Phyton (Austria) Special issue: "APGC 2004"	Vol. 45	Fasc. 4	(471)-(476)	1.10.2005

Glucosylation of Bisphenol A by Various Plant Species

By

N. NAKAJIMA[1)], Y. OHSHIMA, J. S. EDMONDS, M. TAMAOKI, A. KUBO, M. AONO, H. SAJI & M. MORITA

K e y w o r d s : Bisphenol A, glycosyltransferase, phytoremediation, glucoside.

S u m m a r y

NAKAJIMA N., OHSHIMA Y., EDMONDS J.S., TAMAOKI M., KUBO A., AONO M., SAJI H. & MORITA M. 2005. Glucosylation of bisphenol A by various plant species. – Phyton (Horn, Austria) 45 (4): (471)-(476).

The concentration of bisphenol A (BPA) in the culture medium of tobacco BY-2 cells decreased rapidly 2.5 h after the application. Four metabolites of BPA were observed in a methanol extract of the cells. They were determined to be BPA mono-β-D-glucopyranoside and 3 highly glucosylated BPA. These results indicate that tobacco cells uptake BPA, then metabolized to β-glucosides. We also determined BGT activity in cell-free extracts of leaves of 43 plant species. The level of BGT activity correlated with the rate of BPA uptake in *Fabaceae* and *Brassicaceae*. The results indicate that BGT activity may be related to uptake of BPA in both species.

I n t r o d u c t i o n

Bisphenol A (BPA: 4,4'-isopropylidenediphenol) has a weak estrogenic activity and used in the many types of plastics (ASH & ASH 1995). Annual world production of BPA has been estimated as more than 500,000 tons (STAPLES & al. 1998). BPA has been detected in terrestrial and aquatic environments (ALEXANDER & al. 1988) and it has be shown to affect embryonic and postnatal development of mammals (TAKAI & al. 2001), therefore the environmental impact of this compound is of concern (STAPLES & al. 1998). Plants have also been considered as suitable agents for the biodegradation of lipophilic compounds and would probably be more easily controlled in the environment (CUNNINGHAM & OW 1996). However, the ability of plant cells to take up and metabolize BPA had not been investigated earlier. In the present study, we

[1)] The National Institute for Environmental Studies, Onogawa, Tsukuba 305-0053, Japan, Tel, Fax +81-298-2490, e-mail: naka-320@nies.go.jp

have identified 4 types of glucosylated metabolites of BPA in tobacco suspension cultured cells. We determined BPA-glucosyltransferase (BGT) activity, which responsible for production of the metabolites, in the leaves of 43 plants species.

Material and Methods

Cell cultures and BPA tratment

Bisphenol A was supplemented with 10 mg·L^{-1} at initial concentration in two weeks-old tobacco BY-2 suspension cultured cells. For a control BPA was added to freshly prepared culture medium in the absence of cells. They were incubated at 25°C with gentle shaking. Aliquots (10 µL) of the medium were taken and analyzed by HPLC [C-18 column 150 × 3.9 mm (Symmetry, Nihon Waters, Tokyo, Japan), 40°C, 40 % aqueous methanol as eluent at a flow rate of 1 mL·min^{-1}. Absorption at 217 nm was monitored (NAKAJIMA & al. 2002).

Analysis of the metabolites of BPA in BY-2 cells

A portion (50 mL) of a two-week-old BY-2 cultured cells was supplemented with 10 mg·L^{-1} of BPA and incubated at 25°C for 24 h with shaking. At each sampling time, 3 mL of the culture was put into 10 mL of methanol and the mixture was allowed to stand for 24 h. The clear supernatant (50 µL) was then subjected to HPLC using the same conditions as above.

