Removal of Dioxin Derivatives by Salmon Milt DNA

Y. Hamada [b], *X. D. Liu* [a b], *F. Yoshida* [b], *M. Matsunaga* [b], *N. Nishi* [a]

Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810 [a], and Nissei Bio Co., Ltd, Tokyo 130-0026 [b], hamada@idenshi.com; Tel. and Fax: +81-3-5669-7262.

Introduction

Salmon milt is often produced as a by-product with large amount in the marine products industry. Only little salmon milt was used for health food, cosmetics materials, and feed, but most of them were discarded as waste. Salmon milt has even been feared to become an environmental problem. About 10% (w/w) DNA exists in the salmon milt and such DNA can be readily mass procured from the salmon milt. Recently, much attention has been paid to DNA from the new standpoint that DNA can be used as a functional biopolymer[1-3]. DNA is a threadlike double-helixed macromolecule consisting of deoxyribonucleotides. Intercalation is one of the principal modes of association of DNA for small molecules having planar aromatic ring systems. In our laboratory, many experimental results have shown that dioxins could form complexes with the DNA molecule, and that the binding mechanism was like intercalation. However, the water-soluble properties mean that DNA alone is difficult to utilize as an absorber of dioxins in water treatment. Therefore, four novel methods were tried to insolubilized DNA molecule, these are the UV irradiation method, silica sol-gel method, polyacrylamide hydrogel method, and confining DNA molecule by dialysis membrane. Especially, the reusability of the DNA solution by hexane extraction was also examined. In the present study, we report the details about the application of salmon milt DNA as an adsorbent for removal of dioxin derivatives in water treatment.

Methods and Materials

1: We have prepared a novel dsDNA film by using UV irradiation as reported previously[3]. Aqueous dsDNA solution (100μL, 10 mg/ml DNA in H_2O) was applied onto glass plates, dried at room temperature overnight, and then irradiated with UV light (R-52G, Ultraviolet Inc., Upland, CA) at 254 nm for various times. The intensity of UV irradiation was 5600 W/cm^2 at the sample position. The UV-treated DNA applied on glass plate was immersed in water and the DNA film was striped from the glass plate and stored in water. **2:** A 1.0 ml of portion of TEOS (tetraethoxysilane), 4.4 ml of H_2O, and 0.6 ml of 0.1 N HCl were mixed and incubated for two hours until a homogeneous mixture was obtained. The mixture was mixed with another mixed solution containing 6.0 ml of salmon milt DNA buffer solution (0.1 M Na_2HPO_4-NaH_2PO_4, pH 6.0) and 1.8 ml of APTES (3-aminopropyltriethoxysilane) ethanol solution and sonicated for 5 min. After incubation for 6 h, the DNA-silica hybrid gel was obtained. **3:** Polyacrylamide hydrogel was prepared by a free radical reaction mechanism. 0.5 ml of 5.0 mg/ml salmon milt DNA aqueous solution, 0.5 ml of 40% (w/w) mixed solution of acrylamide and methylenebisacrylamide with a weight ratio of 9:1, and 10 μl of 10% (w/w) ammonium persulfate aqueous solution were mixed in a 1.5 ml micro test tube. Then, 2 μl of TEMED was added to initiate the polymerization and the polymerizing mixture was incubated for 4 h to give the DNA

contained polyacrylamide hydrogel. **4:** The dialysis membrane used was a cellulose membrane tube with a molecular weight cut off of 13,000. Prior to use, the dialysis bag was rinsed with water for 6 h and soaked in a 1 mM EDTA solution for 2 h. DNA solutions were loaded into the bags and were dialyzed in distilled water for 24 h.

Results and discussion

1: We examined the stability of the UV-irradiated DNA film in water. More than 80% DNA was insolubilized in the film when the irradiation time was 6 h. The UV-irradiated DNA showed an increase of molecular weight, which suggested the formation of DNA intermolecular cross-links with a three-dimensional network. The CD spectra of the UV-irradiated DNA film in aqueous solution showed the B-form structure as DNA aqueous solution. **2:** The stability of the DNA in the DNA-silica gel (1.0 g) was evaluated by measuring the amount of DNA eluted to distilled water (5.0 ml) with an absorbance at 260 nm. The DNA in the DNA-silica gel was very stable, not more than 0.2% DNA was detected in the solution after elution for 24 h. This DNA-silica gel accumulates Ethidium bromide clearly more than bisphenol A, suggesting that the silica sol-gel method did not induce the transformation of DNA structure. **3:** To examine the stability of DNA in the gel, the resulting gel was incubated in 25 ml distilled water for 24 h, and the amount of DNA in the gel was measured by a phosphorus element analysis. More than 96% DNA was remained in the hydrogel. In contrast with the UV-irradiation method mentioned above, this polyacrylamide hydrogel method has an advantage that could be prepared in large scale. DNA contained hydrogel beads could be directly manufactured by using an inverse suspension polymerization method via free radical reaction mechanism. **4:** To increase the DNA content remained in the composite system, we tried to use DNA aqueous solution together with dialytic membrane. The huge DNA molecule is expected to be kept inside the dialysis membrane, and then form complexes with the small dioxin molecules that permeate from the outside. As our expectation, the dialytic membrane confined more than 99% DNA in the membrane tube, only about 1% DNA was released from the system and the eluted DNA was considered as the DNA with small molecule weight.

