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TAXONOMIC DISTRIBUTION OF PLANT GLUCOSYLTRANSFERASES ACTING ON XENOBIOTICS[1]

IN HONOUR OF PROFESSOR G. H. NEIL TOWERS 75TH BIRTHDAY

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Key Word Index— *O*-glucosyltransferase; *N*-glucosyltransferase; *S*-glucosyltransferase; pentachlorophenol; DDT; chlorinated anilines; 4-chlorothiophenol.

Abstract—*O*-Glucosyltransferase activities for chlorinated phenols, 2,2-bis-(4-chlorophenyl)-acetic acid (a DDT-metabolite) and indole-3-acetic acid were determined in 59 different plant species and 4 plant cell suspension cultures. These enzyme activities were found to be widely distributed in the plant kingdom with certain species showing particularly high activities. Similar observations were made for *N*- and *S*-glucosyltransferase activities acting on chlorinated anilines and 4-chlorothiophenol, respectively. These results indicate that the "green liver" concept previously derived for xenobiotic metabolism in higher plant species is also valid for the *O*-, *N*- and *S*-glucosyltransferases of lower plant species. Due to their enormous biomass, the latter may act as a significant global sink for xenobiotics. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Higher plant species have been shown to metabolize a broad range of xenobiotics, in particular pesticides, air pollutants and industrial chemicals [1, 2]. The metabolite patterns, participating enzyme classes and the responsible genes in plants are quite similar to those known for xenobiotic metabolism in the mammalian liver. Furthermore, these enzyme and gene classes are also similar to those involved in normal plant metabolism of natural compounds. These observations have been integrated into the "green liver" concept of xenobiotic plant metabolism [3, 4]. With few exceptions, this concept is based on observations made with higher plant species, in particular crop plants. In order to further explore the occurrence and evolution of the enzyme classes for xenobiotics, a systematic study emphasizing lower plant species has now been performed. Four cell suspension cultures and 59 plant species from various positions in the plant kingdom have been examined for *O*-, *N*- and *S*-glucosyltransferase activities towards seven xenobiotics and one natural substrate.

RESULTS

A standardized procedure for the preparation of soluble enzyme fractions was developed, and assay procedures for *O*-, *N*-, and *S*-glucosyltransferase activities were adopted from the literature. The distribution of enzyme activities glucosylating pentachlorophenol (PCP), 2,3,4-trichloro- and 2,4,5-trichlorophenol, for the DDT-metabolite, 2,2-bis-(4-chlorophenyl)-acetic acid (DDA) and indole-3-acetic acid are, with the exception of the enzyme for PCP, widely dispersed in the plant kingdom (Figure 1). Particularly high *O*-glucosyltransferase activities were observed for the following compounds: pentachlorophenol, in *Lycopodium annotinum* (11.8 ± 1.4 pkat/mg protein), *Glycine max.* (7.7 ± 1.2 pkat/mg protein), and *Equisetum giganteum* (1.3 ± 0.2 pkat/mg protein); 2,3,4-trichlorophenol, in *Monostroma arcticum* (56.2 ± 4.6 pkat/mg protein), *Polysiphonia urceolata* (51.9 ± 4.3 pkat/mg protein) and *Fontinalis antipyretica* (45.5 ± 4.3 pkat/mg protein); 2,4,5-trichlorophenol, in *Polysiphonia urceolata* (89.2 ± 4.6 pkat/mg protein), *Monostroma arcticum* (85.6 ± 5.7 pkat/mg protein) and *Lemna minor* (65.0 ± 5.4 pkat/mg protein). DDA, in *Ascophyllum nodosum* (22.3 ± 1.4 pkat/mg protein), *Halydris siliquosa* (11.3 ± 2.3 pkat/mg protein), and *Palmaria decipiens* (9.9 ± 1.4 pkat/mg protein); indole-3-acetic acid, in *Ascophyllum nodosum* (69.6 ± 4.6 pkat/mg

[1] Dedicated to Professor G. H. Neil Towers on the occasion of his 75th birthday.

