INHIBITION OF RARE EARTH CATALYTIC ACTIVITY BY PROTEINS

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Abstract. Catalytic action of rare earth element, Ce(IV) to hydrolyze phosphomonoester bonds was confirmed. This effect was considered to suppress abiotic synthesis of nucleotides and nucleic acids in the primitive sea, and hence the origin of life. However, we found that the presence of proteins, especially albumin, strongly inhibited the catalytic action of Ce(IV). This finding was supported by preferential binding of rare earth elements (REEs) to proteins which was revealed using the radioisotopes of these REEs. Consequently, if a large amount of proteins was synthesized in the primitive sea, abiotic synthesis of phosphomonoester compounds, and hence nucleic acids, might have been possible.

1. Introduction

Several recent studies focused on the hydrolysis of DNA, RNA (Komiyama and Sumaoka, 1998) and $3',5'$-cyclic adenosine monophosphate (Sumaoka *et al.*, 1992) by rare earth elements (REEs). We previously found strong dephosphorylating activity of REEs using *p*-nitrophenyl phosphate (*p*-NPP) as a substrate (Akaboshi *et al.*, 1999) Among all the elements examined, the activity of Ce(IV) was the highest, and the activities of Sm and Ho followed Ce(IV). Under ordinary conditions (37 °C – 1 hr), 0.28 μmol of *p*-NPP was hydrolyzed per 1 μmol Ce(IV). The magnitude of this activity was about 1/70.000 that of standard alkaline phosphatase. Kinetic analysis of this reaction showed that the K_m value of Ce(IV) (0.405 mM) was similar to that of the enzyme (0.630 mM), and that the reaction rate (0.28 μmole/μmol hr^{-1}) was retained until at least 16 hr when total hydrolyzed *p*-NPP reached 2.5 μmole μmol^{-1} Ce(IV). Based on these results, we concluded that the reaction was catalyzed by Ce(IV).

These experimental results and the findings that considerably higher concentration of REE might have been dissolved in the primitive sea water (Bowen, 1966; Cloud, 1968), suggest that accumulation of phosphate monoester compounds, such as AMP and GMP, the concentrations of which in the primitive sea were expected to be sufficiently high to produce nucleic acids in the later process of chemical evolution, might have been impossible. Therefore, the origin of life as a consequence of chemical evolution might also have been impossible. However, life on earth developed via chemical evolution. We proposed a working hypothesis that

some mechanism which suppresses the hydrolyzing activity of REEs existed in the primitive ocean.

In order to confirm the hypothesis described above, we searched for a substance with an inhibitory effect on the hydrolyzing activity of REEs. Our study examined the effect of physical and chemical factors on the hydrolyzing activity of REEs, as well as, the implication of these activities in chemical evolution.

2. Experimental Methods

2.1. Chemicals and assay of hydrolyzing activity

All chemicals, other than albumin (SERVA Feinbiochemica GmbH & Co. KG, Lot No. 05102), histone (Washington BioChem. Co., Lot No. M2B859) and gelatin (Sigma Chem. Co., Lot No. 63H0659) used in this study were of analytical grade supplied from Sigma Chem. Co. or Wako Chem. Co. Hydrolyzing activities of both REEs and alkaline phosphatase were assayed using the alkaline phosphatase measuring kit supplied by Kirkegaard & Perry Laboratories Inc. Usually 10 μL of 0.2 μM REE-solutions or 25 pM alkaline phosphatase (Sigma's product), was added to the 5 mL reaction mixture containing 5 mg p-NPP and 4 M of diethanolamine buffer (pH = 10.4). Then the mixtures were incubated for 1 hr at various temperatures. After incubation, the optical density (yellow color derived from liberated p-nitrophenyl) was measured at 410 nm using a spectrometer.

