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Isolation and Identification of Xenobiotic Aryl Hydrocarbon Receptor Ligands in Dyeing Wastewater

PEI-HSIN CHOU,† SABURO MATSUI,† KENTARO MISAKI,‡ AND TOMONARI MATSUDA*,†

Department of Technology and Ecology, Graduate School of Global Environmental Studies, Kyoto University, Sakyo-Ku Yoshida-Honmachi, Kyoto 606-8501, Japan, and Department of Urban and Environmental Engineering, Graduate School of Engineering, Kyoto University, Nishikyo-Ku Kyotodaigaku-Katsura, Kyoto 615-8540, Japan

Dyeing wastewater collected in Kyoto city, Japan, was investigated for the occurrence of aryl hydrocarbon receptor (AhR) ligands by using an AhR-responsive reporter gene assay. Concentrated extracts of wastewater samples elicited a dose-dependent increase in AhR ligand activity, and several hydrophobic HPLC fractions of the extracts were highly effective in inducing AhR ligand activity. Three potential AhR ligands were isolated from these fractions and identified to be Disperse Red 92, Disperse Yellow 64, and 3′-hydroxybenzo[b]quinophthalone by using HPLC and LC−MS/MS. Disperse Red 92, which has also been detected in the treated effluent from a sewage plant receiving the wastewater, is an anthraquinone disperse dye showing weak AhR binding affinity in the assay. Disperse Yellow 64 and 3′-hydroxybenzo[b]quinophthalone are quinoline disperse dyes capable of activating the AhR at nanomolar concentrations. In particular, Disperse Yellow 64 is a highly potent AhR ligand that was 3 times more effective in inducing AhR ligand activity than β-naphthoflavone in the assay. Quinoline disperse dyes are suggested to be a new class of xenobiotic AhR ligands which pose a danger to aquatic biota and human health.

Introduction

Dyeing wastewater contains many hazardous chemicals and has been shown to elicit genotoxicity and mutagenicity (*1*, *2*). Although certain carcinogenic dyes have been prohibited, toxicological data for dyes or dye derivatives are still insufficient, and at least 14 dye products in use in European countries were reported to be mutagenic in 2004 (*3*−*6*). Additionally, while many dyes are not readily biodegradable, ozonation or chlorination have also been shown to increase the toxicity of dyeing wastewater, indicating the formation of more toxic intermediates during the treatment processes (*7*−*10*). Thus, discharging non-treated or inappropriately treated dyeing effluent poses a threat to the receiving water body.

* Corresponding author phone: +81-753-5171; fax: +81-753-3335; e-mail: matsuda@eden.env.kyoto-u.ac.jp.

† Department of Technology and Ecology, Graduate School of Global Environmental Studies.

‡ Department of Urban and Environmental Engineering, Graduate School of Engineering.

Among the toxic dyes that were banned, sudan dyes or methyl yellow have been shown to interact with the aryl hydrocarbon receptor (AhR) (*11*, *12*), a ligand-activated transcription factor that mediates many biological and toxic effects of environmental contaminants such as dioxins or polycyclic aromatic hydrocarbons. The ligand−AhR complex enters the nucleus after ligand binding and dimerizes with the AhR nuclear translocator protein. The heterodimer subsequently interacts with the xenobiotic responsive element and activates the transcription of downstream genes, including those encoding xenobiotic-metabolizing enzymes. The AhR is also involved in many physiological functions, such as cell cycle regulation, apoptosis, development, and reproduction (*13*−*15*). Activation of the AhR by xenobiotic ligands may lead to metabolical detoxification by metabolizing enzymes or altered gene expression that relates to various toxic responses, including teratogenicity, immunotoxicity, or carcinogenicity. Thus, binding of a xenobiotic ligand to the AhR is suggested to be an indication of possible toxicity.

Environmental AhR ligands have been detected in industrial effluent such as pulp and paper mill wastewater or produced water from offshore oil and gas production (*16*, *17*). As for dyeing industries, dioxazine or phthalocyanine dyes have been found to be contaminated with polychlorodibenzodioxins (PCDDs) and polychlorodibenzofurans (PCDFs) due to the use of chlorinated aromatic solvents or chloranil containing high levels of PCDD/PCDFs during manufacturing processes (*18*, *19*). In addition, several synthetic azo dyes or anthraquinone dyes have also been reported to activate the AhR (*11*, *12*, *20*). Since it is presumable that dyeing wastewater contains chemicals capable of binding to the AhR, investigating the occurrence of potential AhR ligands in dyeing wastewater is necessary to provide basic information for hazard reduction.

