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Removal of dissolved estrogen in sewage effluents by β-cyclodextrin polymer

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Abstract

Substances with estrogenic activity are found in effluents of municipal sewage plants and dairy farms. These effluents have the potential to induce feminization in male fish. In this study, cyclodextrin polymers (CDPs) that are insoluble in both polar and nonpolar solvents were selected for the removal of dissolved estrogens in the effluent of a municipal sewage plant. The removal capacity of CDPs was high in the order of β-CDP ≥ γ-CDP » α-CDP. The mechanism for adsorption of estrogens to β-CDP was not only due to a host-guest interaction as molecular recognition by β-cyclodextrin (β-CD), but also due to adsorption by the polymer matrix. β-CDP of 0.2%(w/v) removed 17β-estradiol (E2) of about 70% from 10^{-11} mol/L, and more than 90% from $\geq 10^{-10}$ mol/L. The removal ratios of E2 in the presence of cholesterols, which are contained at higher concentrations than estrogens in sewage effluents and are adsorptive competitor for β-CDP, were about 85% at a cholesterol/E2 molar ratio of 100 and >90% at molar ratios of 0.1, 1, and 10. The effluent from a municipal sewage plant had estrogenic activity corresponding to 5.5×10^{-11} mol E2/L by yeast two-hybrid assay. The estrogens in the effluent were also removed >90% by the β-CDP treatment. Therefore, β-CDP is able to remove dissolved estrogens over a wide range of concentrations in the presence of various contaminants such as wastewaters.

Keywords; endocrine disruption, estrogen, β-cyclodextrin polymer, sewage effluent, steroids removal

1. Introduction

There is currently great interest in the contamination of steroid hormones such as natural estrogens and their synthetic analogs in natural aquatic environments (Hansen et al., 1998; Sarmah et al., 2006; Velicu and Suri, 2009). These compounds affect reproduction and development in wildlife at ng/L concentrations (Hansen et al., 1998; Jobling et al., 1998; Metcalfe et al., 2001; Miles-Richardson et al., 1999). Feminization of fish occurs from exposure to certain estrogens in concentrations as low as 10 ng/L (Kolodziej et al., 2004; Routledge et al., 1998). Velicu and Suri (2009) detected estrone (E1) and estriol (E3) in the environment at concentrations ranging from 0.6 to 2.6 ng/L and from 0.8 to 19 ng/L, respectively. Metcalfe et al. (2001) observed the formation of ova in the testes of Japanese medaka (*Oryzias latipes)* starting at concentrations of 4 ng/L for E2, and 0.1 ng/L for 17α-ethynylestradiol (EE2). The major sources of E2 and EE2 in natural environments are thought to be municipal sewage and animal waste (Kömer et al., 2001; Panter et al., 1998; Sarmah et al., 2006; Shore and Shemesh, 2003). Desbrow et al. (1998) and Johnson et al.(2000) have also found natural and synthetic steroid estrogens such EE2 in the outlets of sewage treatment works. Snyder et al. (2001) also reported that natural estrogens and their synthetic analogues have been found in treated wastewater effluents. Furthermore, Chimchirian et al. (2007) found that the concentration of estrogens in the influent and effluent of three treatment plants ranged from 1.2 to 259 ng/L and 0.5 to 49 ng/L, respectively. Rodgers-Gray et al. (2000) and Routledge et al. (1998) have also reported that hermaphroditic fish have been found downstream from a wastewater treatment plant. Most agricultural wastes have also been shown to contain high levels of estrogenic compounds (Desbrow et al., 1998; Hunag, and Sedlak, 2001; Kömer et al. 2001; Sarmah et al., 2006). Animal waste effluents especially contain large amounts of the compounds (Sarmah et al., 2006; Shore and Shemesh, 2003). These reports also show that certain surface waters can contain concentrations of estrogens that have the potential for endocrine disruption.

Significant concentrations of estrogens in effluents have been attributed to their incomplete removal during the wastewater treatment process (Samir et al., 2006). In the degradation process, E2 is oxidized to E1 (Hanselman et al., 2003; Holbrook et al., 2002), and other estrogens are also degraded to simpler moieties, however EE2 is much more resistant to degradation than E2 (Jürgens et al., 2002). Therefore, the orders of detection frequency and concentration are E1, E3, 17α-estradiol, and E2 (Belfroid et al., 1999; Isobe et al., 2003; Velicu and Suri, 2009). The concentration of environmental estrogens discharged from sewage treatment plants are decreased by dilution, sorption, and degradation in the receiving ecosystem. Jürgens et al. (2002) reported that estradiols and E1 in stream water have half-lives of 0.1 and 11 days, respectively. Some of other estrogens can persist for a significant time in surface water. Therefore, it is important to remove chemically and physically steroid hormones in sewage treatment plants.

