CHARACTERIZATION OF NATURAL AHR LIGANDS IN HEALTH FOODS ESTIMATED BY IN VITRO REPORTER GENE ASSAY

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Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates the toxic and biological actions of many aromatic environmental pollutants such as dioxins. As part of an investigation to clarify the interaction of foods with AhR, we previously reported that excessive intake of foods containing AhR-activators may be conductive to promote dioxin-like toxicity though there would not be a problem following normal intake.[1)] Additionally, it is discussed that the signal transduction of natural AhR ligands, which occurs after AhR activation, should differ from that of dioxins. In this study, we examined the binding ability of fifty extracts prepared from kinds of commercial supplements and health foods containing high concentrations of their respective ingredients to the AhR using reporter gene assay. At the same time, the active sample extracts were fractionated to characterize the AhR active substances, and reversed-phase HPLC analysis was conducted for the active fractions

Materials and methods

1. Extraction and isolation

Fifty supplements and health foods were from drug stores in Japan. The samples were prepared as follows: Tablets were powdered and the contents of capsules and soft capsules were used for sample preparation. The materials (1 g) were homogenized in ethanol/water (4:1) (30 mL) for 10 min and filtered. The filtrates were concentrated under reduced pressure and freeze-dried (total extract). Total extracts were added to water (10 mL), and these solutions were subjected to liquid-liquid partition (each 30 mL) to give three extracts: *n*-hexane, ethyl acetate, and water soluble portions.

2. HPLC conditions

HPLC analysis was carried out using a Shimadzu Prominence system. Conditions were as follows: column, L-column ODS (5 μm, 150 × 2.1 mm i.d.); mobile phase, solvent A was 3% acetic acid and solvent B was acetonitrile (0–30 min, 0–50% B in A; 30–35 min, 50–85% B in A; 35–40 min, 85–85% B in A); injection volume, 5 μL; column temperature, 40°C; flow-rate, 0.3 mL/min; detection, 200–400 nm.

3. Estimation for AhR ligand activity

For the identification of AhR-activating materials, a CALUX assay was used.[2)] When mouse hepatoma (H1L6.1c2) cells are exposed to ligands such as dioxins, luciferase protein synthesis is inducted. The amount of light emitted by the luciferase protein is correlated directly with the dioxin level, and this system is used as a simple dioxin monitoring method (Figure 1).

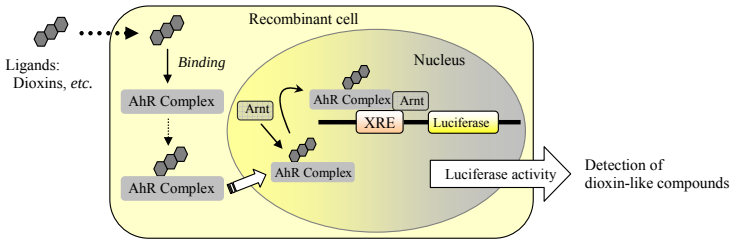

Figure 1. AhR-mediated *in vitro* bioassay (CALUX assay)

Results and discussion

Although most of the samples showed no dioxin-like activity even at a high concentration, some samples exhibited activity at high concentration in the order of mg/mL in dose-dependent manner. In order to characterize the active components of each sample from soybean-related samples (No. 30 and 31), sesame (No. 29), and propolis (No. 26), AhR activity was measured for the respective *n*-hexane, ethyl acetate, and water fractions. The *n*-hexane fraction of the propolis extract sample exhibited AhR activity, and marked AhR activity was noted for the ethyl acetate fractions of the other samples (soybean and sesame extract samples) at 0.1-10 mg/mL (Figure 2).