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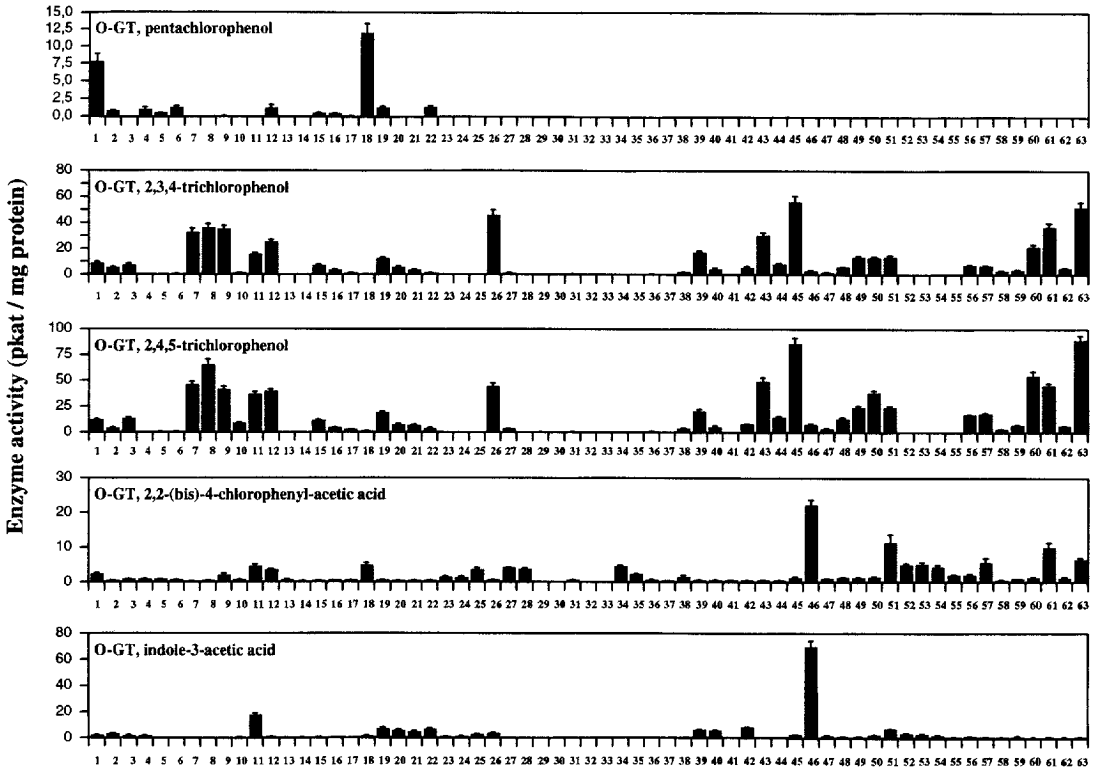