Isolation and identification of metabolites

Two liter of BY-2 cells supplemented with BPA at 10 mg·mL^{-1}, was incubated at 25°C with gentle shaking. The cells were collected and extracted with 1 liter of methanol. The extract was evaporated to dryness, dissolved in 200 mL of 67 % aqueous methanol and the pH adjusted to 10 by the addition of a sodium hydroxide solution. Lipophilic material was removed by three times of extraction with 200 mL of chloroform and the aqueous phase was evaporated to dryness after the pH was adjusted to 4 with acetic acid. The residue was extracted with 200 ml of chloroform at 50°C. The chloroform-soluble material was evaporated to dryness and dissolved in 2 mL of water. The aqueous solution was then subjected to HPLC (as above) with 40 % aqueous methanol as eluent. Three fractions containing metabolites were combined, concentrated. Fraction "A" was re-separated with 23 % aqueous methanol as an eluent. Again the fractions containing metabolites were combined and were then evaporated to dryness. NMR spectrum was obtained from a JNM-ALPHA500 (500 MHz for ^{1}H, JEOL, Tokyo, Japan).

Determination of BPA uptake and BGT activity in various plant species

The name of species for determination of BPA uptake and BGT activity were recored in Tables 1. Four weeks old seedlings were immersed in 1.5 mL of 10 mg·L^{-1} BPA solution, then incubated at 25°C under white light (1000µmol·m^{-2}·s^{-1} of photosynthetically active photon flux density). At each sampling time amount of BPA in the medium was determined by HPLC. For determination of BGT activity, 1g of leaves were homogenized with 5 mL of extraction buffer (50 mM Tris-HCl, pH7.5, 1 mM 2-mercaptoethanol). After centrifugation, low molecular weight substances in the supernatant were removed by gel filtration. The enzyme reaction mixture contained 50 mM Tris-HCl pH7.5, 1 mM UDP-Glucose and 0.5 mM BPA. The reaction was performed at 30°C for 20 min, then stopped with a addition of 60 % of perchloric acid. Precipitate was removed by centrifugation, BPA-*o*-β-D-glucopyranoside in the supernatant was determined by HPLC.

Results and Discussion

Tobacco BY-2 cells uptake BPA and metabolize to glucosides

To determine whether plant cells can absorb BPA, tobacco BY-2 cells

were exposed to 10 mg·$L^{-1}$of BPA in the medium. The BPA concentration rapidly decreased to one third of the initial value after 0.5 h, and to total disappearance by 2.5 h. In the absence of cells there was no reduction. These results indicate that BY-2 cells have the ability to take up BPA. We separated methanol extracts of the cells after BPA was added to the medium. Four metabolites were apparent in the extracts. They were coded A-1, A-2, B and L. Their chemical structure are determined to be BPA di-β-D-glucopyranoside (A-1), BPA β-D-glucopyransyl-(1→4)-[β-D-glucopyransyl-(1→6)] β-D-glucopyranoside (A-2), BPA mono-β-D-gentiobioside (B) and BPA-mono-D-glucopyranoside (L). Our results indicated that tobacco cells uptake BPA, then metabolized to β-glucosides.

BGT may be responsible for BPA uptake in *Fabaceae* and *Brassicaceae*

We determined whether the ability of seedlings to take up BPA correlated with the level of BPA specific glucosyltransferase (BGT) activity in the leaves. In 43 species we analyzed, the ability to absorb BPA varied (Table 1). The highest absorber was *Capsicum annuum* L. We could not detect any absorbtion in three species (*Cucurbita maxima, Canavalia gladiata* and *Lemna minor*). Among the 43 species tested, *Brassicaceae* plants had higher BGT activity (Table 1). We could not detect any activity in three species (*Citrullus vulgaris*, *Eichhornia crassipes*, and *Lycopersicon esculentum*) and could not determine that of *Brassica campestris* L. (pak-choi) by interfering substances. The level of BGT activity did not correlate with the rate of BPA absorption in 3 of 5 families tested. However, significant correlation was observed in *Fabaceae* and *Brassicaceae* plants (Fig. 1A, B). *Arabidopsis* had the highest absorption rate in *Brassicaceae* plants tested but it had the lowest BGT activity. While *Arabidopsis* showed extraodinal nature in the relation to BPA uptake and BGT activity, our results indicate that the level of BGT activity may be responsible for the rate of BPA absorption in *Fabaceae* and *Brassicaceae*.

