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The use of complexes of algae polysaccharides and Ce^{4+} to degrade compounds containing peptides or phosphate ester bonds

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Abstract

Effective degradation of compounds containing peptides or phosphate ester bonds in environment is important. In this paper complexes of algae polysaccharides (APS) and Ce^{4+} were made, and catalysts of the complexes for degradation of compounds containing peptides or phosphate ester bonds were examined. The results show that the complexes could hydrolyze bovine serum albumin (BSA). The complex composed of laminaran and Ce^{4+} could not only significantly hydrolyze BSA but also rapidly convert the supercoil DNA (plasmid DNA form) into the linear form DNA (plasmid DNA form) in neutral pH solution. They could also promote the degradation of chlorpyrifos. The chlorpyrifos residual in neutral pH solution treated by the complex was 1.16 mg/l and the degradation rate was about 94.28% after 48 h, whereas without the complex treatment the residual 9.37 mg/l and the ration 53.82%. The chlorpyrifos residual in spinach from the treatment plot was only1.14 mg/kg on 10 days after spraying the complex, whereas the residual in spinach from the control plot was 3.10 mg/kg.

Keywords: Degradation; Phosphate ester; Bovine serum albumin; Algae polysaccharides; Cerium

1. Introduction

Some of organic compounds in municipal and industrial sewage are polymers, which contain peptides or phosphate ester bond. Organophosphates are widely used as agricultural pesticides after organochlorine pesticides were forbidden. Many organophosphates residues in food have arisen from their extensive agricultural application and industrial emission in the environment. Many toxic organophosphates persist in the environment and tend to accumulate in the body fat of animals occupying a higher trophic level. One reason for the environmental persistence of these compounds is that microorganisms are either unable to degrade them or do so very slowly.

The contaminated environment must impact on food safety. Therefore, effective degradation of the compounds containing peptides or phosphate ester bonds in the environment is very important to us.

Bacteria, plants and aquatic animals take up monomers more easily than those polymers. But cleavage of the peptide or phosphate bond in the polymers takes hundreds of years or more without natural enzyme catalysts. Under communal environment, there are many problems in applying natural enzymes to hydrolyze these bonds. Man has done a lot since the 1990s in order to develop a new tool for hydrolysis of the bonds. It is found that the activity of rare earth elements especially Ce^{4+} had overwhelmingly greater than those of other metal ions on cleavage of peptide or phosphodiester bonds (Kajimura, Sumaoka, & Komiyama, 1998; Komiyama, 1995; Sumaoka, Igawa, & Komiyama, 2000, & Takarada, Yashira, & Komiyama, 2000). However, Ce^{4+} easily forms a gel of metal hydroxide under neutral solution, and this feature imposes limitations to the scope of its application in agriculture and environment sciences. Fortunately, Kajimura A. et al. (Kajimura et al., 1998) found that dextran, some monosaccharides and their ramifications such as lyxose and mannitol could coordinate with Ce^{4+} by oxygen atoms into complexes, which could easily dissolve in water and hydrolyze plasmid DNA (Komiyama, 1995, & Sumaoka et al., 2000).

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Algae polysaccharides (APS) made from brown seaweeds such as *Laminaria* and *Macrocyslis* are abundant marine polysaccharides. The APS can be divided into alginic acid, fucoidan and laminaran according to the composition and structure of APS (Tako, Nakada, & Hongou, 1999; Xiao, Shicui, Song, & Yang, 1999, & Zvyagintseva, et al, 1999). Alginic acid is mainly composed of β-1,4-D-manuronic acid and α-1,4-L-guluronic acid. Fucoidan, a sulfated polysaccharide found in the cell-wall matrix of brown algae, is composed of fucose, galacose, xylose, glucuronic acid. Laminaran, which is mainly composed of water-soluble(1→3),(1→6)-β-D -glucans, is a storage polysaccharide existing in the cell.