Fig. 1. Activities (pkat/mg protein) of the *O*-GT, *N*-GT, and *S*-GT enzyme activities with the indicated substrates. Mean values ± SD ($n = 3$) are shown. The numbering on the abscissa refers to the following cell suspension cultures or intact plant species. **Plant cell cultures** (used as reference systems) 1: *Glycine max.* L. Merr. cv. Mandarin. 2: *Triticum aestivum* L. cv. Heines Koga II; 3: *Zea mays* L. cv. Black Mexican Sweet; 4: *Picea abies* L. Excelsa RJ 80. **Intact plant samples. Spermatophyta.** Dicotyledonae. 5: *Rosa canina* L; 6: *Helianthus giganteus* L.; 7: *Salicornia europaea* L.; 8: *Lemna minor* L. **Ginkgoatae**. 9: *Gingko biloba* L.; 10: *Rhus typhina* L; 11. **Coniferae**. 11. *Sequoiadendron giganteum* Buchh. **Cycadatae**. 12: *Cycas revoluta* L. **Pteridophyta**. Filicatae. 13: *Athyrium filix-femina* (L) Roth.; 14: *Blechnum spicant* (L) Roth.; 15: *Azolla filiculoides* Lamk.; 16: *Selaginella lepidophylla* L. **Psilophytatae**. 17: *Psilotum nudum* L. **Lycopodiatae**. 18: *Lycopodium annotinum* L. **Articulatae**. 19: *Equisetum hyemale* L.; 20: *Equisetum arvense L.* 21: *Equisetum telmateia* Ehrh.; 22: *Equisetum giganteum* L. **Bryophyta**. Musci. 23: *Plagomnium undulatum* (*Hedw*) *B.S.G.*; 24: *Polytrichum formosum* Hedw.; 25: *Sphagnum capilifolium* (*Ehrh.*) Hedw; 26: *Fontinalis antipyretica* Hedw. **Hepaticae**. 27: *Marchantia polymorpha* L.; 28: *Conocephalum conicum* (L.) *Lindb.* **Mycobionta**. Basidomycetes. 29: *Sarcodon imbricatum* L.; 30: *Lactarius deterrimus* L.; 31: *Phanerochaete chrysosporium* Burd. Lichens. 32: *Alectoria samentosa* (Ach.); 33: *Peltigera aphtosa* (L.) Willd. **Chorophyta**. Chlorophyceae. 34: *Zygmena sp* L.; 35: *Chlorella fusca* Beijerinck; 36: *Chara corallina* L.; 37: *Caulerpa mexicana* Harv.; 38: *Ulva lactuca*; 39: *Enteromorpha compressa*; 40: *Enteromorpha bulbosa* (L.) Grev.; 41: *Cladophora rupestris* (L.) Kütz.; 42: *Halimeda opuntia* (L.) Lamour.; 43: *Acrosiphonia sonderi* (Kütz.) Komm.; 44: *Lambia antarctica* (Skottsb.) Delepine; 45: *Monostroma arcticum* Wittr. **Chromophyta**. Phaeophyceae. 46: *Ascophyllum nodosum* (L.) LeJol; 47: *Cytoseira baccata* (Gmel.) Silva; 48: *Laminaria digitata* (Huds.) Lamoureux; 49: *Laminaria hyperborea* (Gunn) Fosl; 50: *Laminaria saccharina* (L.) Lamoureux; 51: *Halydris siliquosa* (L.) Lyngb; 52: *Fucus vesiculosus* L.; 53: *Fucus serratus* L.; 54: *Fucus spiralis* L. **Rhodophyta**. Florideophyceae. 55: *Delesseria sanguinea* (Huds.) Lamoureux; 56: *Chondrus crispus* Stackh; 57: *Plocamium cartilagineum* (L.) Dixon. **Rhodophyceae**. 58: *Porphyra umbilicalis.* J. Ag.; 59: *Cystoclonium purpureum* (Huds.) *Batt*; 60: *Iridaea cordata* Kütz.; 61: *Palmaria decipiens* (A. and E.S.) Gepp, Kylin; 62: *Pantoneura plocamioides* (J. Ag.) Kyl; 63: *Polysiphonia urceolata* (Lightf. ex. Dillw.).

protein), *Sequoiadendron giganteum* (17.6 ± 1.4 pkat/mg protein), and *Enteromorpha compressa* (6.0 ± 0.4 pkat/mg protein).

Similar results, but with much higher specific enzyme activities, were obtained for the *N*-glucosyltransferases glucosylating 3-chloro- and 3,4-dichloroaniline (Fig. 1). The highest activities with 3-chloroaniline were observed for the *Pteridophyta*. The plant species with the highest activities were *Lycopodium annotinum* (210.4 ± 11.4 pkat/mg protein), *Halimedo opuntia* (197.0 ± 6.5 pkat/mg protein) and *Equisetum arvense* (148.0 ± 8.6 pkat/mg protein). For 3,4-dichloroaniline, the most active species were *Selaginella lepidophylla* (390.0 ± 11.4 pkat/mg protein), *Lycopodium annotinum* (265.0 ± 23.4 pkat/mg protein), and *Equisetum hyemale* (227.0 ± 12.5 pkat/mg protein).