2.2. Radioactive isotope production

^{141}Ce, ^{160}Tb and ^{170}Tm were produced by thermal neutron bombardments of ^{140}Ce (Ce(SO$_4$)2H$_2$O, 10 mg) ^{159}Tb (Tb$_4$O$_7$, 46 mg) and ^{169}Tm (Tm(CH$_3$COO)$_3$, 50 mg). For Tb and Tm, thermal neutron irradiation was carried out in a pneumatic tube (thermal neutron flux: 2.34×10^{13} n cm^{-2} sec^{-1}) of KUR (Kyoto University Reactor) for 1 hr, while the hydraulic conveyer (thermal neutron flux; 8.15×10^{13} n cm^{-2} sec^{-1}) was used for 75 hr in the case of Ce. After irradiation, each RI were dissolved in conc. HNO$_3$, and evaporation of HNO$_3$ was carried out at below 100. After dissolving in HCl and repeated evaporating with H$_2$O until complete removal of HCl, the samples were dissolved in 1 mL H$_2$O. Then the mixture of these RI was prepared as follows: Ce; 10 μL, 3.33 MBq, Tb; 10 μL, 8.51 MBq and Tm; 480 μL, 2.36 MBq, and 10 μL of the mixture (total REE content: 0.0013 mmol) was added to 1 mL of aqueous solution of various bio-molecules which contained 2 mg each of DNA, RNA, albumin, gelatin, histone and penta-glycine. Then they were incubated for 0.5–3 hr at 37 °C. After incubation, the molecular fractions were collected by 60%-ethanol precipitation and the radioactivites in the supernatant and precipitation fractions were measured using a Ge(Li)-semiconductor detector coupled to a multichannel analyzer.
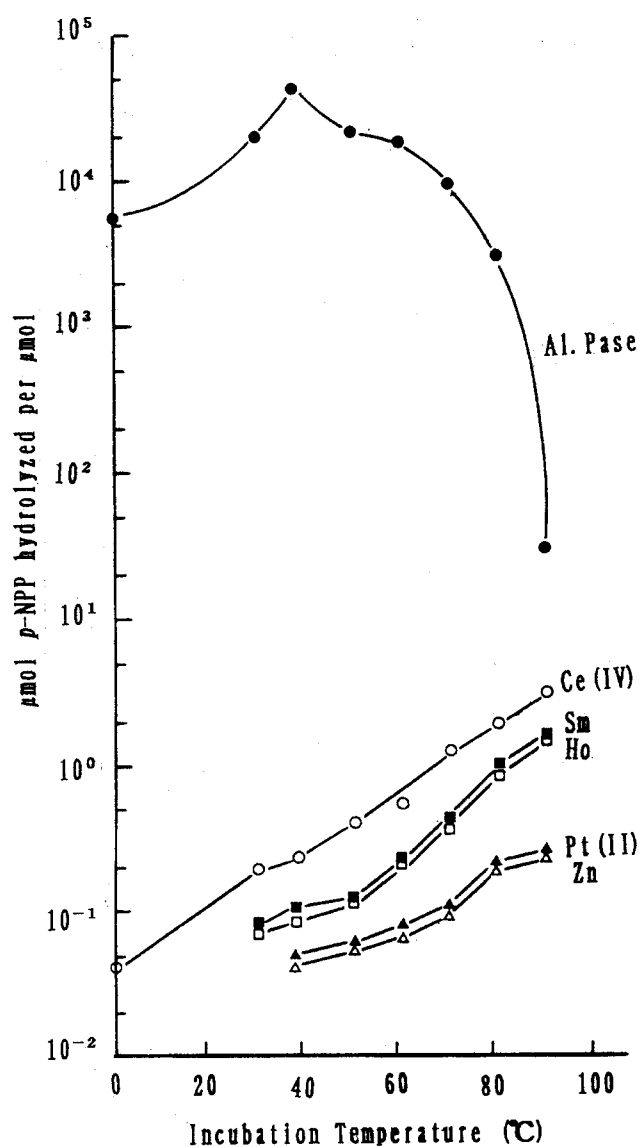
Figure 1. Variations in the hydrolyzing activities of REEs and the other metals with incubation temperature.