Since experiments of highly toxic dioxins need special laboratory facilities or equipment, the dioxin derivatives with low toxicity such as dibenzo-p-dioxin, dibenzofuran and biphenyl were used as a model for this studying in our laboratory. Because these dioxin derivatives don't have any chlorine atoms on the dioxin molecule, further examinations are required to investigate whether the chlorine atoms on the dioxin molecule structure interfere with the intercalation. Using UV-inducted DNA film, 7 PCCD congeners, 10 PCDF congeners and 13 PCB congeners were subjected to an adoption experiment. The amounts of the dioxins removed were measured by GC-MS method at another laboratory and the results are summarized in table 1. In contrast with DD, DF and BP, no decrease was observed in the removal amount, and the result was interpreted as dioxins with chlorine atoms still bound to the DNA molecule.

A simulated experiment was carried out to evaluate the removal of the dioxin derivatives by using DNA solution. An aqueous mixture solution of DD, DF and BP mixed with or without Na^+, K^+, Ca^{2+}, and Mg^{2+} ions was cyclically pumped through a dialyzing tube of the DNA solution. The dialysis time was programmed as 96 h, and the equilibrium concentrations of DD, DF, and BP on both sides of the dialytic membrane were determined by the HPLC method. DNA solution in the dialytic tube has enriched more than 200 times dioxin derivatives from outside. This result showed that DNA solution in dialytic tubes could accumulate dioxins in water. Especially the hollow fiber membrane has been thought as the most promising candidate as it had low cost and higher efficiency in the water treatment.

Table 1. The removal of the various dioxins and the dioxin derivatives by UV-irradiated DNA film.

	Removal amount (%)		Average removal amount* (%)
DD	64.7	PCDD	78.1
DF	60.4	PCDF	81.8
BP	49.8	PCDD	62.3

*Average removal amount of PCDD, PCDF and PCB was calculated from 7 PCCD congeners, 10 PCDF congeners and 13 PCB congeners, respectively. The removal amounts of PCDD, PCDF and PCB were measured by GC-MS method at Shimadzu Techno-Research Inc.

Furthermore, the selective adsorption of DNA-immobilized glass bead column was observed. We examined the removal of dioxin derivatives from aqueous solutions by the DNA-immobilized glass bead column. The removal of these compounds was determined by the absorption spectra of the eluted solution. More than 70% of dibenzofuran, dibenzo-*p*-dioxin, biphenyl, and benzo[*a*]pyrene bound to the DNA-immobilized glass beads, but the DNA-immobilized glass beads did not bind bisphenol A and diethylstilbestrol. This result suggested that DNA selectively binds small molecules with planar molecule structure.

Based on the results above, the using DNA for the water treatment is considered to be practicable. Some automatable DNA water treatment processes were proposed as Fig. 1. The dioxins in polluted water are finally accumulated to the organic solvent such as hexane, and the DNA solution or DNA contained beads were repeatedly used similarly as ion change resin. Such systems were eagerly expected to apply in practice.

In conclusion, we demonstrated that salmon milt DNA is an abundant and readily producible biopolymer. Dioxins might be associated with the DNA by an intercalating mechanism. The applying DNA is an efficient and practicable method for enriching and removing the widely spread dioxins from the environment. Furthermore, application of the DNA polymer to a cigarette filter might reduce the damage of firsthand and secondhand smokes by selectively removing polycyclic aromatic compounds. Molecules of the dioxin derivatives bound in DNA can be removed by extraction with an organic solvent such as hexane and the recycled DNA solution is reusable for further water treatment.

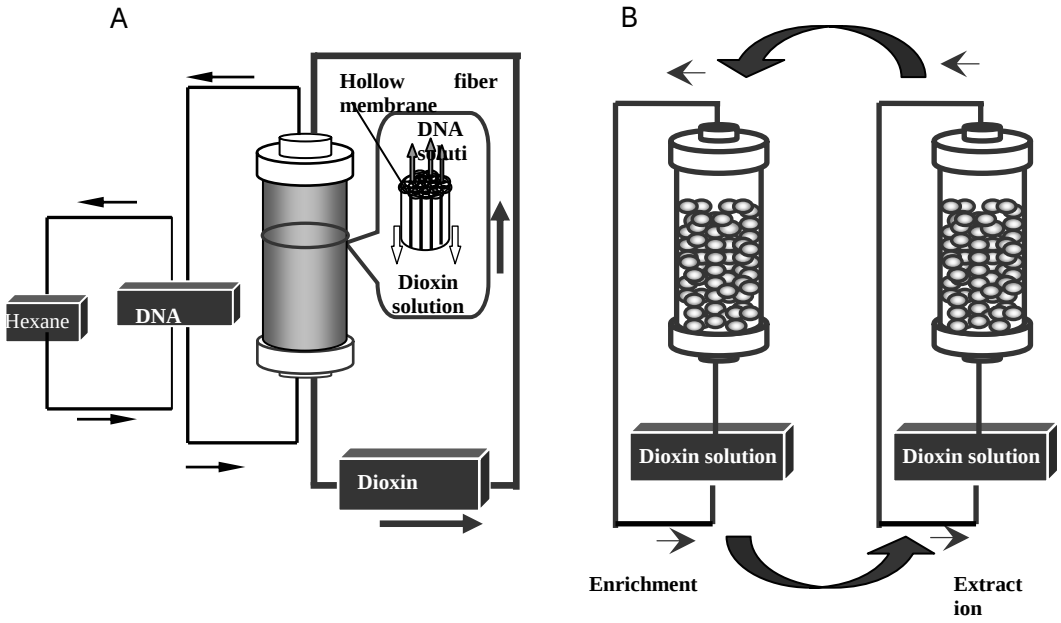

Fig. 1. Schematic diagram of the automatable water treatment processes. (A) The processes of DNA solution combining with hollow fiber membrane; (B) The processes of the column made of DNA immobilized beads.

References:

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