Published information on *S*-glucosides in plants is scarce. The *Brassicaceae* are known to form glucosinolates, but very little information on xenobiotic *S*-glucosides exists [5]. A screening program with 4-chlorothiophenol showed a broad distribution of

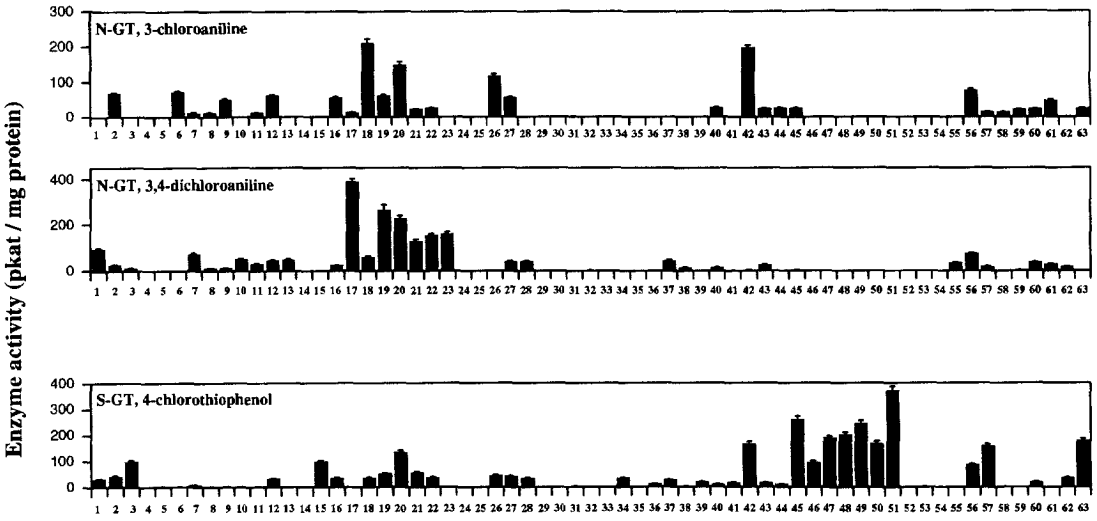

Fig. 1—*continued.*

enzyme activity. Highest enzyme activities were observed with marine macroalgae. The most active plant species were *Halydris siliquosa* (370.0 ± 16.5 pkat/mg protein), *Monostroma arcticum* (259.8 ± 12.3 pkat/mg protein), and *Laminaria hyperborea* (244.3 ± 12.4 pkat/mg protein).

DISCUSSION

The present data show that *O*-, *N*-, and *S*-glucosyltransferases that act on xenobiotic substrates are widespread in the plant kingdom. Standard isolation and assay procedures used were not optimized for every plant species tested. Plant species in which enzyme activity could not be demonstrated could, therefore, still possess such enzyme activities when analyzed under different experimental conditions. However, in the case of cultured soybean cells and PCP, the procedure published here led to 7-fold higher activity than previously reported [6]. The activities for the 2,3,4- and 2,4,5-trichlorophenols showed very similar distribution patterns, indicating that only one *O*-GT isoenzyme converts both compounds. The enzyme activity with pentachlorophenol as a substrate showed very different and much more limited distribution patterns indicating the existence of a different isoenzyme. Distribution patterns of CA and DCA directed *N*-glucosyl-transferases showed both certain similarities and also numerous deviations indicating that there was generally no proportionality. Therefore, it was concluded that at least two isoforms of this enzyme exist. The *S*-glucosyltransferase displayed a unique distribution pattern, with particularly high activity in the *Chromophyta*, suggesting that the enzyme differed from the tested *O*-GT and *N*-GT isoenzymes.