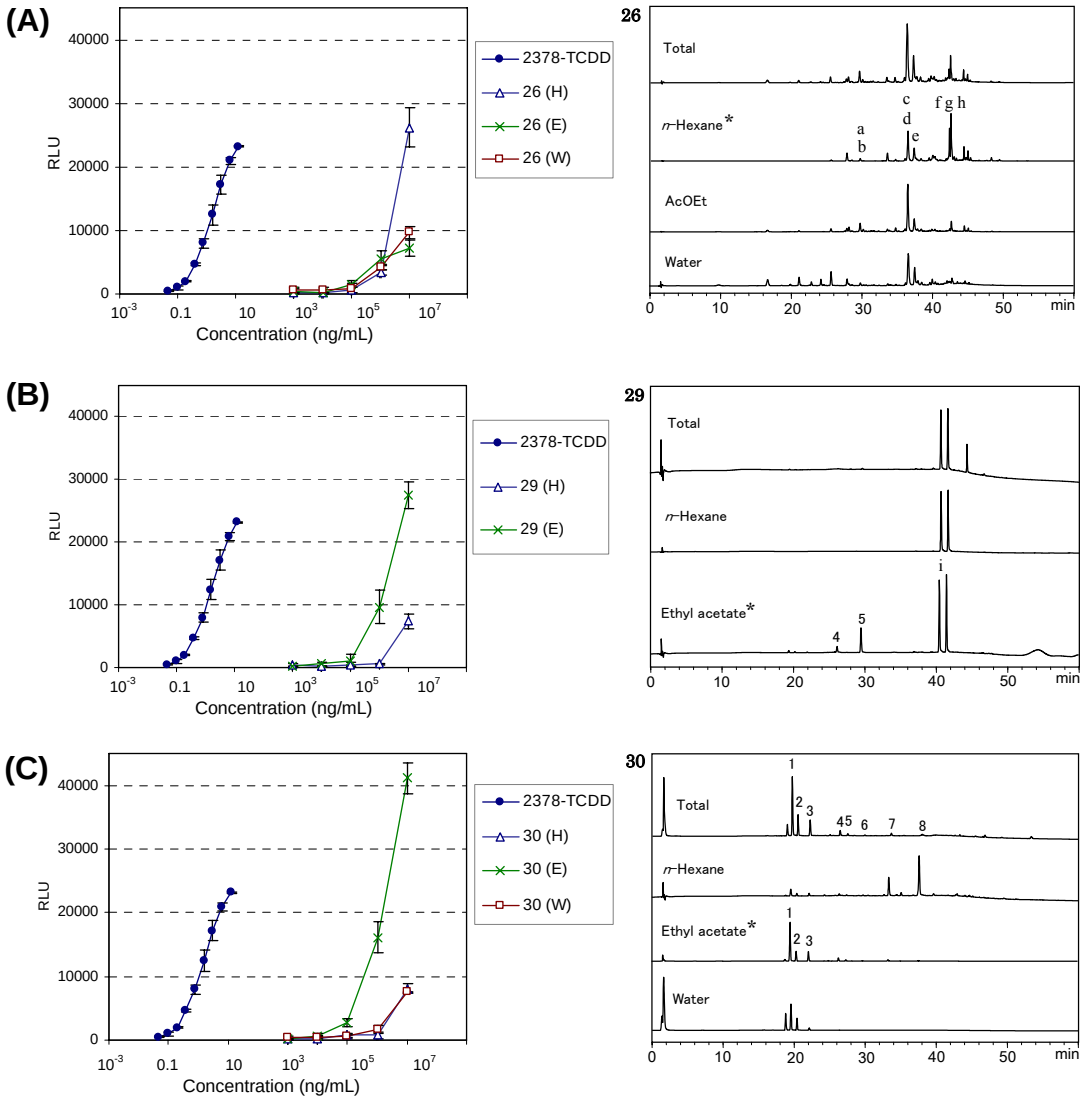

Figure 2 (continued on the following page).