In the previous study (NAKAJIMA & al. 2002), we showed that tobacco cells metabolize BPA to BPA mono-β-D-glucopyranoside (BPAG); here we showed that all the plant leaves used in this study, except for those of 3 species, had at least a low level of BGT activity. These findings suggest that glucosylation might be a common metabolic process of BPA in plants. We demonstrated that BPAG accumulates in the leaves (NAKAJIMA & al. 2002). Therefore, plants may metabolize BPA by a BGT-catalyzed reaction, accumulate BPAG and its highly glucosylated forms in the leaves, and then eliminate it by detaching the leaves. Therefore, identification of the gene encoding BGT would open the way toward improvement of BPA uptake by ectopic expression of BGT in plant cells.

Table 1. Comparison of BPA uptake and BGT activity in leaves of several plant families. Three to five independent experiments were performed according to described in materials and methods. Average of the value and standard devieations (SD) are indicated (n=3-5). N.D.: Not determined, U.D.: Under detectable.

Family	Species	BPA Uptake ($\mu g \cdot h^{-1} \cdot gfw\ leaf^{-1}$)		BGT activity ($nmol \cdot h^{-1} \cdot mg\ protein^{-1}$)	
		average	S.D.	average	S.D.
Brassicaceae	*Raphanus sativus L. hortensis Backer*	1.86	0.11	9.84	0.32
	Brassica rapa L. var capitata L.	2.26	0.33	13.65	0.55
	Brassica oleracea L.var.capitata L	2.66	0.37	19.14	4.50
	Brassica napus L..	2.09	0.19	20.10	2.42
	Raphanus sativus L. var. sativus L.	1.75	0.38	5.54	1.25
	Brassica rapa L. var amplexicaulis	1.56	0.36	6.30	0.37
	Arabidopsis thaliana L.	6.49	2.26	2.60	0.04
	Brassica campestris L (Pak-choi)	3.04	0.77	N.D.	N.D.
	Brassica campestris L. (Tsai-shin)	1.02	0.68	4.75	0.11
Cucubitaceae	*Citrullus vulgaris L*	6.83	1.34	U.D.	U.D.
	Cucurbita maxima L. Duchesne	U.D.	U.D.	1.42	0.18
	Cucumis sativus L.	11.72	2.29	1.06	0.04
	Cucumis melo L. var. makuwa Makino	12.38	4.59	0.39	0.11
	Litchi chinensis	43.10	1.20	0.17	0.04
	Lagenaria leucantha	11.73	2.40	0.69	0.12
	Luffa cylindrica Roem	11.73	2.40	0.87	0.14
Fabaceae	*Vigna angularis Ohwi et.*	29.28	4.05	3.09	0.07
	Vigna sinensis	14.34	2.10	1.29	0.23
	Glycine max	11.06	3.46	0.19	0.04
	Phaseolus vulgaris L.	5.09	3.44	0.06	0.03
	Pisum sativum L.	43.08	12.92	3.48	0.33
	Vicia faba L	5.92	0.83	1.49	0.07
	Canavalia gladiata DC.	U.D.	U.D.	1.31	0.003
Poaceae	*Triticum aestivum L.*	4.81	0.48	1.54	1.14
	Sorghum nervosum Bess	8.49	1.18	0.56	0.01
	Zea mays L.	5.71	1.86	0.21	0.04
	Oryza Sativa L	18.31	3.89	0.46	0.02
	Lagurus ovatus L.	27.90	3.45	17.67	1.98
	Cymbopogon citratus	30.86	15.60	0.23	0.01
	Capsicum annuum L.Var angulosum	7.92	1.12	3.68	0.20

