown in Figure 6, Only 2P is affected even up to 40 h at pH 2 and the peaks of 3′PGA (0.94 ppm) and inorganic phosphate (0.44 ppm) were observable. This results reflects the high selectivity for the Ce^{4+} in promoting the BPG hydrolysis in acidic condition. At pH 2, the signals of phosphate of BPG and the hydrolytic products should shift to the downfield with the decrease of pH value (Russu *et al.* 1990). However, the upfield shift of signal of phosphate of BPG was obviously due to the Ce^{4+} induced shift of BPG, it indicates that Ce^{4+} possesses a higher binding tendency to wards phosphate of BPG and the effect of binding shift is larger than that of pH value. As shown in Figure 7, within 7 h, the hydrolysis at pH 8 is still limited to 2P, since beside the peaks for 2P (3.30 ppm) and 3P (3.98 ppm), only two new peaks appeared, i.e. 3′PGA (4.38 ppm) and inorganic phosphate (3.60 ppm). A longer reaction time led to the appearance of the new peak of 2′PGA (2.60 ppm) which is owing to the hydrolysis of 3P. However, since the ratio between the peaks 3′PGA and 2′PGA is 2.7, the scission of the P-O(2′) bond

Table 1. The effect of Ce^{3+} (18.7 mM) on the hydrolysis of BPG(0.1 mM) in relation with the amountof Ce^{4+} in the reaction mixture at pH 7.4. and 37 °C.

	Percentage of BPG hydrolysis(%)				The amount of Ce^{4+}(%)			
Reaction time (h)	0.0	0.5	2.0	6.0	0.0	0.5	2.0	6.0
In contact with air	0.0	7.5	18.2	48.5	0.0	10.2	28.3	68.0
Degassing the solvent with N_2	0.0	1.8	4.5	10.5	0.0	2.6	3.8	6.5

Values were the mean of three results.

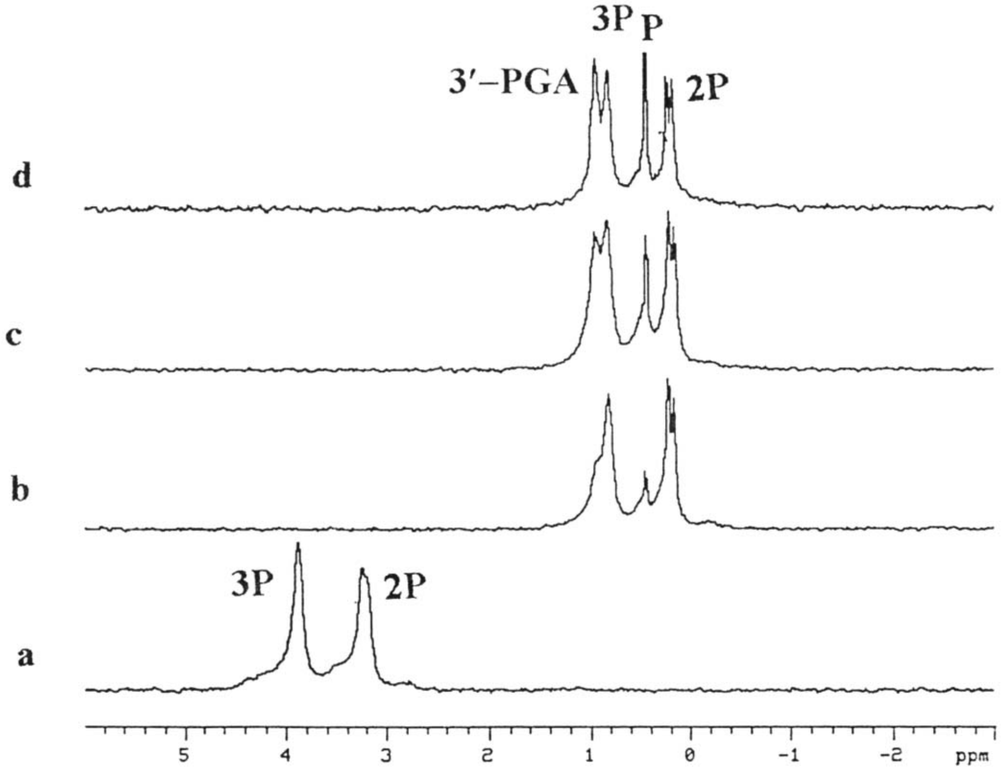

Fig. 6. ^{31}P NMR (161.903 MHz) spectrum of hydrolyzing BPG by Ce^{4+} at 37 °C and pH 2. $[Ce^{4+}]_0 = 30$ mM, $[BPG]_0 = 12$ mM. $[\ ]_0$-initial concentration (a) pure sample of BPG at pH 7.4; (b) incubated for 7 h; (c) 20 h; (d) 40 h.