3. Results

3.1. EFFECT OF DIFFERING TEMPERATURES AND pH ON THE HYDROLYSIS RATE

First we examined the hydrolysis rates by REEs at different temperatures. Experimental results are shown in Figure 1 in which the logarithm of the hydrolysis rate
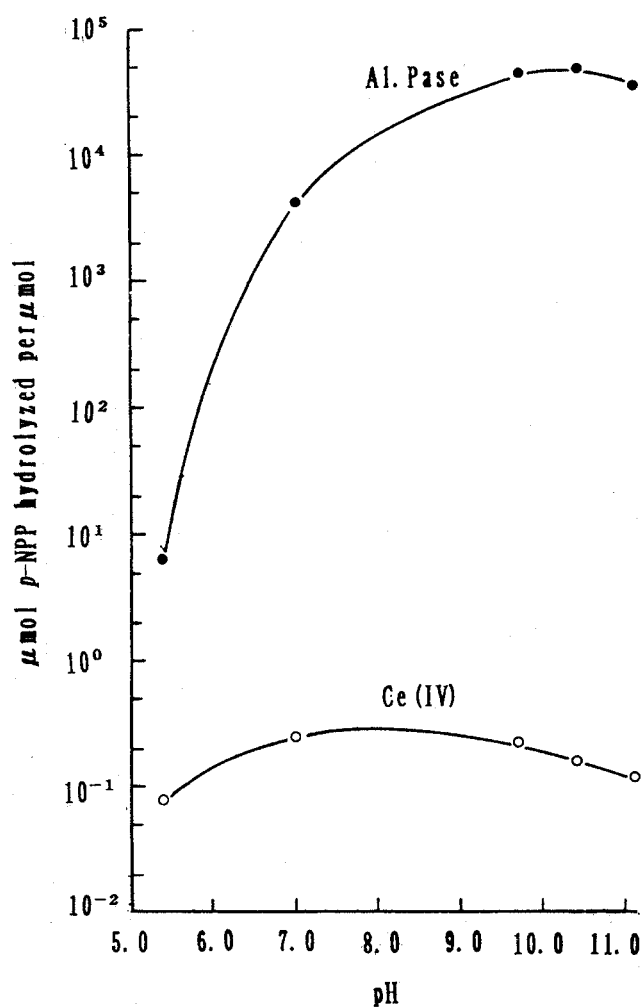
Figure 2. Variation in the hydrolyzing activity of Ce(IV) with different pH.

per hour is plotted against temperature. The rate of hydrolysis by REEs and other metals increased almost exponentially with increasing temperature until at least 90 °C, while that by alkaline phosphatase drastically decreased at lower and higher temperatures than 37 °C.

We could not detect such activities by much more abundant elements Zn, Ca, Mg, etc. (only that by Zn is shown in the figure) and less abundant elements Pd, Pt, Au, etc. (only that by Pt is shown) below 37 °C. From Figure 1, the rate of hydrolysis by Ce(IV) at 20 °C was estimated to be 0.11 μmol μmol^{-1} hr^{-1}. Moreover, it was found that the activity did not change at different pHs, while that of the enzyme was completely absent at pH 5.4 (Figure 2).
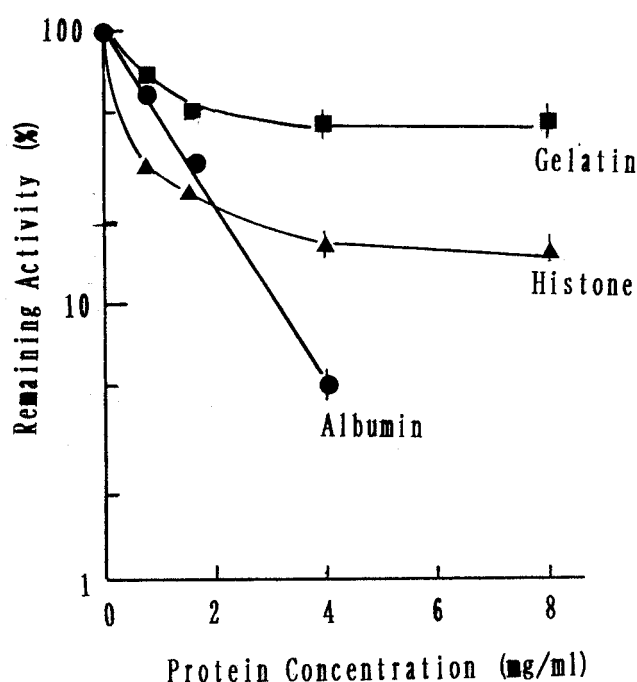
Figure 3. The suppressive effect of various proteins on the hydrolyzing activity of Ce(IV). Values given are means±SD from 3 separate experiments.