In this study, untreated dyeing wastewater was collected in the vicinity of a sewage plant that treats the wastewater together with domestic wastewater. Xenobiotic AhR ligands and mutagens have been detected in the treated effluent of the sewage plant and are suggested to be dyes or dye derivatives (*9*, *10*, *20*). A bioassay-directed analysis, which consists of a yeast-based reporter gene assay for measuring AhR ligand activity, high-performance liquid chromatography (HPLC) for separating target compounds, and HPLC−tandem mass spectrometry (LC−MS/MS) for identifying ligand candidates, was used to investigate potential AhR ligands in the wastewater. The results revealed that several commercial dyes currently used in Japan elicit AhR ligand activity, and quinoline disperse dye is suggested to be a new class of xenobiotic AhR ligands.

Materials and Methods

Reagents. Disperse Red 92 [Sumikaron Brilliant Red S-BLF; C.I. 60752] was kindly provided by Sumitomo Chemicals (Osaka, Japan). 2,3-Naphthalenedicarboxylic anhydride was obtained from Tokyo Chemical Industries (Tokyo, Japan). 3-Methylcholanthrene, 3-hydroxy-2-methyl-4-quinolinecarboxylic acid, and Disperse Yellow 64 [Latyl Yellow 3GB; C.I. 47023] were purchased from Aldrich (WI). Commercially obtained dyes were purified using HPLC to remove impurities before use. Other chemicals were of analytical grade and were purchased from Wako (Osaka, Japan).

Synthesis of 3′-Hydroxybenzo[b]quinophthalone. 3′-Hydroxybenzo[b]quinophthalone (1H-Benz[f]indene-1,3-(2H)-dione, 2-(3-hydroxy-2(1H)-quinolinylidene), CAS 341-34-7) was synthesized according to the method described in ref *21*. In brief, equimolar amounts of 2,3-naphthalenedi-

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carboxylic anhydride and 3-hydroxy-2-methyl-4-quinolinecarboxylic acid were dissolved in nitrobenzene, stirred, and heated overnight under reflux. Nitrobenzene was removed by distillation under reduced pressure, and the target compound was purified repeatedly by using silica gel column chromatography (eluent: hexane/chloroform) followed by recrystallization from methanol. Further purification was performed by HPLC.

Sampling and Extraction. Dyeing wastewater was collected from an open channel containing effluent of dyeing factories in the southern area of Kyoto city, Japan, in July 2003, March 2004, and October 2004. Samples collected in July 2003 and March 2004 (500 mL) were filtered through 0.45 μm glass fiber filters and passed through Sep-pak Plus C18 environmental cartridges (Waters, MA) at a flow rate of 10 mL/min. Each cartridge was washed with 10 mL of water and then eluted with 10 mL of methanol. The eluents were combined, evaporated to dryness in a centrifugal vacuum concentrator (Tomy, Tokyo, Japan), and redissolved in a small amount of dimethyl sulfoxide (DMSO).

Dyeing wastewater was taken again in October 2004 (1500 mL) to obtain sufficient amounts of potential AhR ligands. The wastewater sample was filtered and passed through Sep-Pak Vac 35 cc C18 cartridges (Waters, MA) using a flow rate of 10 mL/min. The cartridges were washed with water and then eluted with 60 mL of 50% methanol in water, 60 mL of 75% methanol in water, and 120 mL of methanol. Three extracts were collected separately, evaporated to dryness, and redissolved in DMSO. Dilution series of all concentrated extracts were prepared in DMSO.

HPLC Fractionation, Quantification, and Ligand Isolation. An aliquot of each concentrated extract was injected into a Shim-pack FC-ODS column (particle size 5 μm, 150 $\times$ 4.6 mm; Shimadzu, Kyoto, Japan) for HPLC fractionation and quantification. The column was eluted in a linear gradient of 10% methanol in water to 100% methanol within 20 min followed by holding at 100% methanol for a further 20 min at a flow rate of 1 mL/min. Fractions were collected every minute for 36 min by using a fraction collector (FRC-10A, Shimadzu, Kyoto, Japan) or manually. Each fraction was evaporated to dryness and then redissolved in DMSO. The HPLC system was equipped with a SPD-M10Avp diode array detector (Shimadzu, Kyoto, Japan), and concentrations of AhR ligand candidates in the extract of wastewater collected in March 2004 were estimated by monitoring the absorption of eluate at 254, 443, and 456 nm.