There are many oxygen atoms in sugar-chain of the APS. The oxygen atoms can bind Ce^{4+}, so it is possible to prepare the complexes of APS and Ce^{4+}. Both of APS and Ce^{4+} have available quantity (Guo, 1998; Hien, Nagasawa, Tham, Yoshii, Dang and Mitomo, 2000; Jiang, Liu, Zhang, & Guan, 1999; Wang, Wang, Wei, Qi, & Zhao, 2003, & Xiao et al., 1999). The oligosaccharide derived from depolymerization of alginates by enzyme or other methods was reported to have novel features such as stimulation of growth of plants. Ce and other rare earth elements have stimulation of growth of plants also. Scientists in China have applied inorganic compounds of rare earth elements and extract from seaweed to agriculture in the form of foliar fertilizer.

The purpose of this study was to elucidate the degradation potential of complexes of APS and Ce^{4+} for some compounds containing peptide or phosphate bond. Plasmid DNA, chlorpyrifos and bovine serum albumin (BSA) were respectively selected for the target compounds owing to plasmid DNA containing phosphodiester, chlorpyrifos containing phosphotriester bond and BSA containing peptide bonds.

2. Experiments

2.1. Chemicals

Fucoidan, laminaran (laminarin), Chlorpyrifos (CAS-No. 2921-88-2, purity 99.2%? and BSA were from Sigma. Alginic acid was purchased from Nacalai Co., LTD (Japan). Plasmid pBR322 DNA (concentration is 6.6 O.D. DNA/ml) was from TOYOBO Co. LTD (Japan). All other chemicals used were of guaranteed grade and from China Chemical Co. LTD (China). The water used was glass doubly distilled water.

2.2. Preparation of homogeneous solutions composed of Ce^{4+} and APS

Homogeneous solutions composed of Ce^{4+} and APS were prepared by the same methods of Kajimura et al. (1998). The required amounts of Ce(NH4)2(NO3)6 were dissolved in 20 m mol/l Hepes buffer. The concentration of the Ce^{4+} ion was 5 mg/ml. The aqueous solutions of alginic acid, fucoidan and laminaran, all concentration was 5 mg/ml, were added dropwise into the Hepes buffer containing Ce^{4+} until small sediment appeared, respectively. The pH of the mixture was immediately adjusted to pH 7.0 by using a small amount of NaOH, and then centrifuged at 10,000*g* for 10 min. The supernatant was collected for the homogeneous solutions composed of Ce^{4+} and APS. The solutions of the complexes did not sediment after 2 weeks. The Hepes buffer of Ce^{4+} (5 mg/ml) began to produce sediments at pH 3.8, when adjusted to pH 7.0, a large amount of gel sediment was formed. The suspension was centrifuged at 10,,000*g* for 10 min, the supernatant was collected for control.

2.3. Catalyst of APS and their complexes with Ce^{4+} for degradation of Plasmid DNA

Three microlitres of plasmid DNA was mixed with 6 μl of the complexes of APS and Ce^{4+}, APS and Ce^{4+}, respectively. The reaction volume was adjusted to 30 μl with Hepes buffer. The hydrolysis of plasmid DNA was carried out at 37 □ for 30 min. The cleavage of plasmid DNA was demonstrated by Agross-gel electrophoresis according to the methods of Sumaoka et al., (2000).

2.4. Degradation of APS and their complexes with Ce^{4+} for BSA

Effects of APS and their complexes with Ce^{4+} on hydrolyzing BSA were tested by conventional method of Wang & Lu (1997). The method is based on the principle that amino acids can react with ninhydrin, create a purple color and give an absorption maximum at 570 nm. The larger the value of OD570 nm, the greater the hydrolysis of BSA. The hydrolyzing reaction system was as follows: 0.5 ml of 1% BSA was mixed with various concentrations of APS and its complexes, respectively. The reaction volume was adjusted to 1.0 ml by water. The hydrolysis of BSA was carried out at 37 □. At an appropriate interval, 0.25 ml aliquot of the reaction solution was taken out and mixed with 0.25 ml of 1% ninhydrin and 0.5 ml of pH 8.0 phosphate acid buffer, then reacted at 100 □ for 15 min. Finally the value of OD 570 nm was determined.