Oishi et al. (2008) showed that the estrogenic activity of E2 was suppressed by forming an inclusion complex with β-CD. However, this complex was not removed from waters because of the highly solubility. Recently, CDPs were produced by cross-linking CDs with epichlorohydrin (Kikuchi et al., 2005; Murai et al., 1998). They are insoluble in both polar and non-polar solvents. Therefore, they were applied for isolation or removal of specific compounds from a mixture (Murai et al., 1998; Kozlowski et al., 2005; Cathum et al., 2006). The effect of β-CDP on removal of dissolved estrogens from sewage effluents containing various contaminants was examined in this study.

2. Materials and Methods

2.1 Materials

α-, β-, and γ-CDPs were synthesized by Aomori Prefectural Industrial Technology Research

Center (Local Independent Administrative Institution) in Aomori prefecture, Japan. The CDPs were prepared by crosslinking CDs with epichlorohydrin under alkali conditions (Kikuchi et al., 2005).

2.2 Adsorptive property of CDPs to E2

Each of α-, β-, and γ-CDPs was added in a 10^{-4} molE2/L solution at 0.2%(w/v). After 1h incubation at room temperature, these solutions were centrifuged for 10 min at 3000 rpm. The UV spectra of the supernatants were measured with a spectrophotometer (Shimadzu, Japan).

2.3 Adsorption capacity of β-CDP to E2

β-CDP was added in 10^{-11}, 10^{-10}, 10^{-9}, and 10^{-8} molE2/L at 0.2%(w/v). Solutions of E2 without the added β-CDP were also prepared as references. After 1h incubation at room temperature, the solutions were centrifuged for 10 min at 3000 rpm. The E2 concentration in the supernatant was measured with an E2 ELISA kit (Tokiwa Chemical Industries Co., Ltd., Tokyo, Japan), and the removal ratio of E2 from each solution was calculated by comparing the treated solution concentration to the reference.

2.4 Effect of cholesterol on removal of E2 by β-CDP

A solution of 10^{-9} mol/L E2 was prepared and divided into six aliquots. Cholesterol was added to each aliquot to give cholesterol/E2 molar ratios of 0, 0.1, 1, 10, and 100. β-CDP was added to each E2 solution at 0.2%(w/v). Reference solutions were prepared without the addition of β-CDP. After 1h incubation at room temperature, the solutions were centrifuged for 10 min at 3000 rpm. The E2 concentration in the supernatant was measured with E2 ELISA kit, and the removal ratio of E2 from each solution was calculated by comparing the treated solution to the reference.

2.5 Removal of dissolved estrogen from a municipal sewage plant effluent by β-CDP

Effluent of a municipal sewage treatment plant was obtained before chlorination and was filtered through GF/C (Whatman International Ltd., England). An E2 solution and mixture of E2 and cholesterol were prepared at 10^{-6} mol/L. β-CDP was added to these solutions at 0.2%(w/v). Reference was prepared without addition of β-CDP. After 1h incubation at room temperature, the solutions were centrifuged for 10 min at 3000 rpm. The estrogenic activities of the supernatants were obtained by yeast two-hybrid assay and were converted into E2 concentrations. These assays were carried out by the Institute of Environment and Resource System Co., Ltd., which is the university-industry collaboration section of Yokohama National University (Yokohama, Japan). The removal ratio of E2 from each solution was calculated by comparing the treated solution to the reference.

3. Results and Discussions

3.1 Adsorptive property of CDPs to E2

E2 has an absorption maximum at 280 nm on a UV spectrum. The absorption intensity of the supernatant treated with each CDP decreased in the following order: β-CDP ≥ γ-CDP » α-CDP (Fig. 1). This shows that these CDPs can remove E2 from waters. β-CDP was the most effective for removal of E2 from waters (Fig. 1). Oishi et al. (2008) previously showed that E2 selectively formed an inclusion complex with β-CD or γ-CD but not with α-CD. In this study, β- and γ-CDPs showed the highest selectivity for adsorption of E2, similar to β- and γ-

CDs. Despite the fact that α-CD did not form a complex with E2, α-CDP adsorbed E2 as shown in Fig.1. This demonstrates that adsorption of E2 by α-CDP is not due to a host-guest interaction through molecular recognition, but rather due to interaction with the polymer matrix. The mechanism for adsorption of E2 to β-CDP or γ-CDP is inclusion complex formation by selective incorporation into the hydrophobic cavity of a β-CD molecule, nonselective incorporation into secondary cavities of the polymer network, and hydrogen bonding with linkers. Therefore, β- and γ-CDPs are attractive material for the removal of E2 molecule from dissolved contaminants.

