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5.4 Radiation Treatment for Endocrine Disrupters in Water

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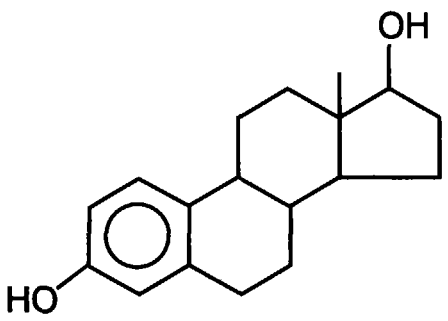
Abstract

The radiation-induced decomposition of a trace amount of 17 β-estradiol (E2) in water was studied as a function of the dose of 60Co γ-rays. Concentration of both E2 and E2 activity were estimated by LC-MS and ELISA, and decreased with an increase in the dose of γ-rays. E2 at 1.8-nM in water was degraded almost completely by irradiation at 10 Gy (= J/kg), but the E2 activity of the same sample still remained, and decreased by 30 Gy to be lower than the threshold level of contamination to induce some estrogenic effects on the environmental ecology.

Introduction

There have been many reports and much evidence on the contamination of water by chemicals with estrogenic properties [1-4]. Exposure to these chemicals induces estrogenic effects on aquatic creatures [5-8]. The artificial chemicals including *o,p'*-DDT, polychlorinated biphenyls (PCBs), alkylphenols and bisphenol-A, with estrogenic properties have been continuously released into the water-environment.

17 β-estradiol (E2: 1,3,5(10)-estratriene-3,17 β-diol) is a steroid hormone produced primarily within the female ovaries and in the male testes. E2 is released into the environmental water from humans and domestic animals. All animals generally produce E2 within their bodies according to their needs and to fulfill their particular purposes. However, E2 entering into the body from outside interferes with the normal processes and creates many deleterious effects. Such an external E2 causes serious problems in aquatic organisms and in animals as an endocrine disrupter. These effects appear above a concentration of about $1x10^{-8}$ g/L (0.03 nM) [7, 9]. Despite their importance, there is limited information available on the decomposition of trace amounts of E2 in water.



Scheme 1
Molecular structure of 17 β-estradiol.

Wastewater containing the chemicals has been mostly treated by the activated sludge and the O_3 process. The activated sludge is often inadequate to absorb trace

amounts of biologically harmful substances. The O_3 process utilizes direct oxidation, or indirect oxidation by generated OH radicals. More remarkable is the treatment of the chemicals by OH radicals, because of their strong electrophilicity. Oxidation of the chemicals by OH radicals generated by ionizing radiations has been well investigated [10-15]. Here, ionizing radiation can produce OH radicals homogeneously at the required concentration in waters. The yield of OH radicals is 2.9×10^{-7} M/Gy (G(OH)=2.8†) for γ-ray irradiation at room temperature [16].

In the present study, we discuss the utilization of ^{60}Co γ-ray irradiation for the decomposition of E2 at extremely lower concentrations than those of the generally used chemicals in water. The reduction of E2 was measured by an LC-MS system. E2 activity of the aqueous solution was evaluated by ELISA (enzyme-linked immunosorbent assay) method before and after γ-ray irradiation.

†G(OH) : G-value is the number of molecules produced per 100 eV absorbed energy.

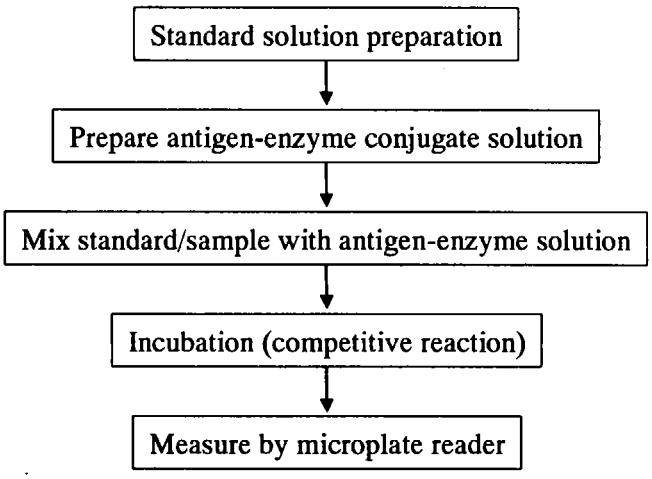
Experimental Section

Reagents

17 ß-estradiol (E2) was obtained from Takeda Chemical Industries, Ltd., Japan. Water was supplied from the ULTRA-PURE WATER SYSTEM (MILLIPORE, Milli-Q PLUS). To study the decompositions by ^{60}Co γ-ray irradiation, an aqueous E2 solution was prepared at 1.8, 0.7, and 0.18 nM concentrations at natural pH. The aqueous E2 solutions at around 5 mL were poured into small glass vials saturated with air. All the aqueous E2 solutions were irradiated with γ-rays at different doses from 1 to 100 Gy (= J/kg) at the room temperature (25 ℃).