2.5. Degradation of complexes of laminaran and Ce^{4+} for chlorpyrifos

The degradation of chlorpyrifos in vitro was carried out in sealed glass tubes. The reaction system was as follows: chlorpyrifos was dissolved in solvent (including 50% acetone and 50% water). 5 ml of the chlorpyrifos solution is mixed with distilled water to obtain concentrations of 100 mg/l. Forty millilitre of the complex of laminara and Ce^{4+}(5 mg/ml) were mixed with 10 ml of chlorpyrifos

(100 mg/l). The reaction was carried out at 25 ? and pH 7.0. The sample was taken out at an appropriate interval respectively for analysis. Immediately each sample was extracted using ethyl acetate. The extract was filtered through Whatman No. 1 filter paper, containing 2 g of sodium sulfate, into a conical flask, then dried by vacuum-drying at 25 □ and dissolved using acetone to a definite volume for gas chromatography.

The degradation of chlorpyrifos in vivo was carried out on spinach. A spinach (Boza 10) garden representing general spinach gardens was selected in Agricultural Science Academe of Qingdao (Shandong Province, China) during March 2004. Six rows of the plant were selected at random for the experimental plots, with an area of 1.0× 16.7 m^2 each. The spinach was sprayed with chlorpyrifos (from Dow AgroSciences, the purity is 480 g/l) according to the manufacturer instructions. One day later, three plots were selected at random for treatment, and sprayed with the complex of laminaran and Ce^{4+} (5 mg/ml). Another three plots were sprayed with the same volume of water as a control. Both the control and the active treatment were repeated three times, and all of the pots received the same management. Spinaches were pulled out at random from the same plot on the spraying day, and 1, 4, 7 and 10 days after spraying the complex, then cleaned, aired and weighed.

Analysis and identification of the chlorophrifos residues in the spinach were conducted according to procedures outlined in the GB/T 5009.20-2003 (Chinese national standard method). After homogenizing in a polytron for 2–3 min, 20,000*g* of the homogenized sample was blended with 60 ml 60% acetone aqueous for 30 min on an oscillator, filtered under reducing pressure, and then washed with 20 ml 60% acetone aqueous. 15 g of solid sodium chloride was added, vibrated for 2 min, and then the acetone ether was collected. The step was repeated twice by 15 ml acetone. The acetone extract was concentrated in rotary evaporator with reducing pressure to produce the final extract in acetone. The concentrated extract was cleaned up prior to the gas chromatographic determination by passing it through activated florisil. The chlorophrifos residues in the spinach are expressed on a fresh weight basis.

2.6. Analysis of chlorpyrifos

The determination of the chlorpyrifos was done by gas chromatography (GC) (HP6890) with flame photometric detector (FPD), using glass capillary column (30 m× 0.32 mm ID) (19091J-413), according to GB/T 5009.20-2003. Injection port and detector temperatures were maintained at 250? and 220□, respectively. Helium was used as a carrier gas (17 ml/min). The column temperatures were programmed as follows: the initial temperature of 80 □ with a residence time of 1 min was increased at a rate of 15 □/min to 170 □ with a residence time of 1 min. From 170 □ to 235 □ at a rate of 10 □/min was used with constant temperature until the samples completely poured out (about 25 min). Samples of 1 μl of the extract was injected, and quantization of the insecticide was performed by automatic integration of the peak areas, and certified standard of chloropyrifos was used for external calibration.

2.7. Statistical analysis

All experiments described here were repeated three or four times; similar results and identical trends were obtained each time. The degradation rate (%) of chloropyrifos was defined as (the content before treatment—that after treatment)/that before treatment×100. All data are expressed as means ± SD for observation of the indicated number (*n*).