is still preferential. The degree of upfield shift was small is related to the large amount of hydroxocerium complexes formed at pH 8.

In order to verify the reactive species in Ce^{4+} promoted BPG hydrolysis, the influence of pH in presence of $Ce(SO_4)_2$ was studied. A higher degree of hydrolysis was attainable in both low and high pH. The mixture for the BPG hydrolysis by Ce^{4+} at pH 7.4 involve some precipitation, presumably because of formation of the metal hydroxide. However, the BPG hydrolysis occurs over a wide pH range (from 1 to 10), and thus a detailed kinetic analysis has been carried out below pH 3 where the mixtures are homogeneous. The pseudo first-order rate constant is determined to be 0.65 h^{-1} and 3.1 h^{-1} respectively in presence of $Ce(SO_4)_2$ (18.7 mM) at pH 2 and pH 1, 37 °C. In order to clarificate the reactive species for the hydrolysis of BPG by Ce^{4+} at high pH, we studied the hydrolysis of BPG by gel phase and liquid phase of Ce^{4+} at pH 7.4. Interestingly, the gelatinous precipitates contribute much to the hydrolysis of BPG and the liquid phase of Ce^{4+} showed no activity at pH 7.4. Compared with Ce^{4+}, other lanthanide ions (Lu^{3+}, Eu^{3+}, La^{3+}) can not form gelatinous precipitates under working condition (pH 7.4, Hepes buffer). However, they are readily precipited when th pH value of the solution