The methanol extract of wastewater sample taken in Oct 2004 was also fractionated by a Wakosil-II 5C18HG column (particle size 4.2−4.7 μm, 20 $\times$ 50 mm; Wako, Osaka, Japan) eluted using a flow rate of 2.5 mL/min with gradient elution, which started at 75% methanol in water and increased to 100% methanol within 5 min followed by holding at 100% methanol for another 25 min. AhR ligand candidates in the fractions collected from retention times of 9−10 min and 18−21 min were further isolated and purified for several times using the Shim-pack FC-ODS column and the Wakosil-II 5C18HG column.

AhR-Dependent Reporter Gene Assay. AhR ligand activity was measured by a reporter gene assay using the recombinant yeast YCM3 strain (*22*), which contains the human AhR and AhR nuclear translocator (Arnt) expression construct and a pTXRE5-Z (LacZ) reporter plasmid responding to the ligand-induced AhR/Arnt complex. DMSO that showed no activity in the assay was use as a vehicle for dissolving and diluting test samples. The bioassay was basically carried out as described in ref *23*. In brief, the yeast was grown in a synthetic glucose medium (2% glucose, lacking tryptophan) at 30 °C for 18 h. One μL of a test sample was mixed with 200 μL of synthetic galactose medium (2% galactose, lacking tryptophan) and 5 μL of the saturated culture in a 96-well microplate, and subsequently incubated at 30 °C for another 18 h. After the incubation, cell density was determined by reading the absorbance at 595 nm (A_{595}) using a microplate reader (Bio-Rad Laboratories, CA), and 10 μL of the cell suspension was transferred into a new microplate and mixed thoroughly with 140 μL of Z-buffer (*22*) and 50 μL of *o*-nitrophenyl-β-D-galactopyranoside solution (4 mg/mL, made in Z-buffer). The absorbance at 405 nm (A_{405}) was measured after incubating at 37 °C for 60 min, and the β-galactosidase activity (reported as LacZ units) was calculated by the following formula: LacZ units = $1000 \times A_{405}/(A_{595} \times 0.01$ (mL of cell suspension added) $\times$ 60 (minutes of reaction time)). Each sample was tested in triplicate, and data are shown as mean values $\pm$ standard deviation. β-Naphthoflavone (β-NF) was used as the positive control. β-NF equivalent concentration was calculated using the dose−response curve of β-NF.

LC/ESI−MS/MS. Potential AhR ligands isolated from the dyeing wastewater were analyzed by an electrospray ionization tandem mass spectrometry (ESI−MS/MS). Experiments were carried out using a Micromass Quattro Ultima Pt mass spectrometer (Waters, MA) equipped with a Shim-pack FC-ODS column and a SPD-10Avp UV−vis detector (Shimadzu, Kyoto, Japan). The column was eluted in a linear gradient of 10% methanol in water to 100% methanol within 20 min followed by holding at 100% methanol for another 40 min at a flow rate of 0.4 mL/min. Data acquisition was performed in the positive ion mode. The experimental conditions were set as follows: capillary voltage 3.5 kV, cone voltage 35 V, ion source temperature 130 °C, desolvation temperature 380 °C, desolvation gas flow rate 700 L/hr. Argon was used as the collision gas, and the collision energy was set at 25 eV for acquiring MS/MS spectra.

Results

Detection of AhR Ligand Activity. Concentrated extracts of dyeing wastewater samples collected in July 2003 (Jul-03 sample) and March 2004 (Mar-04 sample) showed a dose-dependent increase in AhR ligand activity measured by using the yeast-based reporter gene assay (Figure 1A). Significant increases in the activity were detected when the concentration factor of both extracts was as low as 0.004 ($p < 0.05$, *t*-test), indicating that even the diluted extracts equivalent to the concentrations of 250-fold diluted dyeing wastewater were able to induce AhR signaling in the assay. Figure 1B shows the AhR ligand activity of HPLC fractions of the two extracts. High AhR ligand activity was induced by the 23rd to 28th and 33rd to 34th fractions of the Jul-03 sample, and the 23rd to 28th, 31st, and 34th to 35th fractions of the Mar-04 sample. Activation of the AhR was not detected in the polar fractions collected from 0 to 20 min of both extracts.