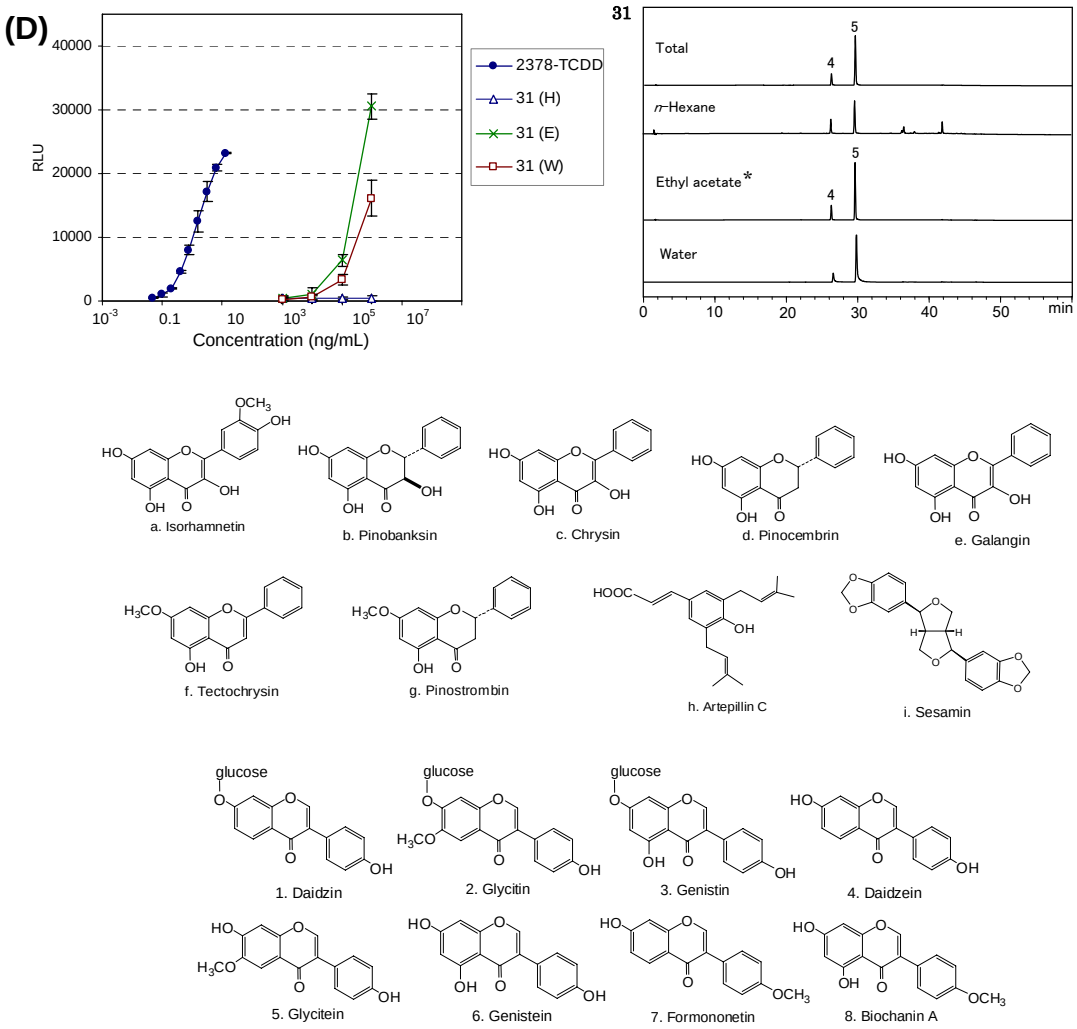

Figure 2. Concentration-response curve and RP-HPLC profiles of active samples and TCDD for the induction of luciferase activity in the CALUX assay.
(A) Propolis (No. 26), (B) Sesame (No. 29), (C) Soy-bean related samples (No. 30), (D) Soy-bean related samples (No. 31)
H, *n*-Hexane fraction; E, Ethyl acetate fraction; W, aqueous fraction
a, isorhamnetin; b, pinobanksin; c, chrysin; d, pinocembrin; e, galangin; f, tectochrysin; g, pinostrombin; h, artepillin C; i, sesamin
1, daidzin; 2, glycitin; 3, genistin; 4. daidzein; 5, glycitein; 6, genistein; 7, formononetin; 8, biochain A
Signals in HPLC were detected at 254 nm except sample 26 (at 280 nm). Sample 29 did not provide an aqueous fraction for analysis. * AhR activated fraction

HPLC analysis of the active fractions of sesame and soybean-related samples identified isoflavones, such as daidzein and glycitein. The *n*-hexane fraction of the propolis product which showed AhR activity contained eight compounds such as tectochrysin and pinocembrin. Among these compounds, tectochrysin showed remarkable

AhR activation (Figure 3). AhR-activated compounds identified in this study are reported as beneficial constituents. Also, they do not have accumulation characteristics such as the dioxin. Most recently, several papers reported that AhR activation may be involved in novel physiological functions.[3)-6)] Therefore it is suggested that natural AhR ligands characterized in the present study may play some beneficial regulatory role in human.

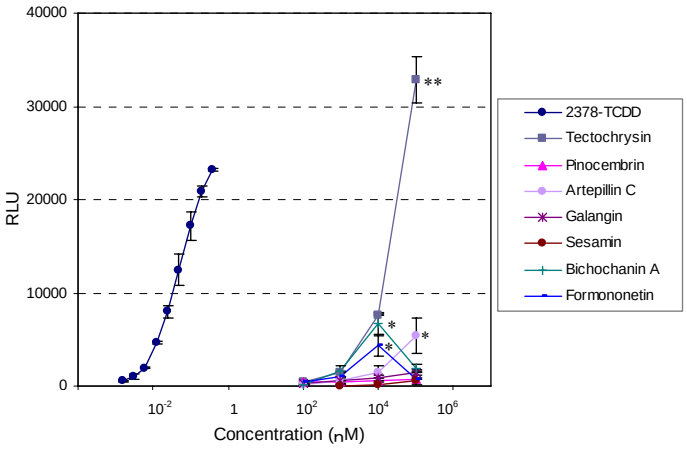

Figure 3. Concentration-response curve of selected compounds and TCDD for the induction of luciferase activity in the CALUX assay
Each point represents the mean of at least three replicate analyses. Results are expressed as means ± SD, and asterisks indicate statistically significant differences (*p<0.05, **p<0.01).

Acknowledgements

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References

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