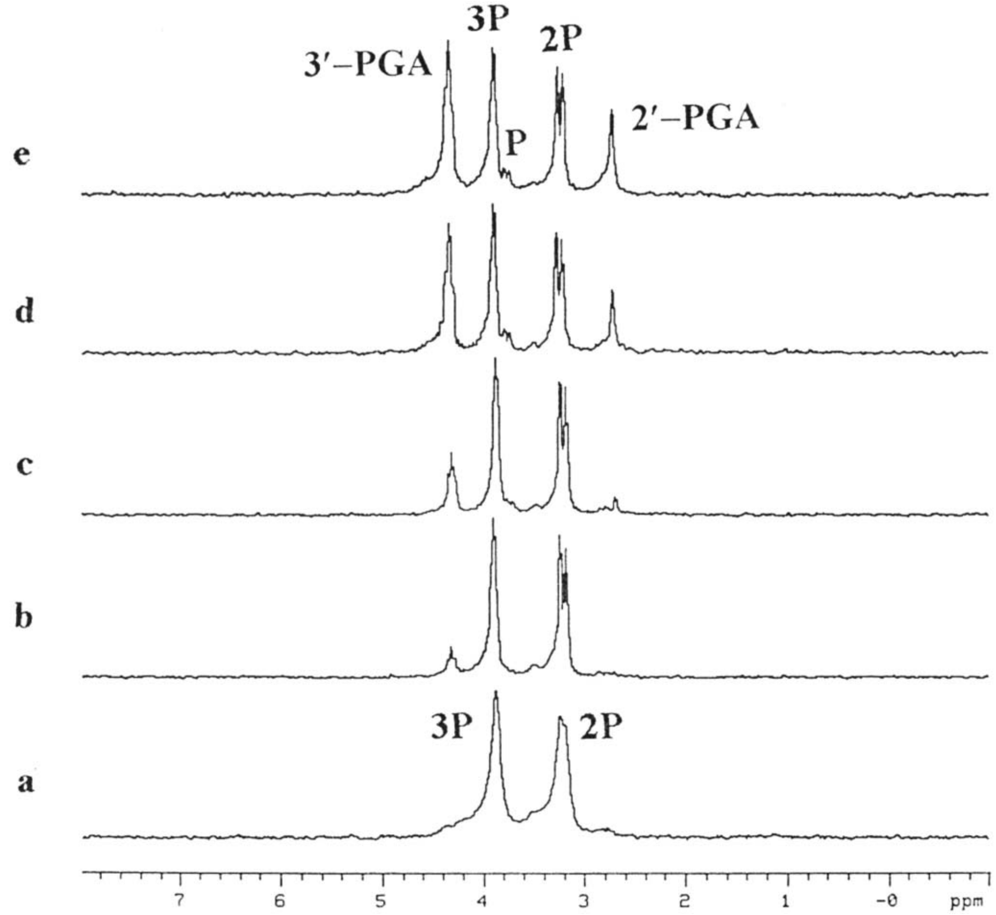

Fig. 7. ^{31}P NMR (161.9 MHz) spectrum of hydrolyzing BPG by Ce^{4+} at 37 °C and pH 8. $[Ce^{4+}]_0 = 30$ mM, $[BPG]_0 = 12$ mM. $[\ ]_0$-initial concentration (a) pure sample of BPG at pH 7.4; (b) incubated for 7 h; (c) 20 h; (d) 40 h; (e) 60 h.

was adjusted to 9. In such way, the gelatinous precipitates of these lanthanides contribute less to the hydrolysis. the results of mentioned above combined with the results of trivalent lanthanide (Lu^{3+}, Eu^{3+}, La^{3+}) are all inactive under acidic condition indicate that both aquocerium(IV) (acidic condition) and hydroxocerium(IV) (basic condition) are the possible reactive species.

Since the sulfate complexes of Ce^{4+} was known to be quite stable (Sillen & Martell 1971), we expected that the influence of increasing concentrations of sulfuric acid on Ce^{4+} promoted BPG hydrolysis is different from those with HCl and $HClO_4$. The results (Figure 8) showed that with the increase H_2SO_4 concentration, the increased concentration of SO_4^{2-} decreased both the concentration of aquocerium(IV) and degree of hydrolysis. On the other hand, the increasing concentration of HCl and $HClO_4$ promoted the hydrolysis further due to increased protonation of SO_4^{2-} and increased Ce^{4+} concentration. Meanwhile,

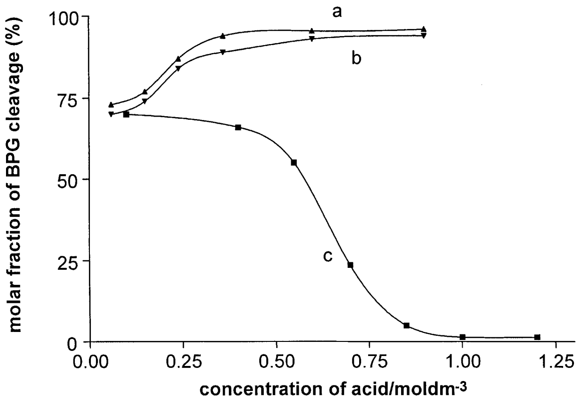

Fig. 8. Influence of concentrations of different acid on hydrolyzing BPG by $Ce(SO_4)_2$. $[Ce(SO_4)_2]_0 = 18.7$ mM; $[BPG]_0 = 0.1$ mM; 0.5 h; $[\ ]_0$-initial concentration (a) $HClO_4$; (b) HCl; (c) H_2SO_4.

Table 2. Effect of Ce^{4+} on BPG hydrolysis at pH 7.4, 37 °C; $[Ce^{4+}]_0 = 9.8$ mmol/L; $[BPG]_0 = 0.1$ mmol/L; $[\ ]_0$-initial concentration.

Reactiontime (h)	Percentage of BPG cleavage (%) $Ce(NH_4)_2(NO_3)_6$	$Ce(SO_4)_2$
0.5	9	8.3
1.5	13	9.2
3	55	43.5
5	83	70.6
10	92.8	89

Data are the average of three experiment results.

Table 3. Effect of Ce^{4+} on the hydrolysis of BPG at pH 1.5, 37 °C; $[Ce^{4+}]_0 = 18.7$ mmol/L; $[BPG]_0 = 0.1$ mmol/L; $[\ ]_0$? initial concentration.