3. Results and discussion

3.1. Catalyst of APS and its complexes with Ce^{4+} for degradation of plasmid DNA

Fig. 1 depicts the hydrolysis of plasmid DNA at 37 □ and pH 7.0 by APS and complexes composed of APS and Ce(NH4)2(NO3)6. The APS and Ce^{4+} show no measurable activity for plasmid DNA hydrolysis (Fig. 1, right). In contrast, the complexes of APS and Ce^{4+} show measurable activity for plasmid DNA hydrolysis under the comparable condition when the APS bound Ce^{4+} and formed homogenous solutions (Fig. 1, left). The complex composed of laminaran and Ce(NH4)2(NO3)6, in the homogenous solutions, show obviously activity for plasmid DNA hydrolysis (Fig. 1, bane 5). The complex of laminaran and Ce^{4+} could rapidly convert the supercoil DNA (plasmid DNA form□) into linear form DNA (plasmid DNA

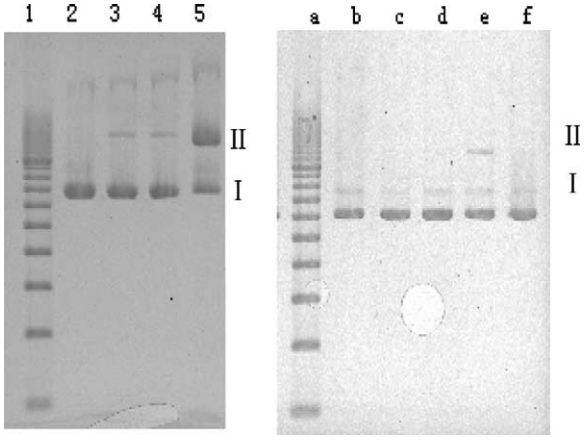

Fig. 1. Catalyst of APS and their complexes with Ce^{4+} for degradation of plasmid DNA. Reaction conditions: pH 7.0, 37 □ for 90 min. a and 1—DNA mark (500bp DNA ladder), b and 2—Plasmid DNA, c—Plasmid DNA + Ce^{4+}, d—Plasmid DNA + fucoidan, e—Plasmid DNA + alginic acid, f—Plasmid DNA + laminaran; 3—Plasmid DNA + fucoidan/Ce^{4+}, 4—Plasmid DNA + alginic acid/Ce^{4+}, 5—Plasmid DNA + laminaran/Ce^{4+}.

form□), the cleavage ratio was over 60%, at 37 □ and pH 7.0 for 90 min.

3.2. Degradation of APS and its complexes with Ce^{4+} for BSA

0.65 mg/ml of fucoidan, alginic acid and laminaran had no activity hydrolyzing on BSA at 37 □, pH 7.0 and within 2 h. After 4 h, the fucoidan appeared to hydrolyze BSA, but not significantly (Fig. 2).

Takarada et al. (2000) reported that Ce^{4+} had a significant hydrolysis effect for peptide. But the Ce^{4+} ion readily form metal hydroxide gel at neutral pH solution. When Ce(NH4)2(NO3)6 was added to a pH 7.0 Hepes buffer, polymeric aggregation of hydroxide rapidly took place and white precipitates were formed. However, it was found that APS greatly suppressed the precipitate formation and provided homogeneous solution composed of Ce(NH4)2(NO3)6 and APS. The complexes composed of APS and Ce^{4+} significantly hydrolyzed BSA, and the activity of complex of laminaran and Ce^{4+} for hydrolyzing BSA was the greatest of the complexes (Fig. 3).

3.3. Catalyst of complex of laminaran and Ce^{4+} for degradation of chlorpyrifos

Organophosphates mostly contained phosphate ester bond such as chlorpyrifos. The result that the complex of laminaran and Ce^{4+} can cleave plasmid DNA suggests that the complex could also hydrolyze organophosphate esters, such as organophosphate. But with regard to this it has not been reported so far.