More generally, valid conclusions on isoenzyme specificities are only justified after extensive enzyme purification. The soybean glucosyltransferase isoenzymes for PCP and DDA have been separated and were shown to be distinct [6]. Nevertheless, both activities co-occurred at 0.3–9.5 pkat/mg protein in cell-free extracts from spruce needles and from cell suspension cultures of maple, beech, spruce, pine, soybean, parsley, bean and wheat [7]. The detection of both xenobiotic *O*-GTs in eight different plant species supports the present results which indicate a broad taxonomic distribution of *O*-, *N*- and *S*-GT isoenzymes. Six distinct soybean *O*-GT and *N*-GT isoenzymes with values of ~50 kDa and pI 4–6 have been characterized for quercetin, pentachlorophenol, DDA, 6-hydroxy-bentazone, chlorinated anilines and metribuzin [4]. No comparable characterization of GT-isoenzymes has been performed in other plant species.

It was previously postulated that aquatic plants are devoid of glucosyltransferases [8], which was confirmed here for the substrate PCP. However, as shown in our studies, numerous aquatic plant species, such as *Lemna minor* or the marine macroalgae, possess high glucosyltransferase activity towards other xenobiotics, forcing a revision of this statement.

In summary, the present results extend the "green liver" concept to lower plant species. Considering the huge biomass attributed to lower plant species [9] it is an important challenge to analyze the metabolic sink strength of lower plants for cleaning our environment.

EXPERIMENTAL

Chemicals

[U-^{14}C]-DCA, [U-^{14}C]-PCP, 4-chlorothiophenol, 3-chloroaniline, PMSF and PVP were purchased from Sigma (St. Louis, Deisenhofen). [Ring-U-^{14}C]-DDA was purchased from Pathfinder (St. Louis, MO), and

UDP-[U-^{14}C]-glucose from Amersham and Buchler. Amberlite XAD-4 was from Serva (Heidelberg). Sephadex G-25 (PD-10) was a product of Pharmacia (Freiburg). All other chemicals used were research grade commercial materials. Radiochemical purities higher than 98% were determined in all cases by TLC [10].

General methods

TLC was carried out on precoated silica gel G60 plates (Merck No. 5554) using the following solvent systems: 1. Ethylacetate/acetic acid/water, 63:1:2 (v/v/v); 2. Butanol-1/acetic acid/water, 12:3:5 (v/v/v). Radioactivity on TLC plates was determined with a TLC Linear Analyzer (Berthold). In addition, liquid scintillation counting was employed [6, 10]. The following buffers were used: A. 0.1 M sodium phosphate, pH 6,4; B. 0.05 M sodium phosphate, pH 7.1; C. 0.02 M Tris–HCl, pH 7.0; D. 0.2 M Tris–HCl, 2 mM $MgCl_2$, pH 7.5. E. 0.1 M potassium phosphate, pH 6.5.

Plant materials

Marine macroalgae were collected on the eastern mud flats of the North Sea island of Helgoland (Germany) and along the coasts of Neuharlingersiel, Norddeich and the island of Spiekeroog, all in the North Sea, during the summer seasons of 1993 and 1994. Algal species were determined as described [11–13]. Antarctic algae were provided by Dr. C. Wiencke of the Alfred-Wegener-Institute for Polar and Marine Research in Bremerhaven (Germany). Other plant species used were mainly collected in the region of Murnau and Garmisch-Partenkirchen (Bavaria), or donated by the Botanical Gardens, Munich [10]. Plant species were determined as described [14, 15]. Plant cell suspension cultures were maintained as previously described [16].