Though the composition of wastewater samples varied between months, several peaks showing similar UV spectra and HPLC retention times were detected in both extracts. Compounds considered to be potential AhR ligands were chosen for further isolation and identification due to the following reasons. A red compound (R1) observed in the 25th fraction drew attention because its retention time and UV spectrum were similar to those of an AhR ligand candidate detected in the treated sewage effluent in our previous studies (*20*). In addition, a yellow compound (Y1) was chosen because it was detected in the 33rd to 34th fractions of the Jul-03 sample and the 34th to 35th fractions of the Mar-04 sample that elicited high AhR ligand activity. A second yellow compound (Y2) observed in the 31st fraction of the Mar-04 sample was also selected because it was the major compound in the fraction eliciting high AhR ligand activity. Figure 1C shows the HPLC chromatogram at UV absorbance 254 nm of the Mar-04 sample.

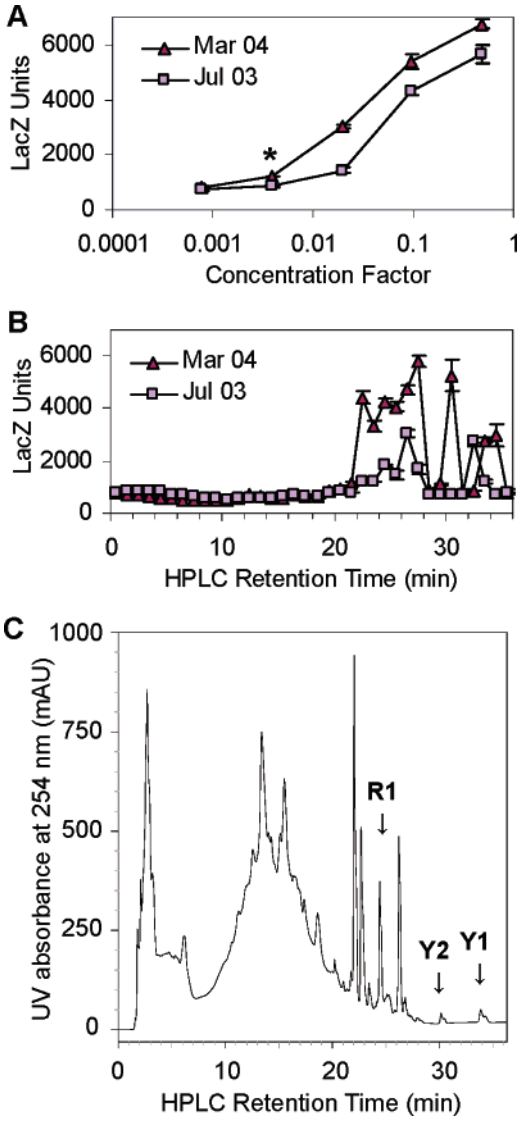

FIGURE 1. (A) AhR ligand activity of the serial dilution of the Jul-03 sample and Mar-04 sample. The asterisk indicates the lowest concentration factor eliciting a significant increase in AhR ligand activity (0.004 for both samples; $p < 0.05$, *t*-test). (B) AhR ligand activity of the HPLC fractions of the Jul-03 sample and Mar-04 sample (final concentration factor 1.2). (C) HPLC chromatogram (UV absorbance at 254 nm versus retention time) of the Mar-04 sample. Peaks indicated as R1, Y1, and Y2 are three AhR ligand candidates observed in fractions eliciting AhR ligand activity.