Chlorpyrifos, an organophosphate insecticide, is chemically known as 0-0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothionate. It is a white granular crystalline material

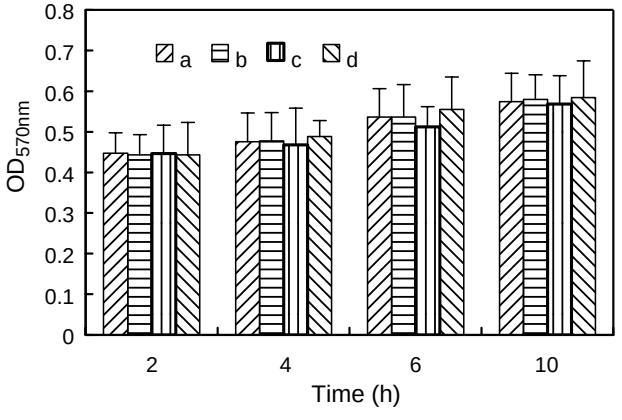

Fig. 2. Effects of APS on degradation of BSA. a—BSA, b—Laminaran, c—Alginic acid, d—Fucoidan The final concentration of alginic acid, fucoidan and laminaran in the reaction liquid was the same as 0.65 mg/ml. The reaction was processed at 37 □ and pH 7.0 for different time, then taken a certain size to be colored with ninhydrin at 100 □ for 15 min, finally measured at 570 nm with UV/Vis spectrophotometer. Values shown are means ($n=4$) ± standard errors.

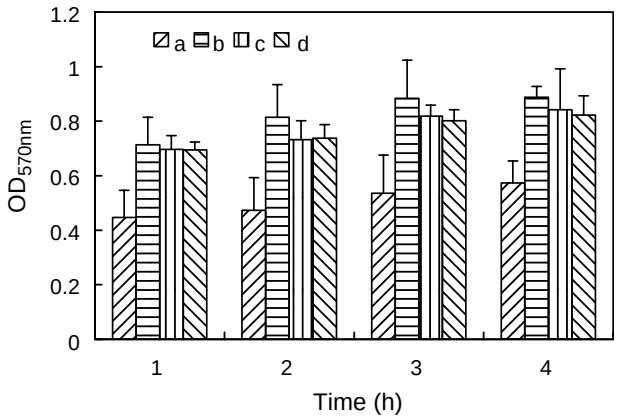

Fig. 3. Effects of the complexes of APS and Ce^{4+} on degradation of BSA. b— BSA, b—Laminaran/Ce^{4+}, c—Alginic acid/Ce^{4+}, d—Fucoidan/-Ce^{4+}The final concentration of the complexes in the reaction liquid was the same as 1.25 mg/ml. The reaction was processed at 37 □ and pH 7.0 for different time, then taken a certain size to be colored with ninhydrin at 100 □ for 15 min, finally measured at 570 nm with UV/Vis spectrophotometer. Values shown are means ($n=4$) ± standard errors.

with a molecular weight of 350.59. Its empirical formula is C9H11Cl3NO3PS. Chlorpyrifos has broad-spectrum insecticidal activity. It is used extensively in agriculture and urban pest control. Therefore we selected chlorpyrifos and examined the effect of the complex of laminaran and Ce^{4+} on hydrolysis of chlorpyrifos. Fig. 4 shows that the complex can effectively degrade chlorpyrifos. The result from Fig. 4 depicts that chlorpyrifos could degrade itself at 25 □ and pH 7.0. The chlorpyrifos concentration was 20.09 mg/l at 0 h, after 48 h 9.37 mg/l, the degradation rate was about

