IN VITRO ASSESSMENT OF INTERACTION OF HUMIC SUBSTANCES WITH 2,3,7,8-TCDD

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Introduction

Humic substances (HS) are ubiquitous natural products of decomposition of dead organic matter. Generally, HS can be divided into three groups: humic acids (HA), fulvic acids (FA) and humins. In the aquatic environment, HS form approximately 50-70% of dissolved/natural organic matter (D/NOM)[1], which is present in most natural freshwaters at concentrations ranging from 0.5 to 50 mg L^{-1}, and organic carbon in sediments can even reach over 20% dry weight[2,3]. Biological effects of HS can be both of indirect and direct nature. Indirect effects such as both toxicants and nutrient controlling via interaction with various HS structures have been known for a long time. However, also direct physiological effects such as changes of both heat shock proteins expression and biotransformation enzymes activity, or hormone-like effects in animals, have been found shown recently[4]. In a previous *in vitro* study, we described the interaction of HS with aryl hydrocarbon receptor (AhR)[5], which has been also confirmed *in vivo*[6]. In this paper, we summarize our new findings on interaction of both AhR-active and -inactive HS with 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin (TCDD). The possible mechanisms are discussed.

Material and methods

HS isolated from different matrices were purchased from Fluka (Switzerland) - HA-Fluka, Sigma Aldrich (Czech Republic) - HA sodium salt, and IHSS (USA) - remaining nine HS samples. HS were dissolved in 0.05M NaOH to achieve final concentrations 50 and 150 mg L^{-1}. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was purchased from Dr. Ehrenstorfer (Germany). Experiments were carried out using TCDD dissolved in ethanol.

H4IIE-*luc* (rat hepatocarcinoma) cells stably transfected with luciferase gene under control of AhR were used for determination of AhR activation. Cells were grown and maintained in DMEM medium containing 10% fetal calf serum (both PAA laboratories, Austria) at 5% CO_2 and 37 ^{o}C. Once the cells reached about 70% confluence they were passaged and seeded into a sterile 96-well plate at density 15,000 cells/well. After 24 h, the cells were exposed in triplicates to the tested samples (50 and 150 mg L^{-1} HS) or reference compound (dilution series 0.1-100 pM TCDD) or mixtures of TCDD and HS (500 pM TCDD with 150 mg L^{-1} and 3.7 pM TCDD with 50 mg L^{-1}) for 24 h at 37 °C (final vehicle concentration was 0.5% v/v). Cells exposed to DMEM with 0.5% EtOH and 0.5% 0.05M NaOH were used for the appropriate vehicle controls. Intensity of luciferase luminescence was measured using Promega Steady Glo Kit (Promega, Germany) after 24 h exposure. At least three independent assays have been conducted for each concentration tested.

Results

Of the 11 HS tested, 5 elicited a significant ($p<0.05$) AhR-mediated activity in H4IIE-*luc* cells. All of the active samples were HA, while no FA and no NOM samples showed any significant activity (Figs 1 and 2, the first column from each couple). None of the 5 AhR-active HS were able to completely activate AhR even in extreme concentrations; their highest activation reached 40 to 70% of $TCDD_{max}$-activation (not depicted). This type of AhR-activation suggests that AhR-active HS behave as partial agonists of AhR. In case of simultaneous exposure of excessive concentrations of HS (150 mg L^{-1}) together with high TCDD concentration (500 pM - AhR is activated to the highest level), we observed inhibition of AhR-activation by 5 HS samples and no significant influence by 6 HS samples (Fig. 1, the second column from each couple); 4 out of 5 inhibiting samples were from the group of AhR-active samples. In another case, simultaneous exposure of environmentally relevant concentration of HS (50 mg L^{-1}), together with medium TCDD concentration (3.7 pM, i.e. EC_{50} for AhR activation), we observed enhancement of AhR-activation by 5 HS samples and no influence by 6 HS samples (Fig. 2, the second column from each couple).