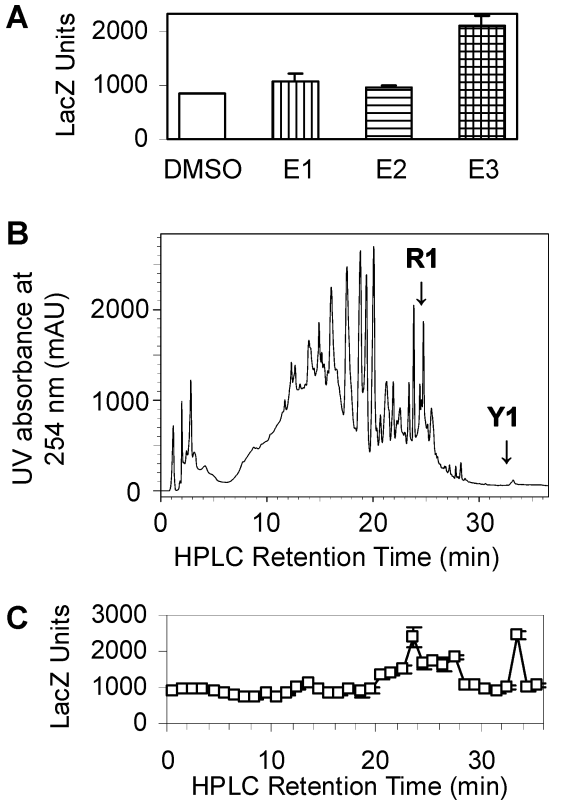

FIGURE 2. (A) AhR ligand activity of DMSO (solvent blank) and three extracts of the Oct-04 sample, E1 (50% methanol in water), E2 (75% methanol in water), and E3 (100% methanol) (final concentration factor 0.6). (B) HPLC chromatogram (UV absorbance at 254 nm versus retention time) of E3. R1 and Y1 are AhR ligand candidates detected in fractions eliciting AhR ligand activity. (C) AhR ligand activity of the HPLC fractions of E3 (final concentration factor 4.9)

Isolation of Potential AhR Ligands. The wastewater sample taken in October 2004 (Oct-04 sample) was used to obtain sufficient amounts of potential AhR ligands for chemical identification. Since the polar HPLC fractions of the Jul-03 sample and Mar-04 sample were incapable of inducing AhR ligand activity, hydrophobic compounds were separated from hydrophilic ones by eluting each cartridge with three eluents as described in the Materials and Methods section. Figure 2A shows the AhR ligand activity of the three corresponding extracts, referred to as E1 (50% methanol in water), E2 (75% methanol in water), and E3 (100% methanol). E3 elicited the highest activity and R1 and Y1 were observed in its HPLC chromatogram (Figure 2B). Figure 2C shows the AhR ligand activity of the HPLC fractions of E3. The β-NF equivalent concentration of AhR ligand activity elicited by each HPLC fraction (final concentration factor: 4.9) was calculated and summed up to be 12.9 nM, which was close to that of the 4.9-fold concentrated extract (estimated to be 12.5 nM), suggesting that the loss in AhR ligand activity during the fractionation step was very low.

E3 was further fractionated to obtain R1 and Y1, and the two compounds were isolated from the fractions collected in retention times of 9−10 min and 18−21 min, respectively. In addition, Y2 was separated from the concentrated extract of the Mar-04 sample. These AhR ligand candidates were purified using HPLC and were then analyzed by LC−MS/MS and the yeast-based reporter gene assay.

Identification of Xenobiotic AhR Ligands. A base peak at m/z 497 was observed in the full-scan MS spectrum of R1, and the MS/MS spectrum of the base peak ion showed a major fragment ion at m/z 330 (Figure 3A). The mass of R1 was suggested to be approximately 496, which corresponded to that of a commercial anthraquinone disperse dye, Disperse Red 92 (DR 92; $C_{25}H_{24}N_2O_7S$, molecular weight 496.53; Figure 3B). The UV−visible spectrum of R1 (λ_{max}: 254, 519, and 553 nm in methanol; Figure 3A) was also similar to that of DR 92 (*24*). Thus, DR 92 was obtained commercially and analyzed by HPLC and LC−MS/MS. R1 was identified to be DR 92 due to the identical HPLC retention time, UV−visible spectrum, and MS/MS spectrum.

