Effects of lanthanide ions on hydrolysis of phosphatidylinositol in human erythrocyte membranes

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Abstract **The effects of lanthanides on the hydrolysis of phosphatidylinositol in human erythrocyte membranes were studied. ^{3}H-inositol labeling chromatography and HPLC were used to determine inositol 1, 4, 5-triphosphate and diacylglycerol separately, the hydrolytic products of phosphatidylinositol due to the reaction of lanthanide ions with human erythrocyte membranes. The unhydrolyzed phosphatidylinositol in membranes was also determined. The results indicate that the hydrolysis of phosphatidylinositol can be promoted by lanthanides (La^{3+}, Ce^{3+}, Y^{3+}, Tb^{3+}) and the effects of La^{3+} and Ce^{3+} are stronger than those of Y^{3+} and Tb^{3+}.**

Keywords: **lanthanids ions, phosphatidylinositol, second messengers.**

Although the mechanism of lanthanides' effects on promoting plant growth and inducing apoptosis has been explored for long, it is still not well understood up to now. By means of HPLC, Matsumura[1] found that the reaction of liposome of phosphatidylinositol (PI), one of phosphatidylinositols, with lanthanides generated diacylglycerol (DAG). This suggested that lanthanides are active in promoting PI hydrolysis. It was well documented that under the catalysis of phospholipase C, phosphatidylinositols (including PI, phosphatidylinositol-4-phosphate (PIP), phosphatidylinositol-4,5-bisphosphates(PIP_2)) were hydrolyzed to diacylglycerol (DAG) and inositol phosphates (including inositol 1-phosphate (IP), inositol 4, 5-bisphosphates (IP_2), inositol 1, 4, 5-trisphosphates (IP_3)). DAG and IP_3, as the intracellular second messengers, trigger the DAG/PKC and IP_3/Ca^{2+} signal transduction system and consequently lead to cell proliferation or apoptosis. In the present work, using human erythroctye membranes as the experimental model, we investigated the effects of lanthanides on this system by ^{3}H-inositol labeling chromatography[2, 3] and HPLC method. The results show that lanthanides do have the phospholipase C-like activity.

1 Materials and methods

(i) Materials. The instruments used in the experiment encluded LG10-2.4A centrifuge (Beijing Medical Centrifuge Factory), low temperature and high speed centrifuge J2-21M (Beckman), Wallac 1410 liquid scintillation counter (Pharmacia), chromatography column (8 mm×20 cm), high performance liquid chromatography instrument (Watson), 996 detector and 510 pump. Tris (Sigma), TritonX-100 (Bio-Rad), Diphenyloxazole (PPO, Fluka), Dowex 1×8 formate resin (Bio-Rad), ^{3}H-inositol (Shanghai Nuclear Technology Exploration Cooperation), choline chloride (Sigma), methanol and acetonitrile were chromatographically pure, other reagents are analytically pure. Lanthanide oxide (purity$>$99.9%) was dissolved in concentrated hydrochloric acid and heated to expel the excess HCl and then diluted with de-ionized water to a certain concentration. The fresh human erythrocytes were provided by the Red Cross Blood Center of Beijing.

(ii) Methods

(1) Determination of hydrolytic products (IP, IP_2, IP_3) of phosphatidylinositol after reaction of lanthanide ions with human erythrocyte membranes. Human erythrocyte membranes were isolated from fresh human blood according to ref. [4] as opal "ghosts". The "ghosts" were suspended in lysis solution (5 mmol • L^{-1} Tris-HCl, pH 7.8) and kept at −20℃. The protein concentration was determined by Lowry method.

To 2 mL of ghosts suspension (the protein concentration was 2.3 g • L^{-1}), 20 μL ^{3}H-inositol (1 Ci • L^{-1}) was added and then incubated for 3 h at 37℃. The membranes were washed with lysis solution for six times. To each of 0.5 mL labeled membranes, one of the lanthanides (La^{3+}, Ce^{3+}, Y^{3+} or Tb^{3+}) was added to make the final concentration of lanthanides to be 3×10^{-3} mol • L^{-1} respectively. The control was run in the same way, but lysis solution was used in substitution for lanthanides. All these samples were incubated at 37℃ for 24 h. Then they were centrifuged at 10000 *g* for 10 min. The supernatant was collected and pH was adjusted to 8.0 with 1 mol • L^{-1} NaOH, and diluted to 5 mL with lysis solution.