3.2 Adsorption capacity of β-CDP to E2

Estrogens affect reproduction and development in wildlife at the concentration of ng/L order (Hansen et al., 1998; Jobling et al., 1998; Kolodziej et al., 2004; Metcalfe et al., 2001; Routledge et al., 1998). They were detected with concentrations ranging from 10^{-11} to 10^{-9} mol/L in surface waters and dairy farm effluents (Sarmah et al., 2006; Velicu and Suri, 2009). Therefore, the removal efficiency of E2 by β-CDP was measured in the range of 10^{-11} to 10^{-8} molE2/L. The removal ratio was about 70% at 10^{-11} molE2/L and was more than 90% at $>10^{-10}$ molE2/L (Fig. 2). From these results, a high removal efficiency of E2 by β-CDP is expected over a wide range of estrogen concentrations.

3.3 Effect of cholesterol on removal of estrogens by β-CDP

Human and all other species of animals excrete different species of steroids. They are not only restricted to estrogen hormones but also originate from foods, medicines and their metabolites. Cleavage of the steroid ring barely occurs during the activated sludge treatment process. Therefore, different types and proportions of steroids including estrogens usually coexist in effluent of wastewater treatment plants. Cholesterols do not have estrogenic activity, but are able to form inclusion complexes with β-CD (Kwak et al., 2002). In general, cholesterols were contained at higher concentrations than estrogens in wastewaters. Therefore, competition for adsorption to β-CDP may occur between estrogens and other steroids. E2 and cholesterol were selected as representatives of estrogens and other steroids, respectively. Figure 3 shows the removal ratio of E2 by β-CDP from solutions where the cholesterol/E2 molar ratios were 0, 0.1, 1, 10, and 100. The removal ratio of E2 was lowest (about 85%) at 100 molar ratio and >90% at 0.1, 1, and 10 (Fig. 3). These results show that β-CDP is able to remove low concentrations of E2 in the presence of other steroids.

3.4 Removal of dissolved estrogen from a municipal sewage plant effluent by β-CDP

The estrogenic activity of this effluent corresponded to 5.5×10^{-11} molE2 /L and were removed at >90% by treatment with β-CDP (Table 1). On the other hand, the removal ratio of E2 from pure E2 solution of 10^{-11} mol /L was about 70% (Fig. 2). The difference in these removal ratios is due to that in the assay methods. Estrogens of differing species and estrogenic activities can coexist in wastewaters. E2 is easily oxidized to E1 by microorganisms during an activated sludge treatment process. Estrogenic activity was the highest in E2, followed by E1 and E3. The activity of E2 is about 1.5-fold of E1 based on yeast two-hybrid assay (Jürgens et al., 2002). The effluent is also contaminated with synthetic estrogen analogs which are much more resistant to degradation than natural estrogens. Therefore, the concentration of estrogens in the effluent from a municipal sewage plant was estimated as that of E2 from determination of the estrogen activity using a yeast two-hybrid assay. The reference prepared with an 10^{-6} molE2/L was estimated to be $>0.765 \times 10^{-6}$ molE2/L by this assay (Table 1). This indicates that this assay is able to evaluate with the high reliability.

Cholesterol/E2 molar ratios of up to 100 did not affect to the removal efficiency of E2 by β-CDP (Fig. 3). In this yeast two-hybrid assay, β-CDP treatment also showed a high removal ratio of E2 in the presence of cholesterol (Table 1). From these results, β-CDP is able to remove a variety of steroids including estrogens over a wide range of concentrations. Figure 1 indicates that γ-CDP has also the same removal capacity of steroid hormones as β-CDP.

5. Conclusions

β-CDP had a high capacity for removal of dissolved estrogens compared with α-CDP. The mechanism for adsorption of estrogens to β-CDP was thought to be not only due to a host-guest interaction as molecular recognition by β-CD, but also due to the polymer matrix. When treated with 0.2%(w/v) β-CDP, the removal ratios of E2 were about 70% at 10^{-11} molE2/L and more than 90% at $\geq 10^{-10}$ molE2/L. When cholesterol and E2 were mixed at 0, 0.1, 1, 10, 100 molar ratios, the removal of E2 was about 85% at 100, and >90% at 0.1, 1, and 10. The estrogenic activity of the effluent from a municipal sewage plant corresponded to 5.5×10^{-11} molE2/L by yeast two-hybrid assay, and the treatment by β-CDP removed estrogens of >90%. Therefore, β-CDP has high adsorption capacity to estrogens in the coexistence of adsorptive competitors.