3.2. SUPPRESSIVE EFFECT OF VARIOUS BIOMOLECULES ON THE HYDROLYZING ACTIVITY

We initially examined the effect of montmorillonite (100 mg of purified clay material, kindly donated from Dr A. Shimoyama, Tsukuba University, was added to the reaction mixture) and synthetic sea water (product of Yashima Chem. LTD., Aqua Marine-S was employed and whole reaction was carried out in the sea water) on the hydrolyzing activity of Ce(IV). Only slight acceleration (3–5%) of the reaction was observed by these modifications. Trace amounts of unexpected REE impurities may be responsible for the acceleration. On the other hand, addition of amino acids including di- and tri-glycine, bases, nucleosides and starch to the reaction mixture did not alter the reaction rate. However, we found that some proteins such as albumin, histone and gelatin showed a strong inhibitory effect. Figure 3 shows the suppressive effect of various proteins on the hydrolyzing activity of Ce(IV). The addition of 20 mg albumin into 5 mL of reaction mixture almost completely suppressed (5%) the dephosphorylating activity of Ce(IV). The dose-survival curves of the hydrolyzing activity by Ce(IV) in the presence of histone and gelatin consisted of two components, suggesting the complexity of the interaction. The values for maximal inhibition were 16 and 45% for histone and gelatin, respectively. The presence of these proteins did not affect the dephosphorylating activity of

alkaline phosphatase. Neither an increase nor a decrease in the inhibitory effect was observed by the trypsin digestion of these proteins, while 2.66~106.5 Gy of ^{60}Co-γ-irradiation (both degradation and polymerization of the proteins was supposed to occur in this dose range) resulted in a slight decrease in the activity depending on the dose. This result suggests that the interaction between REEs and peptide chains or the functional groups on the side chains of the long peptide structure caused this phenomenon.

3.3. BINDING KINETICS OF REEs WITH PROTEINS

To confirm this, we produced radioactive isotopes (RI) of several REEs and examined the binding kinetics of REEs with these molecules. Figure 4 shows the radioactivity in the precipitated materials as the % of the total radioactivity. All REEs tested (Figures 4A, Ce; 4B, Tb and 4C, Tm) bound to proteins in preference to nucleic acids and pentaglycine. The rate of binding of REEs to proteins was so rapid that 1 or more hours under these conditions was sufficient to the complete process. Nearly 100% of Tm, 80–90% of Tb and 50–70% of Ce added bound to these protein molecules after 1 hr-incubation at 37 °C. Among three proteins, albumin showed the highest affinity to REEs, while gelatin showed the lowest. This result supported the above findings that proteins, especially albumin showed a strong inhibitory effect on hydrolysis by Ce(IV). Binding of REEs with proteins might inactivate the ability of REEs to hydrolyze phosphomonoester bonds. Concerning the number of REEs combined to albumin molecules, the results demonstrated that nearly all REE molecules added (0.0013 mmol) combined to 2 mg of albumin molecules, namely 0.000044 mmol as protein (about 0.016 mmol as amino acid). This finding suggests that not only the terminal sites of the protein molecules but also the functional groups of side chains play a role in the binding. Furthermore, REEs seem to recognize their counterparts in their combination. Namely, Ce distribution in the DNA fraction was higher than that in the RNA fraction, while Tm tended to distribute in the RNA fraction.