The Dowex 1 × 8 chromatography columns were equilibrated for 5 h with 60 mmol • L^{-1} ammonium formate solution containing 5 mmol • L^{-1} borax. The samples prepared as above were applied to the columns. Inositol phosphates, including IP, IP_2 and IP_3 were eluted with 10 mL eluting solution Ⅰ (0.1 mol • L^{-1} formate and 0.2 mol • L^{-1} ammonium formate), Ⅱ (0.1 mol • L^{-1} formate plus 0.4 mol • L^{-1} ammonium formate) and Ⅲ (0.1 mol • L^{-1} formate plus 1.0 mol • L^{-1} ammonium formate) respectively. The eluants were collected as 2 mL fractions. After adding 10 mL Biofluor (dimethylphenylene / TritonX-100=2∶1 (*v*/*v*)), containing 0.5% diphenyl-oxazole), the radioactivity of each fraction (0.5 mL) was counted.

(2) Determination of phosphatidylinositol remained in erythrocyte membranes after reaction with lanthanides. Human erythrocyte membranes were prepared and labeled according to the method described above. Lanthanides

(La^{3+}, Ce^{3+}, Y^{3+} or Tb^{3+}) were added separately to the labeled membranes to the concentration of 2×10^{-3} mol • L^{-1}. To run the control, lysis solution was added in place of lanthanides. After being incubated at 37℃ for 24 h, the samples were extracted by 2.5 mL extractant (chloroform/methanol/HCl (12 mol •L^{-1})=50/100/1 (*v/v/v*)) for 40 min with continuously shaking. A portion (0.5 mL) of the lower phase was removed into scintillation vial. The extraction was repeated twice and finally 1.0 mL of solution were collected for radioactivity measurement after adding 10 mL of biofluor.

(3) Determination of DAG released from erythrocyte membranes under the action of lanthanides. To 200 μL of human erythrocyte membranes prepared as above, one lanthanide (La^{3+}, Ce^{3+}, Y^{3+} or Tb^{3+}) was added and then diluted to 3 mL with 5 mmol • L^{-1} Tris-HCl (pH 8.0). The final concentration of lanthanide ion was 1×10^{-3} mol •L^{-1}. After incubation at 37℃ for 30 h, the mixture was extracted by 2 mL chloroform twice and then dried with nitrogen. The residue was dissolved in 150 μL chloroform by shaking for 2 min. A sample of 50 μL was applied to HPLC (ODS column), and eluted with methanol/acetonitrile/water (93.5/1.5/5, *v/v/v*) mixture containing 25 mmol • L^{-1} choline chloride. The elution rate was kept at 1 mL/min, and the elution was monitored by measuring the absorbency at 210 nm.

2 Results

The results of the hydrolytic products of phosphatidylinositol (IP, IP_2, IP_3) after the action of lanthanide ions on human erythrocyte membranes are shown in table 1, figs. 1 and 2.

Table 1 Effects of La^{3+}, Ce^{3+}, Y^{3+} and Tb^{3+} on the hydrolysis of phosphatidylinositol in erythrocyte membrane (*n*=2)

Sample	Radioactivity/Bq		
	IP	IP_2	IP_3
Buffer	8.2±1.6	1.3±0.28	0.87±0.026
La^{3+}	6.9±1.8	7.1±0.46	1.8±0.13
Ce^{3+}	6.8±1.8	1.2±0.025	1.8±0.26
Y^{3+}	7.3±2.3	1.5±0.38	0.97±0.026
Tb^{3+}	7.8±1.8	0.95±0.026	0.85±0.026

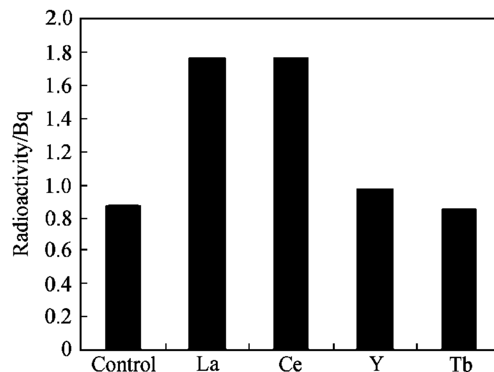


Fig. 1. Effects of La^{3+}, Ce^{3+}, Y^{3+} and Tb^{3+} on IP_3 released from erythrocyte membranes.