Reactiontime (h)	Percentage of BPG cleavage (%) $Ce(NH_4)_2(NO_3)_6$	$Ce(SO_4)_2$
0.5	14.7	5.3
2	20.0	7.8
4	27.1	10.3
7	41.7	22.1
10	52.8	30.6

Data are the average of three experiment results.

the BPG hydrolysis promoted simply by hydrogen ions was not measurable. Thus, we speculate that the concentration of aquocerium(IV) is the major factor determining BPG hydrolysis in presence of $Ce(SO_4)_2$.

In addition, we studied the difference between $(NH_4)_2Ce(NO_3)_6$ and $Ce(SO_4)_2$ under acidic and basic conditions. As shown in Table 2 and Table 3, the hydrolysis was accelerated more significantly by $Ce(NH_4)_2(NO_3)_6$ than by $Ce(SO_4)_2$ in both conditions. Due to the higher stability constant of sulphato complex of Ce^{4+}, the concentration of aquocerium(IV) and hydroxocerium(IV) in $Ce(NH_4)_2(NO_3)_6$ solution are higher than that of $Ce(SO_4)_2$ in acidic and basic condition respectively. The results are also in support of the postulation that the aquo- and hydroxo-cerium(IV) are the acting species in acidic and basic conditions.

Discussion

In previous studies, Stankiewicz *et al.* (Stankiewicz 1989) have reported that vanadium(IV) cation stimulates the hydrolysis of BPG and thus increase the oxygen affinity of hemoglobin. The products of BPG hydrolysis were reported to be inorganic phosphate and 3-phosphoglycerate They suggested a mechanism involved in which the activated α-hydroxy phosphate is attacked by the carboxylate group of 2,3-BPG intramolecularly. This mechanism was based to explain the specificity for hydrolysis at the 2-position. Recently, Komiyama *et al.* studied the catalytic hydrolysis of phosphatidylinositol (PI) by lanthanides in basic conditions and found that YCl_3 was of the highest activity (Matsumura & Komiyama 1994). Of the two P-O bonds in the PI, only the one in the diacylglycerol side was cleaved. They proposed a mechanism, in which the lanthanide ions bind to the phosphate group, and the hydrolysis is ascribed to the intramolecular attack by the 2-hydroxyl group in the inositol. However, with this mechanism, the high-activity of YCl_3 and the preferential scission of the P-O bond in the diacylglycerol side can not be well explained. They also succeeded in demonstrating the first non-enzymatic hydrolysis of linear DNAs by use of lanthanide metal(III) salts and their complexes, Ce(III) is the most active. Then, they and Chin *et al.* (Nakasaki & Chin 1994) independently found that the Ce(IV) ion formed *in situ* is responsible for catalysis. The argument was finally supported by the effective catalysis of Ce(IV) $(NH_4)_2(NO_3)_6$ for DNA hydrolysis. The activity of Ce(IV) was found to be especially high. The mechanism for oxidative cleavage was ruled out and the hydrolysis was ascribed to the intramolecular attack by Ce(IV)-bound hydroxide ion (Komiyama *et al.* 1994; Komiyama *et al.* 1995; Sumaoka *et al.* 1994). In addition, catalytic hydrolysis of phosphate monoesters (Kluger & Cameron 2002) diester (Franklin 2001; Komiyama *et al.* 1995) and triester (Hay & Govan 1990), by lanthanide complexes was also reported and the catalytic species was ascribed to be hydroxolanthanide species too.