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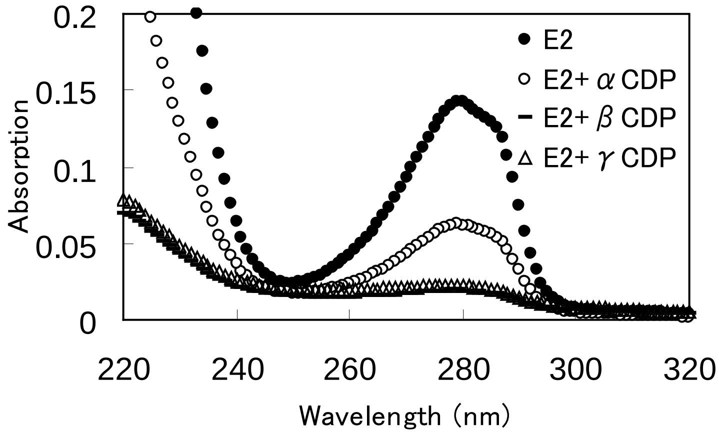

Fig.1 UV spectrum of E2 in the presence of α-, β-, and γ-CDPs

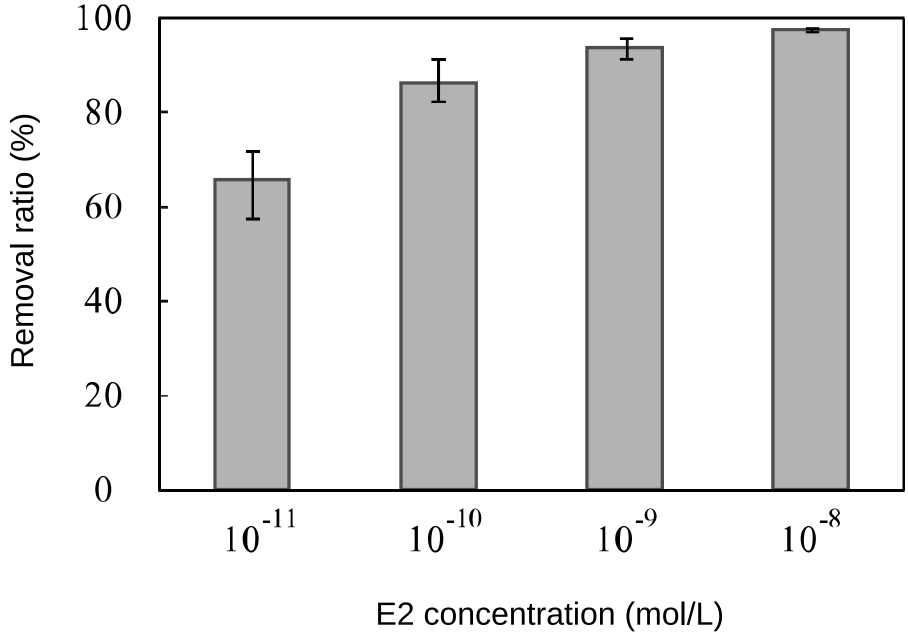

Fig.2 Removal ratios of E2 by β-CDP from different dilute E2 solution

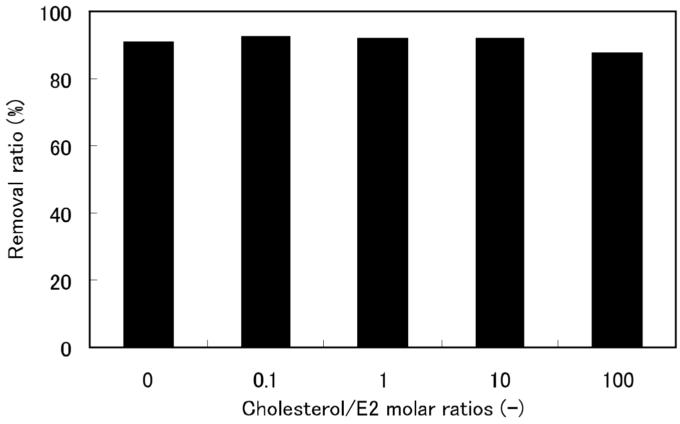

Fig.3 Effects of cholesterol to removal of E2 by β-CDP

Table 1 Removal of dissolved estrogen by β-CDP from a wastewater treatment effluent

Sample	β-CDP (%)	E2* (10^{-9} mol/L)	Removal ratio (%)
10^{-6} E2 mol/L	—	767	—
	0.2	40	94.8
10^{-6} E2 + Cholesterol mol/L	0.2	38.9	94.9
Effluent form sewage work	—	0.055	—
	0.2	<0.004	>91.9

*The value was estimated as E2 from estrogenic activity of yeast two hybrid assay