E2 decomposition measurement by LC-MS system

Aqueous E2 solution at 1.8-nM concentration was analyzed by a Liquid Chromatography-Mass Spectrometric (LC-MS) system (JEOL, JMS-LC mate) using a column switching method for condensing E2. The sample solutions were injected at a 10-fold volume of the maximum injection volume of 500 μL and condensed within a reversed phase column (GL Science, Inertsil ODS-2). Water was used as an eluent, and its flow rate was 1.0 mL/minute for condensation. The eluent was then changed from water to pure methanol



Scheme 2
Flowchart for measurements of 17 β-estradiol activity of the sample solutions.

for analysis. The condensed samples were extracted by methanol as an eluent and separated with a reversed phase column (Shodex, RSpak DE-613). All the eluted compounds were allowed to flow through the UV detector into the mass spectrometer. An APCI (Atmospheric Pressure Chemical Ionization) ion source was selected for mass-spectrometry, and used in the positive mode for selected ion monitoring. Calibration curves of E2 concentration were drawn using standard sample solutions under the same conditions for the irradiated samples.

Evaluation of E2 activity by ELISA method

The E2 activities of the aqueous E2 solutions before and after γ-ray irradiation were estimated by ELISA method using a 17 ß-estradiol ELISA Kit (Takeda Chemical Industries, Ltd., E2 ELISA Kits). All operations of treatment procedure were performed at room temperature and shown in Scheme 2. E2 was condensed for the measurement of concentrations lower than 0.18 nM of E2 in the solution. The C-18 bonded cartridge was first washed with methanol followed by pure water. The aqueous E2 solution was passed through the cartridge. The cartridge containing E2 was washed with pure water and hexane, respectively. E2 was then eluted with 5-mL dichloromethane. The elutes were completely evaporated to dryness with a gentle stream of N_2 gas and dissolved in 10-% (v/v) methanol water (100 μL). A Microplate Reader (Bio Rad Model–550) was used for measurements of the optical density of the obtained sample solutions at 450 nm. Calibration curves of the E2 activity were drawn using standard sample solutions just before measurement of the irradiated samples.

Results and Discussion

E2 decomposition measurement by LC-MS system

A mass spectrum was obtained by the LC-MS system in the positive mode of the APCI ion source, and a peak identified to be E2 was observed at a mass number (*m/z*) of 255 amu, which comes by H_2O-release from protonated ions of E2 (255 = 272 + 1 - 18). Figure 1 shows the chromatograph recorded on the LC-MS by monitoring of the selected ion mass at 255 of E2 in the aqueous solution at 1.8-nM concentration before the irradiation. The peak observed at retention time of 18 minutes was assigned to E2 itself, and the area of this peak was linearly proportional to the concentration of E2. The system peaks by the six-port switch and eluent change were observed at retention time of 9 and 16 minutes.

Concentrations of E2 were accordingly estimated from the area of the peaks in the chromatograms before and after irradiations. The concentration of E2 in water decreased exponentially with an increase in the γ-ray dose, as shown in Figure 2. The γ-ray irradiation of 10-Gy reduced the concentration of E2 more than one order of magnitude. The concentration of E2 decreased to less than 0.05 nM at the dose of 10 Gy from the initial concentrations of 1.8 nM or lower initial concentrations of the sample solutions. This concentration is the threshold level, and not resulting any effect on the environmental

ecology [7, 9].

The concentration of OH radicals produced at 1 Gy is estimated to be 290 nM assuming *G*-value of 2.8 [16]. Because the concentration of OH radicals is adequately large compared with E2 (1.8 nM), the concentration of OH radicals can be assumed to be constant during the irradiation, and the degradation of E2 should be expressed by an exponential curve versus the dose for stochastic reasons as shown in Figure 2.

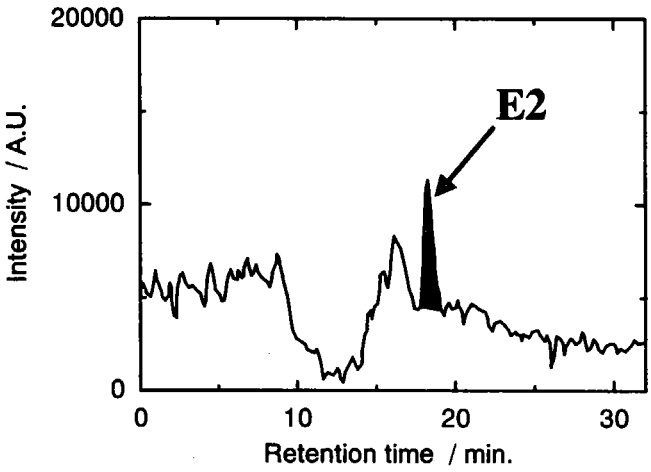


Figure 1

Mass chromatogram at 255 *m/z* of 17 β-estradiol in water after -ray irradiation. Initial concentrations were 1.8 nM