Enzyme preparation

Three existing methods [17–19] were combined in order to prepare microsomal and soluble enzymes in the same preparation. Enzyme extraction was performed at 4°C. Plant material (cultured cells, whole algae or leaves), usually 25–30 g fresh weight, was ground to a fine powder with liquid nitrogen in a mortar. This powder was transferred to another precooled mortar and extracted for 10 min with 50 ml buffer A, containing 20% (w/v) glycerol, 14 mM DTE, 20 mM ascorbic acid, 1% insoluble PVP (pre-swollen in water), 1 mM EDTA and 1 mM PMSF, final pH 6.0. After passing the extract through a 50 μm nylon net, cell debris was removed by centrifugation at $10{,}000 \times g$ for 20 min. In order to remove phenolic compounds, 10% (w/w) Amberlite XAD-4 was added to the supernatant while stirring. After a second filtration step, the extract was adjusted to pH 7.0 and centrifuged at $100{,}000 \times g$ for 60 min. The supernatant was defined as the soluble fraction, the pellet representing the microsomal fraction. In order to remove inhibitors, soluble enzymes were precipitated from the initial supernatant by solid ammonium sulfate fractionation. The pellet of the 35–80% ammonium sulfate fraction was suspended in 1.5 ml buffer C, containing 5 mM DTE, followed by desalting on Sephadex G-25 (PD-10). The soluble fraction was immediately assayed for enzyme activities. Protein was determined using bovine serum albumin as a protein standard [20].

Enzyme assays

The *O*-, *N*- and *S*-glucosyltransferase activities were assayed according to published procedures [6, 21]. Assay mixtures contained 30 μl buffer D (*O*-GT assays) or buffer E (*N*- and *S*-GT assays), 20 or 40 μl of 20 mM UDP-glucose, 10 μl ethanolic substrate solution (see below) and 100 μl enzyme (0.3–0.8 mg protein/ml). Salicin (2-hydroxymethylphenyl-β-D-glucopyranoside) and 4-nitrophenyl-β-D-glucopyranoside (20 μl each of 12 mM stock solutions) were routinely included in order to protect the products from degradation by endogenous β-glucosidases [21]. The final test volume was 200 or 220 μl.

The substrate stock solutions in ethanol had the following concentrations: 0.2 mM [^{14}C-]DCA, 0.4 mM [^{14}C-]PCP, [^{14}C-]DDA, 0.9 mM 2,3,4- 2,4,5-TCP, CA and 4-chlorothiophenol). Unlabelled UDPG (20 mM; 20 μl) was used for the [^{14}C]-labelled aglycones (PCP, DDA, DCA), while [^{14}C]-UDPG (20 mM) was used with non-radioactive aglycones (2,3,4- and 2,4,5-TCP, IAA and 4-chlorothiophenol; 40 μl) and CA (20 μl). A 100 μl aliquot of enzyme solution was generally used except for the two chlorinated anilines (50 μl enzyme).

Samples were incubated for 60 min at 30°C. The reactions were stopped by adding 10 μl 1 M H_3PO_4 (*O*-GT assays), and ethylacetate (400 μl for *O*-GT and *S*-GT assays; 200 μl for *N*-GT assays), followed by shaking for 20 min at room temperature. After centrifugation ($5000 \times g$, 5 min) for phase separation, quantification of products was done by TLC of the organic phase, and by liquid scintillation counting of the organic and aqueous phases. Specific activity is expressed as pkat/mg protein. Values are generally given as means $\pm$ SD derived from three replicate determinations.

Product identification by TLC was adopted from previous reports [6, 21]. The product of PCP conjugation was located at R_f 0.35, of 2,3,4-TC and 2,4,5-TC at R_f 0.45–0.47, of DDA at R_f 0.50–0.57 and of IAA at R_f 0.44 (all in solvent system 1). DDA-acylglucoside migrated at R_f 0.5–0.57 (solvent system 2). Further product R_f values in solvent system 1 were 0.06 and 0.05 for CA and DCA, and 0.74 for 4-chlorothiophenol, respectively. The latter conjugate was further characterized by its complete cleavage by crude almond β-glucosidase (Sigma, G 0395).

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