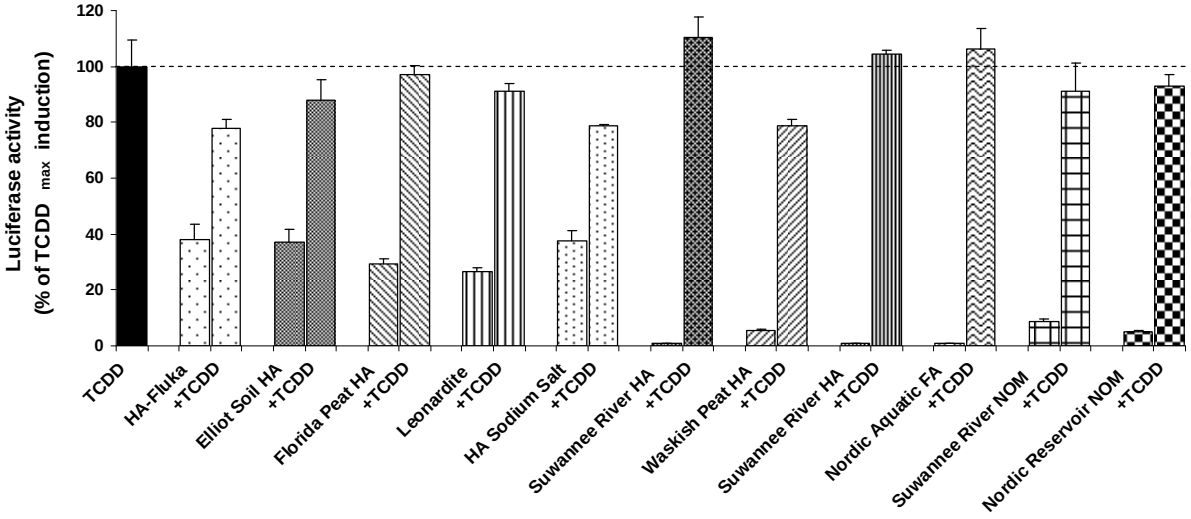

Figure 1: AhR-mediated activity of 11 samples of HS determined in H4IIE-*luc* cells; black column and the dash line at the level of 100% correspond to c_{TCDD}= 500pM; the first column from each couple - c_{HS}=150 mg L^{-1}; the second column from each couple - co-exposure HS and TCDD. Values represent the mean ± SD of triplicate determinations.

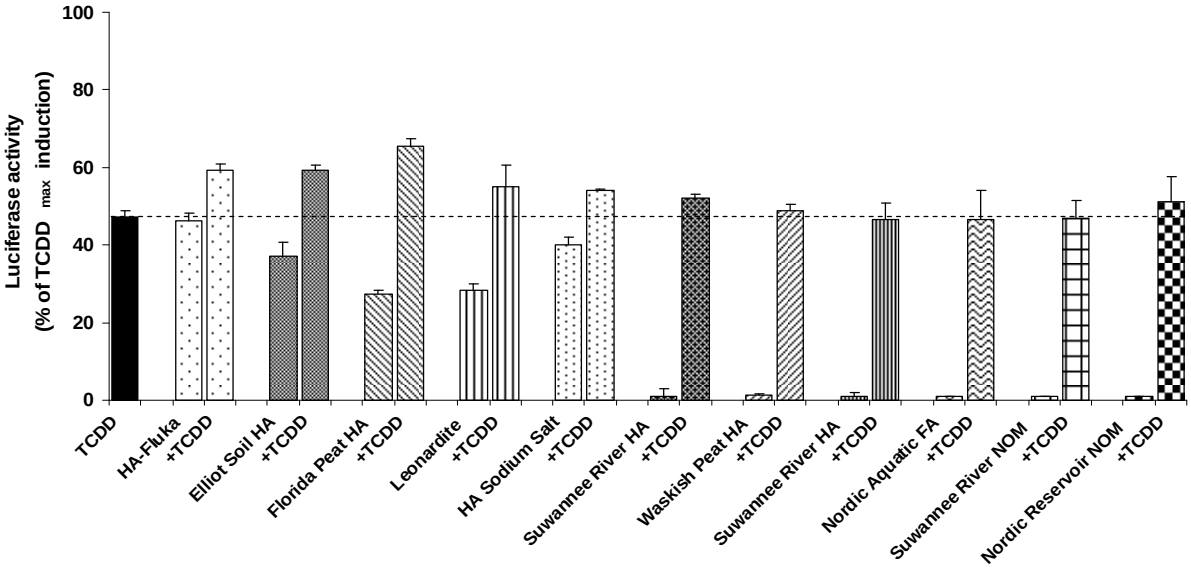

Figure 2: AhR-mediated activity of 11 samples of HS determined in H4IIE-*luc* cells; black column and the dash line at the level of cca 50% correspond to c_{TCDD}=3.7 pM; the first column from each couple - c_{HS}=50 mg L^{-1}; the second column from each couple - co-exposure HS and TCDD. Values represent the mean ± SD of triplicate determinations.

Discussion

According to the "receptor-ligand interaction theory" the partial agonists (probably AhR-active HS) of appropriate receptor (AhR) should suppress the ability of full agonist (TCDD) to activate this receptor[7]. Thus, according to this theory, AhR-active HS could relatively weakly bind to AhR, but block binding of possible dioxin-like pollutant to AhR, which would protect organism against consequent adverse physiological effects. Our results depicted in the Fig. 1 and Fig. 2 support this theory, because we have observed both *in vitro* suppression (at excessive TCDD and HS concentrations), and addition (at lower TCDD and HS concentrations) of TCDD-caused AhR-mediated effect by AhR-active HS.

Nevertheless, it is also possible that observed effects at excessive HS concentrations could be caused by the sorption of TCDD onto HS, thanks to well known strong sorption ability of hydrophobic compounds onto HS[8]. For example, TCDD exerts sorption partition coefficient (log K_{OC}) of 6.1. Fava and Picollo[9] have described analogous results, where high HS amount have decreased aerobic biodegradation of PCBs in soil, whereas lower HS amount have stimulated this aerobic biodegradation of PCBs (compared to humics-free soil). According to this explanation, we could expect different influence of HS on dioxins in sediments (high HS content) and water column (lower HS content). To decide which mechanism is responsible for the observed changes of TCDD-mediated AhR-activity in the mixture with HS, it is necessary to conduct more experiments. These experiments will be aimed to evaluate prolonged incubation of HS with TCDD prior to the cell exposure, because the probable sorption of TCDD onto HS (or another type of interaction) is time dependent.

Acknowledgments

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