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Degradation of Estrogen Conjugates Using Titanium Dioxide as a Photocatalyst

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Estrogen conjugates (estradiol-3-glucuronide, -17-glucuronide, estrone-glucuronide and -sulfate) were subjected to photodegradation using titanium dioxide immobilized on glass beads as a catalyst. Their time courses were measured by HPLC and compared with those of the unconjugated estrogens. Estradiol, its 17-glucuronide and estrone, which have an unconjugated phenolic hydroxy group at the C-3 position, were almost completely degraded by UV irradiation within 4 h. On the other hand, significant amounts of estradiol- and estrone-3-glucuronide (*ca.* 20%, 25%) and estrone sulfate (*ca.* 90%), which were conjugated at the 3-hydroxy group, remained after a 6.5 h irradiation. These results supported the hypothesis that the photodegradation of estrogens was initiated at the phenolic hydroxy group.

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Introduction

In recent years, there has been increasing concern over the endocrine disrupting effect of the endogenous estrogens and xenoestrogens in environmental water through the excretions of humans, domestic and farm animals. Among the endogenous estrogens, estradiol (E_2) shows the highest estrogenic potential followed by estrone (E_1) which are found in many water systems, such as rivers and the effluent from sewage treatment plants.[1-5] It is noted that estrogens are mainly excreted as conjugates such as glucuronide and sulfate.[6,7] Although the estrogen conjugates do not have estrogenic activities, they can act as the hormone precursors by deconjugation during the process of sewage treatment using activated sludge. It is difficult to completely remove these substances from environmental water by the usual methods using sewage treatment,[4,8] and therefore the development of novel technologies is necessary to overcome this problem.

Photodegradation using a semiconductor photocatalyst as typified by titanium dioxide (TiO_2) is one of the safe, effective and promising environmental cleanup technologies. It was reported that E_2 in an aqueous solution was photodegraded and mineralized to carbon dioxide using TiO_2 particles or films under UV light irradiation.[9,10] The mechanism of the photocatalytic oxidative degradation of E_2 suggested that the phenolic hydroxy moiety of E_2 was initially attacked by the hydroxyl radical, which was generated by the UV irradiation of TiO_2 in water.[9,11,12] However, it has not been clarified that the estrogens conjugated at the phenolic hydroxy group are able to be degraded by photocatalytic degradation using TiO_2.

In this paper we describe the time courses of the photodegradation of estrogen conjugates using TiO_2 immobilized on glass beads as a catalyst, which were compared with those of the unconjugated estrogens (Fig. 1). The reaction was monitored by measuring the remaining substrate by UV-HPLC.

Experimental

Materials and reagents

E_2 and E_1 were donated from Teikoku Hormone Mfg. (Kawasaki, Japan). E_2 3-glucuronide (E_23G), E_2 17-glucuronide (E_217G) and E_1 glucuronide (E_1G) were purchased from Sigma-Aldrich (St. Louis, MO, USA). E_1 sulfate (E_1S) was prepared in our laboratory from E_1 by the usual procedure using the chlorosulfonic acid-pyridine complex.

The photocatalyst, BL2.5DX (diameter, 2.5 mm; membrane thickness, 1.0 μm; TiO_2 immobilized on glass beads), was purchased from Photo-Catalytic Materials (Komaki, Japan).

Oasis HLB cartridges (60 mg, 3 ml) (Waters, Milford, MA, USA) were successively conditioned with methanol (2 ml) and water (2 ml) prior to use.

Apparatus

HPLC was performed using a PU-980 pump (JASCO, Tokyo, Japan) equipped with a J'sphere ODS-H80 column (4 μm, 150 × 4.6 mm i.d.) (YMC, Kyoto, Japan) and a SPD-10A UV detector (Shimadzu, Kyoto). E_23G and E_1S were monitored at 275 nm and 269 nm, respectively. The other compounds were

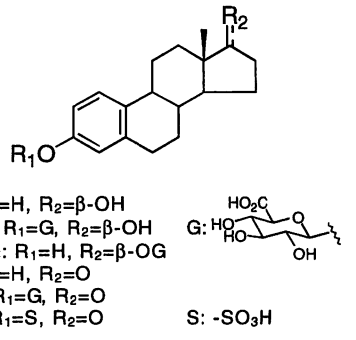

Fig. 1 Structures of E_2, E_1 and their conjugates.

This paper is dedicated to Professor Heinz G. Floss (Washington Univ., WA, USA) on the occasion of his 70th birthday.

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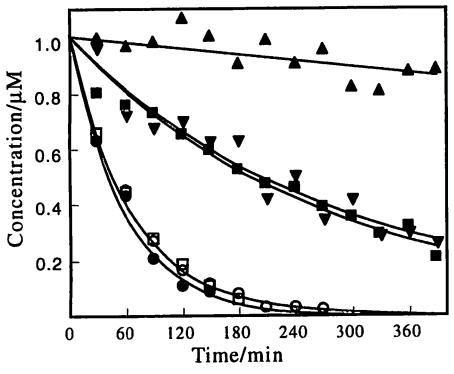

Fig. 2 Photocatalytic degradation of estrogens using photocatalyst under UV irradiation (n = 2, mean). ●, E_2; ○, E_1; ■, $E_2$3G; □, $E_2$17G; ▼, E_1G; ▲, E_1S.

monitored at 280 nm. The flow rate and column temperature were set at 1.0 ml min^{-1} and 40°C, respectively. As a mobile phase, methanol-water (7:3, v/v) was used for the determination of unconjugated estrogens [E_2 (t_R 4.3 min) and E_1 (t_R 4.6 min)], and methanol-water containing 5 mM of HCO_2NH_4 was used for the determination of conjugated estrogens {E_1S (1:1, v/v; t_R 5.3 min), E_2G [2:3, v/v; t_R 7.3 min (3G), 7.8 min (17G)] and E_1G (2:3, v/v; t_R 7.9 min)}.