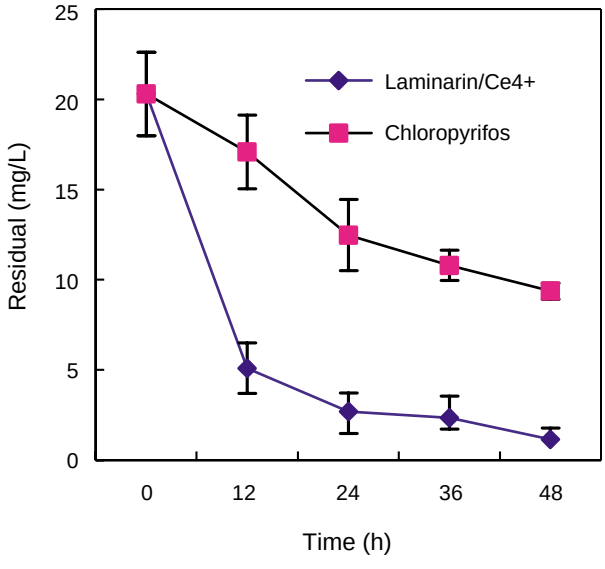

Fig. 4. Catalyst of complex of laminarin and Ce^{4+} for degradation of chloropyrifos. The reaction system was as followed: 40 ml of the complexes of APS and Ce^{4+} (5 mg/ml) was mixed with 10 ml of chloropyrifos (100 mg/l). The reaction was carried out at 25 □ and at pH 7.0. The sample was taken out at an appropriate interval respectively and detected by gas chromatograph. Values shown are means ($n=3$) ± standard errors.

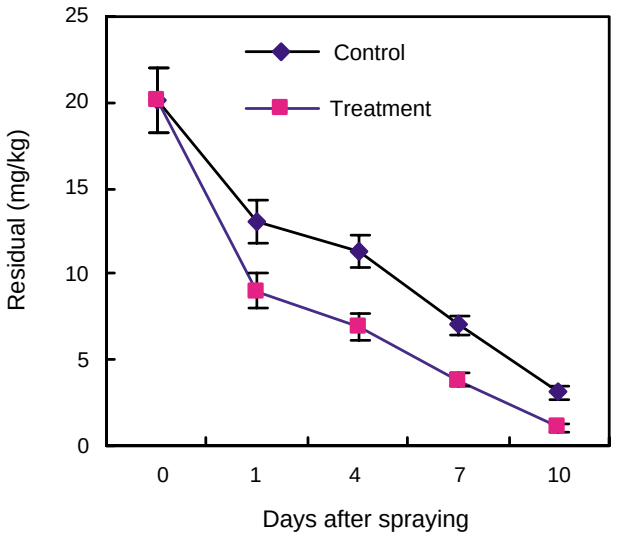

Fig. 5. Catalyst of complex of laminaran and Ce^{4+} for degradation of chloropyrifos in spinach. The treatment = the plots sprayed with the complex of laminaran and Ce^{4+} (5 mg/ml); The control = the plots sprayed with the same volume water. The sample was taken out at an appropriate interval respectively and the chloropyrifos residual in spinach was detected by gas chromatograph. Values shown are means ($n = 3$) ± standard errors.

53.82%. Compared with chlorpyrifos degradation itself, the complex of laminaran and Ce^{4+} could obviously promote the degradation of chlorpyrifos and the pesticide was almost degraded completely after 48 h. The chlorpyrifos residual treated by the complex was 1.16 mg/l and the degradation rate was about 94.28% after 48 h.

The chlorpyrifos residual in the spinach sprayed with the complex of laminaran and Ce^{4+} were evidently lower than those from control. According to Fig. 5, the chlorpyrifos residual in spinach from the treatment plot decreased by 31.13% when compared with that in spinach from control plot on 1 day after spraying the complex. It decreased by 38.30, 43.31, and 63.22%, respectively on 4 days, 7 days and 10 days after spraying the complex. The chlorpyrifos residual in spinach from the treatment plot was only 1.14 mg/kg on 10 days after spraying the complex, whereas the residual contain in spinach from the control plot was 3.10 mg/kg under the comparable condition.

It has been reported that both of APS and Ce^{4+} have novel features such as stimulation of growth of plants. Our experiments found that laminaran and Ce^{4+} have other new functions, which complex of laminaran and Ce^{4+} could degrade compounds containing peptides or phosphate ester bonds. The results of this study may be applied to agriculture to enhance crop output and to guarantee food safety.

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