Evaluation of E2 activity by ELISA method

Degradation of E2 by γ-ray irradiation was confirmed by LC-MS. However, we must confirm the decrease in the E2 activity of the sample solutions after the irradiation. The E2 activities of sample solutions before and after the irradiation were estimated by ELISA method. This method indicates E2-equivalent concentration, which is the total concentration molecules having the E2 activity, and does not indicate the real E2 concentration as measured by LC-MS. Therefore, if products from the irradiation have no E2 activity, the reduction curves of the E2-equivalent

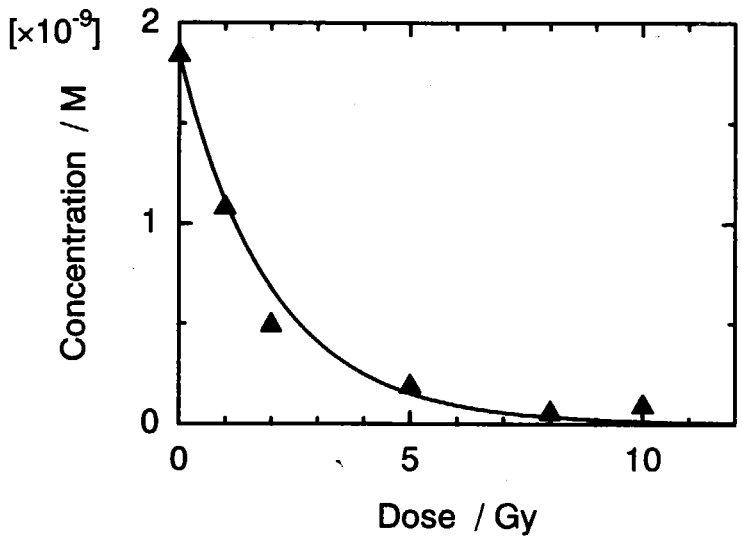


Figure 2

Decomposition of 17 β-estradiol (1.8 nM) in water measured by LC-MS system after γ-ray irradiation.

concentration should be the same as that of real E2 concentration in water after the irradiation. The E2-equivalent concentrations of E2 at initial concentrations of 1.8, 0.7, and 0.18 nM are decreased as a function of dose as shown in Figure 3. The E2-equivalent concentration decreased to almost zero at a dose of 30 Gy for the initial concentrations of 1.8 nM or lower. By comparing the decrease in the real concentration and the E2-equivalent concentration, it is obvious that they are not in agreement. A possible interpretation of this fact is tried as that the primary products, *e.g.*, OH-adducts, from E2 by γ-ray irradiation have the E2 activity, and these products are decomposed by further irradiation.

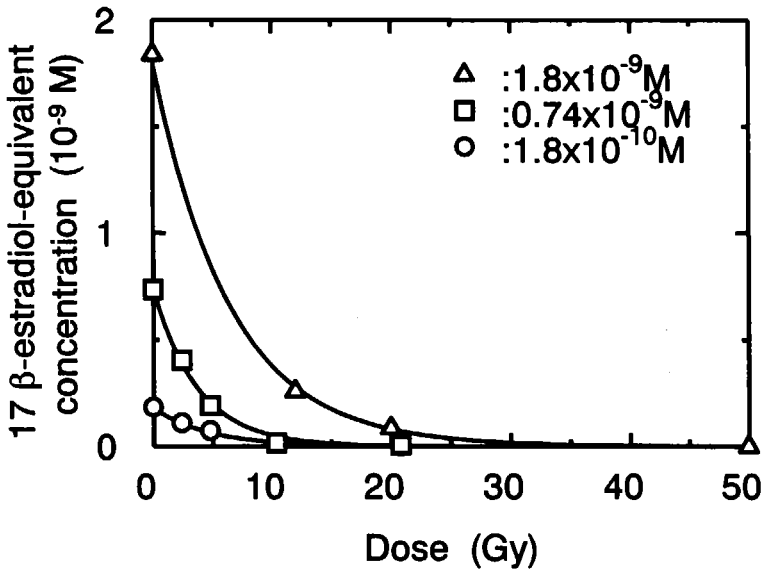


Figure 3
17 β-estradiol-equivalent concentration of sample solutions measured by ELISA (enzyme-linked immunosorbent assay) method after γ-ray irradiation.

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