4. Discussion

At the beginning, we estimated the amount of phosphate monoester bonds which had been hydrolyzed in the primitive sea. Assuming that the decomposition rate for phosphate monoester compounds at 20 °C by REEs was 0.11 μmole/μmole-REE hr^{-1} and that the REE concentration in the primitive ocean was higher than 100 pmol/kg, namely 0.1 nM, (La; 37.2, Ce; 5.97, Nd; 26.8, Sm; 5.02, Dy; 7.03 and the others) (German *et al.*, 1995), REEs dissolved in the sea water can hydrolyze 0.011 nmol (0.11 nM in 1 L-sea water) of phosphate monoester bonds per 1 hr, and hence 96.4 μM yr^{-1}. (As to the element composition of the primitive ocean: Literatures describe as follows. The primal ocean probably had a slightly different
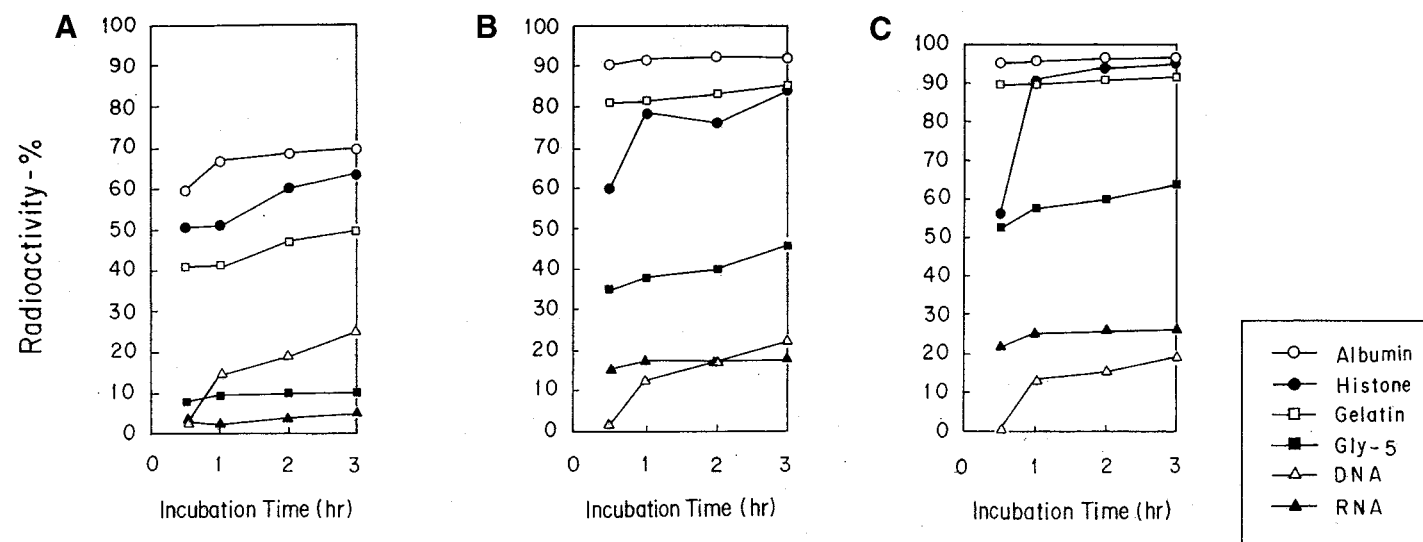
Figure 4. Radioactivities retained in the precipitated fractions of various biomolecules treated with the RI-mixture (4A, Ce; 4B, Tb and 4C, Tm).

composition from the modern sea, which has been modified by many cycles of erosion and sedimentation. It is thought that these two processes have been in dynamic equilibrium for at least 0.5 aeons, so that the composition of the ocean has remained the same for that length of time (Bowen, 1966). The composition of the primitive seas, although not exactly known, was probably not markedly different from that of the present composition (Cloud, 1968)).

Therefore, sea water containing about 10 mM of accumulated phosphomonoester bonds might lose these bonds within 100 yr. This concentration corresponds to the solubility limit in water of various bases, nucleosides and nucleotides. We measured the solubility of several nucleotides, and found that 200 mg of citidine $2'(3')$-monophosphate (free salt) could hardly dissolved in 63 mL of water (namely, solubility; 9.8 mM) or 51 mL of synthetic sea water (12.2 mM) at 20 °, while 1 g of guanosine-$5'$-monophosphate sodium salt dissolved in 9.2 mL water (266.3 mM) and 100 mg of the same compounds in 50 mL of synthetic sea water (4.9 mM).

These findings also indicate that chemical evolution and hence life on earth were impossible unless marked prebiotic synthesis of phosphomonoester compounds occurred or these compounds rapidly escaped from the system of synthesis.However, the present results revealed that if a large amount of protein was synthesized in the primitive sea, abiotic synthesis of phosphomonoester compounds, and hence nucleic acids, might have been possible. According to Lahav (1999), the origin of life process may be divided into a first stage of prebiotic synthesis of the building blocks of biology and their simple interactions, and a second stage characterized by the emergence of more complex chemical entiies distinguished by central attributes of life, such as metabolic cycles and template-directed synthesis (Lahav and Nir, 1997). The present paper adds an important step to the first stage by suggesting that at least polypeptides or protein-like substances may have been produced prior to nucleic acids.

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