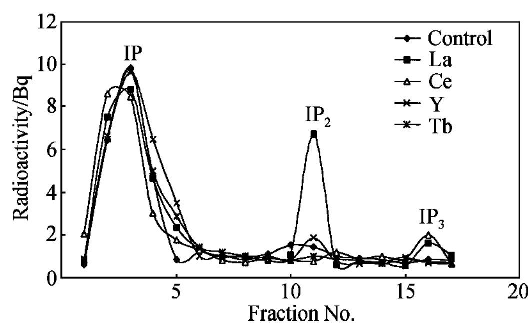
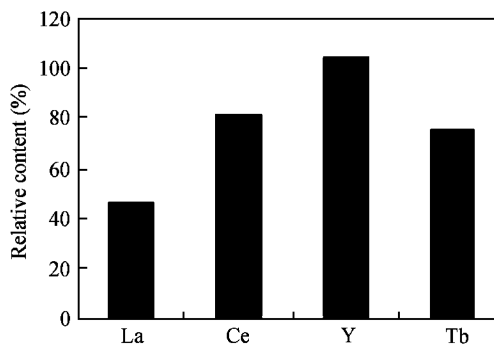


Fig. 2. Effects of La^{3+}, Ce^{3+}, Y^{3+} and Tb^{3+} on the amount of inositol phosphates (IP, IP_2, IP_3) released from erythrocyte membranes.



$$\text{Relative content (\%)}=\frac{\text{Sample}-\text{blank}}{\text{Control}-\text{blank}}\times 100\%.$$

Fig. 3. Effects of La^{3+}, Ce^{3+}, Y^{3+} and Tb^{3+} on relative content of phosphatidylinositol remained in erythrocyte membranes ($\bar{X}$ ± S. D., n = 4).

The results given in figs. 1 and 2 showed that lanthanides, like phospholipase C, promoted PI hydrolysis producing IP_3 in human erythrocyte membranes. The level of IP_3 was increased after treatment with any lanthanide studied. The effects of light lanthanides such as La^{3+} and Ce^{3+} were stronger than those of heavy lanthanides such as Y^{3+} and Tb^{3+}.

The decreases of phosphatidylinositol remained in human erythrocyte membranes after treatment with lanthanides were shown as relative content to the control in fig. 3. In consistency with the results shown in fig. 1, La^{3+} was shown to be the most active, Ce^{3+} and Tb^{3+}, weaker, and Y^{3+} almost inactive.

The amount of DAG released from erythrocyte membranes hydrolyzed under the action of La^{3+}, Ce^{3+}, Y^{3+} or Tb^{3+} is given in table 2 and fig. 4.

The results given in table 2 and fig. 4 reveal that the lanthanides are able to cleave the phosphomonoester bond in phosphatidylinositol like phospholipase C. The peak of DAG appeared at retention time 40 min, which is consistent with the reported value[1]. Based on the integrated area of each peak, La^{3+} is the most active one among the

lanthanide ions studied and the activity sequence is $La^{3+} > Ce^{3+} > Y^{3+} > Tb^{3+}$ (table 2).

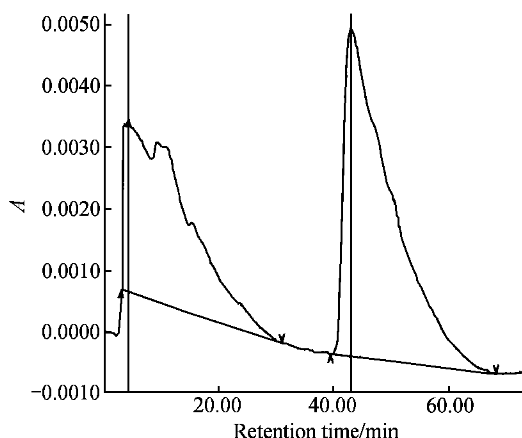


Fig. 4. HPLC pattern for DAG released from erythrocyte membranes by La^{3+} promoted hydrolysis.

Table 2 DAG released from erythrocyte membranes by La^{3+}, Ce^{3+}, Y^{3+} and Tb^{3+} promoted PI hydrolysis

Sample	Value of peak (AU)
Buffer	—
La^{3+}	0.005
Ce^{3+}	0.0007
Y^{3+}	0.0003
Tb^{3+}	0.0003

3 Discussion

The IP_3 and DAG generated from phosphatidylinositol and the changes in total amount of inositol phosphates in human erythrocyte membranes under the action of lanthanides were determined by two methods. All the results showed that lanthanides, like phospholipase C, can induce phosphatidylinositol hydrolysis in membrane. However, the activities of the individual lanthanide was different. The catalytic abilities of light lanthanides such as La^{3+} and Ce^{3+} were stronger and heavy lanthanides such as Y^{3+} and Tb^{3+} were comparatively weak. Based on the present results, it cannot be excluded that the effect of lanthanides on the activity of enzymes relating to PI metabolism, beside direct hydrolysis of phosphatidylinositol. The lanthanides probably interfere with the PI metabolism by directly affecting the enzymes' activities. The present results might be an integrated effect of nonenzymatic and enzymatic mechanisms.

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