The mass of Y1 was suggested to be approximately 339 because a base peak at m/z 340 was observed in the full-scan mass spectrum. In addition, fragment ions generated from the base peak were detected at m/z 323 and 155 (Figure 4A). 3′-Hydroxybenzo[b]quinophthalone (3′-HB[b]QP; $C_{22}H_{12}NO_3$, molecular weight 339.34; Figure 4B), a quinoline disperse dye which was studied as a potential substitute for the toxic dye cadmium yellow in Japan during the 1970s, was suggested to be the candidate of Y1 due to its similar molecular weight

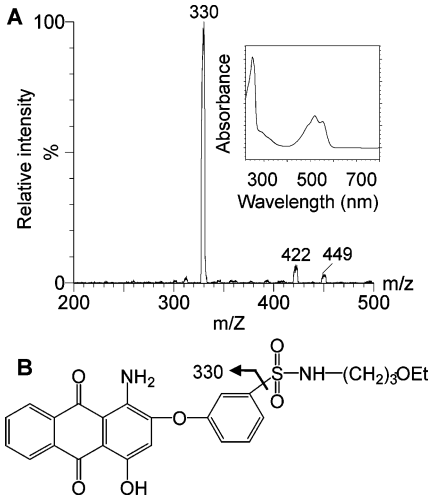

FIGURE 3. (A) MS/MS spectrum of the $[M + H]^+$ ion 497 of R1 and the UV−visible spectrum of DR 92 in methanol (λ_{max}: 254, 519, 553 nm). (B) Chemical structure of DR 92.

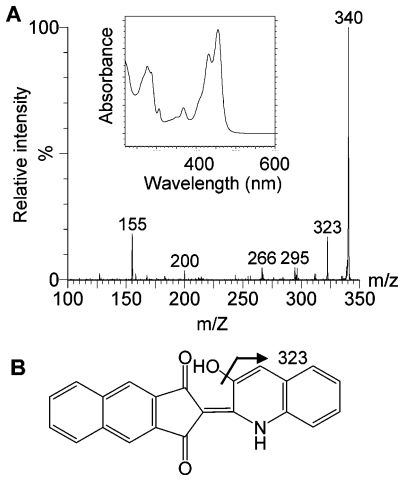

FIGURE 4. (A) MS/MS spectrum of the $[M + H]^+$ ion 340 of Y1 and the UV−visible spectrum of 3′-HB[b]QP in methanol (λ_{max}: 456, 432, 276, 286, 368 nm). (B) Chemical structure of 3′-HB[b]QP.

and UV−visible spectrum (*21*). 3′-HB[b]QP was synthesized and then subjected to HPLC and LC−MS/MS analysis. Y1 was confirmed to be 3′-HB[b]QP because the HPLC retention time, UV−visible spectrum (λ_{max}: 456, 432, 276, 286, and 368 nm in methanol; Figure 4A), and MS/MS spectrum of 3′-HB[b]QP were identical to those of Y1.

Y2 exhibited a characteristic isotope pattern for bromine in its MS spectrum (peaks at m/z 368 and 370 corresponding to ^{79}Br and ^{81}Br isotopes, respectively), and λ_{max} at 443, 422, and 285 nm were observed in its UV−visible spectrum (Figure 5A). The MS/MS spectrum of the base peak at m/z 368 showed major fragment ions at m/z 350, 289, and 245 (Figure 5B). A monobrominated quinoline disperse dye, Disperse Yellow 64 (DY 64; $C_{18}H_{10}BrNO_3$, molecular weight 368.18; Figure 5C), was considered to be the candidate of Y2. Commercially obtained DY 64 was purified and then analyzed by HPLC and LC−MS/MS. Y2 was confirmed to be DY 64 because identical data were obtained in the analyses (Figure 5A and B).

AhR Ligand Activity and Concentrations of Potential Ligands. Figure 6 shows the dose−response curves of AhR ligand activity of the three potential AhR ligands isolated from the dyeing wastewater (DR 92, 3′-HB[b]QP, DY 64) and two typical AhR ligands (3-methylcholanthrene (3-MC) and β-NF). DY 64 is the most potent AhR ligand identified in the