A VL-4LC black light lamp (365 nm, 4 W) (Vilber Lourmat, Cedex, France) was used as the light source.

Photodegradation of estrogens

The ethanol solution of estrogen was diluted with water to 1 μM (the ethanol concentration was less than 0.3%, v/v). Photocatalytic glass beads (*ca.* 13.6 g) were spread to cover the bottom of a glass petri dish (diameter, 8 cm), and the estrogen solution (1 μM, 15 ml) was placed in the dish. The petri dish was placed in a light shielded box [22.5 (wide) × 13.5 (depth) × 16 cm (height)], and irradiated by a black light lamp 16 cm away for 6.5 h. Five hundred microliters of solution was sampled every 30 min and then subjected to an Oasis HLB cartridge. After washing with water (2 ml), estrogen was eluted with methanol (1.5 ml) and evaporated under a N_2 gas stream. The residue was dissolved in methanol (50 μl) and an aliquot was applied to an HPLC.

Recovery of estrogens

The estrogen solutions (1 μM, 500 μl) were subjected to an Oasis HLB cartridge, concentrated and then analyzed by HPLC as described above which were used as the 0 min illuminated samples. The peak areas of the estrogens in these samples were compared with those of the expected amount of authentic estrogens, and the obtained recovery rates (*ca.* 80%) were used for correction of the concentration in the following experiments.

Results and Discussion

The time courses of the photocatalytic degradations of the estrogens using the photocatalytic glass beads were monitored by measuring the remaining substrate by UV-HPLC. An aliquot of the reaction mixture was subjected to the solid-phase extraction, concentrated and then analyzed by HPLC, because the UV detector did not have enough sensitivity to detect estrogens without pretreatment.

The results of the photocatalytic degradation are shown in Fig. 2 (n = 2, mean). E_2, $E_2$17G and E_1, which have an unconjugated phenolic hydroxy group at the C-3 position, were almost completely degraded after 4 h of UV irradiation by first-order kinetics. Their degradation rate constants were 1.7×10^{-2} min^{-1} [coefficient of determination (r^2) = 0.984], 1.5×10^{-2} min^{-1} (r^2 = 0.991) and 1.5×10^{-2} min^{-1} (r^2 = 0.991), respectively. On the other hand, the degradation rates of $E_2$3G, E_1G and E_1S, which are conjugated at the 3-hydroxy group, were much lower than those of the above estrogens. $E_2$3G and E_1G were degraded slowly by first-order kinetics [3.6×10^{-3} min^{-1} (r^2 = 0.967) and 3.4×10^{-3} min^{-1} (r^2 = 0.938), respectively], and *ca.* 20 and 25% of the substrates remained after a 6.5 h irradiation, respectively. In the case of E_1S, *ca.* 90% of the substrate still remained at the end of the irradiation and its degradation order was unclear. E_2 was almost quantitatively recovered from the reaction mixture under the control conditions, such as no UV light or catalyst. These data showed that the adsorption of estrogen on a photocatalyst was negligible and the photocatalyst effectively functioned.

As described above, the hydroxyl radical was generated when TiO_2 was irradiated by UV light in an aqueous solution.[11,12] The oxidation of the phenolic hydroxy moiety of estrogens by the hydroxyl radical was regarded as the initiation of the photocatalytic degradation of the estrogens.[9] The results described in this paper supported the above hypothesis and showed that the 3-hydroxy group of estrogen is important for the photodegradation. The catalytic effect of the other photocatalysts on the degradation of the estrogens conjugated at the 3-hydroxy group and the investigation of the formed degradation products are now in progress in our laboratory.

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References

1. Y. Nakamura, K. Onda, C. Takatoh, and A. Miya, *Bunseki Kagaku*, **2003**, *52*, 107.
2. K. Majima, T. Fukui, J. Yuan, G. Wang, and K. Matsumoto, *Anal. Sci.*, **2002**, *18*, 869.
3. G.-G. Ying, R. S. Kookana, and Y.-J. Ru, *Environ. Int.*, **2002**, *28*, 545.
4. F. Tilton, W. H. Benson, and D. Schlenk, *Aquat. Toxicol.*, **2002**, *61*, 211.
5. H. M. Kuch and K. Ballschmiter, *Fresenius J. Anal. Chem.*, **2000**, *366*, 392.
6. K. Wright, D. C. Collins, and J. R. K. Preedy, *Steroids*, **1979**, *34*, 445.
7. K. Shimada, K. Mitamura, and T. Higashi, *J. Chromatogr. A*, **2001**, *935*, 141.
8. T. A. Ternes, H. Andersen, D. Gilberg, and M. Bonerz, *Anal. Chem.*, **2002**, *74*, 3498.
9. Y. Ohko, K. Iuchi, C. Niwa, T. Tatsuma, T. Nakashima, T. Iguchi, Y. Kubota, and A. Fujishima, *Environ. Sci. Technol.*, **2002**, *36*, 4175.
10. H. M. Coleman, B. R. Eggins, J. A. Byrne, F. L. Palmer, and E. King, *Appl. Catal. B: Environ.*, **2000**, *24*, L1.
11. C. Richard and P. Boule, *New J. Chem.*, **1994**, *18*, 547.
12. N. Watanabe, S. Horikoshi, H. Kawabe, Y. Sugie, J. Zhao, and H. Hidaka, *Chemosphere*, **2003**, *52*, 851.