The site-selectivity to 2′-phosphate and the upfield shift of 2′-phosphate signal shed much light on the mechanism of lanthanides promoted BPG hydrolysis. We reported that the difference in the hydrolysis of 3′-mononucleotides and 5′-mononucleotides by lanthanides was related to the 2′-hydroxyl group in 3′-mononucleotides (Zhu *et al.* 1997). For the later cases, we proposed a mechanism including an intermediate with seven member chelate ring formed by the binding of lanthanide ions to the phosphate group and the adjacent 2′-hydroxyl group. Then the cleavage was affected by the intramolecular attack by the Ln^{3+}-bound hydroxide ion. Under acidic condition, the function of charge neutralization by Ce^{4+} play important role in the hydrolysis of 3′-mononucleotides. In the present case, from the influence of pH on the BPG hydrolysis in presence of Ce^{4+} and other lanthanides ions, we noted that the activity of Ce^{4+} is higher in both high and low pH, but the higher activity of trivalent lanthanides was observable only in basic condition. This indicates that the possible reactive species in basic conditions is the hydroxolanthanides species and that in acidic conditions is hydrated Ce^{4+}. The mechanism of the lanthanides promoted BPG hydrolysis is likely following a similar mechanism we proposed for 3′-mononucleotides.As shown in Figure 9, the lanthanide ions chelate the 2′-phosphate and the carboxylate group to form an unstable seven-membered ring structure, then the intramolecular attack by the Ln^{3+}-bound hydroxide ion. The inactivity of Sc^{3+} can be explained by its higher stability of hydoxo species (Sillen & Martell 1971). That 18.7 mM hydrogen peroxide has no effect on BPG hydrolysis indicates the hydrolysis is not related to the reactive oxygen species. The effects of hydrolysis BPG by Ce^{3+} and Ce^{4+} is furthermore in line with the recent observation of Chin *et al.* and Komiyama, even redox-active Ce^{4+} ions act as hydrolytic catalysts for the hydrolysis of DNA, the reaction involves a complex between the DNA and Ce^{4+} . All the kinetic and ^{31}P NMR evidence indicates that the hydroxide ion bound to the Ce^{4+} intramoleculayly attacks the phosphate of the DNA, which coordinates to the Ce^{4+} ion. As to the BPG hydrolysis, the activation of BPG by complex formed with Ce^{4+} is not remarkable. The ^{31}P-chemical shift change (0.3 ppm) is considerably amaller than those (2.3 ppm) observed for the co-ordination of phosphoesters to the Ce^{4+} complex (Komiyama *et al.* 1995). However, the activation of BPG by complexs formed with Lu^{3+} and La^{3+} is obviously, the ^{31}P-chemical shift change is greater than that observed for Ce^{4+}. The lower activity of trivalent lanthanides than Ce^{4+} in acidic conditions indicates that Ce^{4+} ions neutralize the negative charge of oxygen of the PO_4^{3-} and weakens the phosphomonoester bond of BPG more effectively. The higher effect of Ce^{4+} than the trivalent lanthanide ions in basic conditions is also attributed to the charge neutralization of Ce^{4+}. The involvement of carboxylate attributes to the site-specificity.

The nature of lanthanide (including Ce^{4+}) promoted phosphomonoester is still not clear. It might be different from the effect on phosphodiester hydrolysis. The latter case is a catalytic process, in which the efficiency is high and the turnover is efficient. However, for BPG, the process is likely not catalytic, since the percentage of hydrolysis depends on the concentration of lanthanide ions greatly, the working concentration is much higher than a catalytic one and the efficiency is rather low even in mole ratio Ce:BPG higher than 1:1 (Figure 4) Furthermore, the dependence on BPG concentration also disproves the catalytic nature of this reaction. The further study is under way to clarify the reaction mechanism.

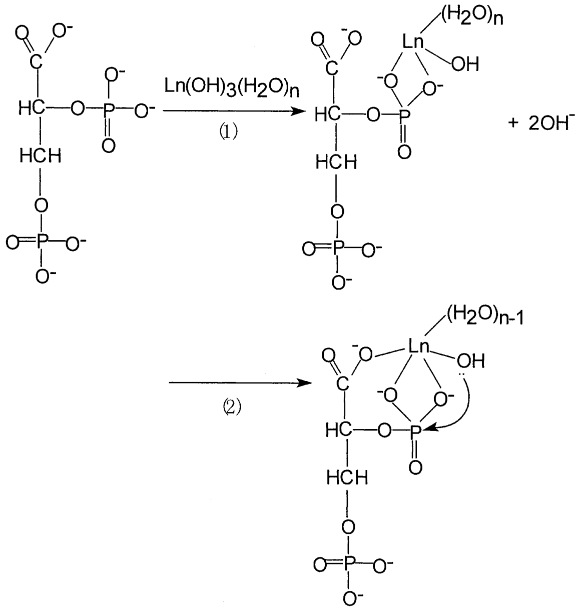

Fig. 9. Proposed mechanism for the BPG hydrolysis by $LnCl_3$.

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