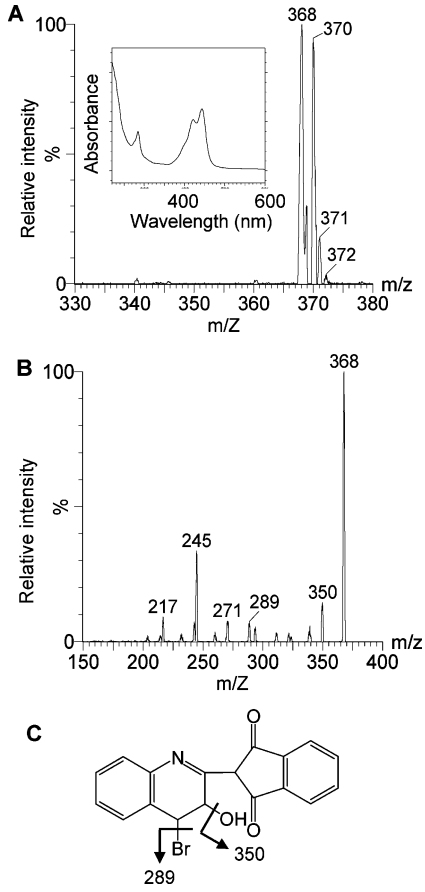

FIGURE 5. (A) MS spectrum and the UV spectrum in methanol (λ_{max}: 443, 422, 285 nm) of Y2. (B) MS/MS spectrum of the $[M + H]^+$ ion 368 of Y2. (C) Chemical structure of DY 64.

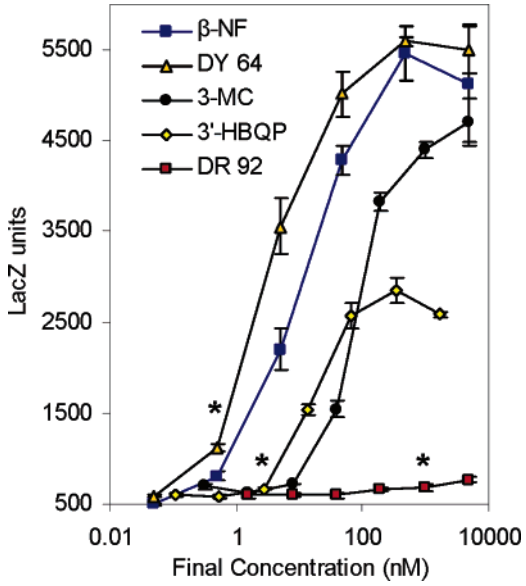

FIGURE 6. Dose−response curves of AhR ligand activity elicited by DR 92, 3′-HB[b]QP, DY 64, 3-MC, and β-NF. The asterisk indicates the lowest concentration eliciting a significant increase in AhR ligand activity (DY 64 0.5 nM; 3′-HBQP 2.7 nM; DR 92 971 nM; $p < 0.01$, *t*-test)

dyeing wastewater and was capable of inducing AhR signaling in the assay at the concentration of 0.5 nM ($p < 0.01$). The effective concentration of DY 64 that induced lacZ units equivalent to 50% of the maximal response (EC_{50}) was approximately 3 nM. 3′-HB[b]QP induced significant increase

in AhR ligand activity at the concentration of 2.7 nM ($p < 0.01$) and was as potent as 3-MC when the concentration was lower than 100 nM. However, it was incapable of inducing maximal activity in the higher concentration range. DR 92 showed weak AhR binding affinity and elicited significant increase in AhR ligand activity when the concentration was higher than 971 nM ($p < 0.01$). It also failed to induce maximal activity.

The concentrations of DY 64, 3′-HB[b]QP, and DR 92 in the Mar-04 sample were estimated to be 34.5, 8.7, and 639 nM by using HPLC analysis. The β-NF equivalent concentration was calculated to be 201, 2.6, and less than 1 nM, respectively. DY 64 and 3′-HB[b]QP accounted for approximately 22% and 3% of the AhR ligand activity elicited by the wastewater extract, suggesting that other unidentified AhR ligands may contribute to 75% of the activity.

Discussion

Quinoline Disperse Dyes as Novel AhR Ligands. Quinoline disperse dyes are suggested to be a new class of xenobiotic AhR ligands because two quinoline disperse dyes, DY 64 and 3′-HB[b]QP, were identified as potent ligands capable of activating the AhR at nanomolar concentrations in the yeast-based reporter gene assay (Figure 6). 3′-HB[b]QP showed AhR ligand activity comparable to that of 3-MC in the low concentration range (from approximately 3−100 nM), and DY 64 elicited higher AhR ligand activity than β-NF and 3-MC at all concentrations. The EC_{50} of DY 64, β-NF, and 3-MC was approximately 3, 10, and 100 nM, respectively, indicating that DY 64 was 3 and 33 times more effective than β-NF and 3-MC, respectively, in the assay.