	Nicotiana tabacum L.	3.28	0.30	0.58	0.10
Solanaceae	*Lycopersicon esculentum Mill.*	16.71	1.93	U.D.	U.D.
	Solanum melongena	38.60	9.28	0.27	0.02
	Capsicumannuum L.	49.31	5.44	1.76	0.57
	Physalis alkekengi L.val.francheti Makino.	23.66	1.99	9.82	1.34
	Lemna minor L	U.D.	U.D.	0.23	0.01
	Sesamum indicum L.	22.36	3.78	1.14	0.12
	Ricinus communis L.	4.30	0.65	6.88	0.96
Others	*Gossypium indicum L.*	5.11	0.46	7.80	0.74
	Daucus carota L.	16.73	4.71	0.08	0.27
	Helianthus annuus L.	6.30	1.78	0.12	0.04
	Echinodorus amazonicus	13.15	3.32	0.02	0.01
	Eichhornia crassipes Solems-Laub.	9.11	1.93	U.D.	U.D.

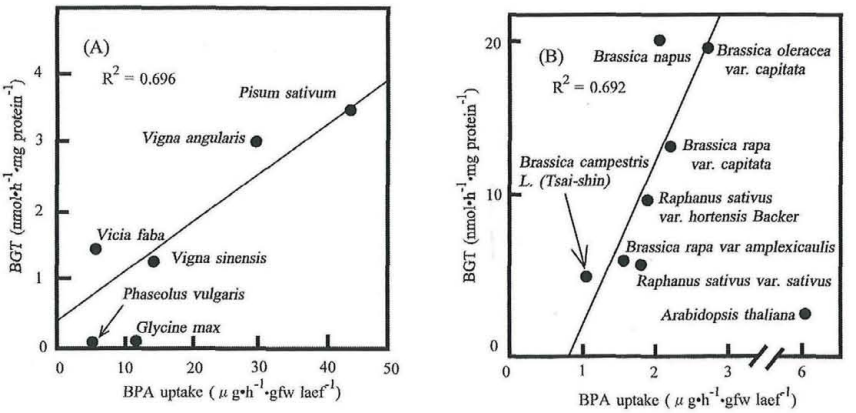

Fig. 1. Correlation between BPA uptake and BGT activity in leaves of *Fabaceae* (A) and *Brassicaceae* (B).

Acknowledgements

We thank M. MARUO for the preparation of the tobacco BY-2 cultured cells. This work was partly supported by Grant-in-Aids for Scientific Research from Japan Society for the Promotion of Science.

References

ALEXANDER H.C., DILL D.C., SMITH L.W., GUINEY P.D. & DORN P.B. 1988. Bisphenol A: acute aquatic toxicity. - Environ. Toxicol. Chem. 7: 19–26.

ASH M. & ASH I. 1995. Handbook of plastic and rubber additives. - Gower, Hampshire, UK.

CUNNINGHAM S.D. & OW D.W. 1996. Promises and prospects of phytoremediation. - Plant Physiol. 110: 715–719.

NAKAJIMA N., OHSHIMA Y., SERIZAWA S., KOUDA T., EDMONDS J.S., SHIRAISHI F., AONO M., KUBO A., TAMAOKI M., SAJI H. & MORITA M. 2002. Processing of bisphenol A by plant tissues: glucosylation by cultured BY-2 cells glucosylation/translocation by plants of *Nicotiana tabacum*. - Plant Cell Physiol. 43: 1036–1042.

STAPLES C.A., DORN P.B., KLECKA G.M., BLOCK S.T. & HARRIS L.R. 1998. A review of the environmental fate, effects, and exposure of bisphenol A. - Chemosphere 36: 2149–2173.

TAKAI Y., TSUTSUMI O., IKEZUKI Y., KAMEI Y., OSUGA Y., YANO T. & TAKETANI Y. 2001 Preimplantation exposure to bisphenol A advances postnatal development. - Reprod. Toxicol. 15: 71–74.