The yeast bioassay is suggested to be a useful screening tool since the yeast HSP90 homolog and other cofactors in the yeast have been reported to interact with the human AhR properly to generate a receptor competent in signal transduction (*25*). However, it is important to note that the results of this bioassay are not always correspondent with those of other experimental systems using mammalian cells or in vivo assays. The discrepancy may be explained by the species difference and the metabolic degradability of different AhR ligands. For instance, indiruin, a potent AhR ligand isolated from human urine, has been shown to be 50 times more potent than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the yeast bioassay (*23*) and capable of inducing CYP1A1 and CYP1A2 mRNAs in human hepatoma cell line HepG2 at concentrations 10 times lower than that of TCDD (*26*). Nevertheless, it has also been reported to be less potent than TCDD in other studies using experimental animals or mammalian cell lines (*27*). In contrast to TCDD, indirubin is easily metabolized by the induced enzymes such as CYP1A1 (*26*), which may be the main reason for the difference in toxicity elicited by these two ligands.

The potent AhR ligand DY 64 isolated from the wastewater has been reported to be genotoxic using a SOS chromotest in the absence of S9 liver homogenate (*28*). In addition, it has also been detected as a sensitizing agent causing allergic contact dermatitis (ACD) in humans (*29*, *30*). It is possible that the activation of AhR is involved in the ACD responses caused by DY 64, because AhR-mediated transcriptional activation has been suggested to relate to inflammatory disorders caused by PAH exposure using transgenic mice expressing a constitutively active form of mouse AhR (*31*).

Possible Occurrence of Xenobiotic AhR Ligands in the Environment. The dyeing wastewater investigated in this study is treated by biological treatment and post-chlorination in a sewage treatment plant, but the identified AhR ligands are considered to be poorly biologically or chemically degraded following conventional treatment. Although DR 92 have been reported to be efficiently removed by using the fungus *Cunninghamella polymorpha* in 72 h (*32*) and over 90% of DY 64 has been eliminated by a good settling activated sludge from a wastewater treatment plant (*33*), the major mechanism of removal was suggested to be the adsorption of dyes to fungal cells or sludge instead of biodegradation (*32*, *33*). The presence of these potential AhR ligands in the treated sewage effluent or in the activated sludge is presumable, and actually DR 92 has been detected in the treated sewage effluent in our previous study (*20*), and DY 64 has also been found in the drying sludge of a municipal waste treatment plant receiving textile wastewater (*34*). Chemical treatment such as chlorination has also been shown to be incapable of treating certain disperse dyes appropriately (*35*). In addition, 3′-HB[b]QP has been reported to be highly resistant to photodegradation (*21*, *36*), and no chemical degradation was detected in the electrochemical treatment of DY 64 using aluminum electrodes (*37*).

The quinoline disperse dyes are suggested to have significant potential to adsorb to sediment rather than being degraded in the environment due to their hydrophobic property. The water/organic carbon partition coefficient (K_{oc}) of DY 64 has been reported to be $\log K_{oc} = 5.9$, which suggested that DY 64 and the more hydrophobic dye 3′-HB[b]QP are very likely to partition into sediments (*38*). The hypothesis is further supported by the detection of DY 64 in the sediment of a creek below a municipal waste treatment plant receiving textile effluent (*34*). However, the bioconcentration factor (BCF) in fish for DY 64 has been reported to be low (log BCF 0.70) due to its low lipid solubility (*39*). It has also been reported that disperse dyes did not bioaccumulate in fish because of their low bioavailability in water and the aggregation tendency that hampered the transport across membranes (*40*).

Degradation of quinoline disperse dyes in anoxic sediment has also been indicated to be very low (*34*, *41*). The mean half-lives of azo or anthraquinone disperse dyes to be degraded in sediment soaked in lake water have been reported to range from 2.6 h to 15 days. However, a much longer half-life has been observed for the degradation of DY 64, which required at least 240 days (*34*). Another structurally related quinoline dye, Solvent Yellow 33, has been shown to be stable in the sediment as well, with a half-life of 82−200 days (*41*). Thus, DY 64 and 3′-HB[b]QP are suggested be refractory and persistent in the environment. Their occurrence in the water environment, especially for the highly potent AhR ligand DY 64, may pose a danger to the aquatic biota and human health since their toxicological data are still insufficient. Further studies concerning their environmental behavior and toxicological data are necessary.

Acknowledgments

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