GLUCURONIDATION OF HYDROXYLATED POLYCHLORINATED BIPHENYLS (PCBS)

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Introduction

Some PCB congeners undergo phase I metabolism to form hydroxylated metabolites, that are not readily excreted.[1] Though these metabolites would be expected to be more hydrophilic, compared to the parent compounds, the estimates of their log P values suggests these compounds are still lipophilic enough to be retained in the cells. For example, the estimated log P value for 4-OH-3,3',4',5-tetraCB is only 0.4 log values lower than the parent compound,[1] hence it would not be expected to be easily eliminated. Unconjugated hydroxylated metabolites of PCB 77 (3,3',4,4'-tetraCB) including 6-OH, 5-OH, and 4-OH tetrachlorobiphenyls have been detected in liver and adipose tissue [2] suggesting their inability to be further conjugated. Hydroxylated PCBs have also been detected in the blood from wildlife as well as from humans.[3,4] Some metabolites like the 4-hydroxy metabolites of PCB 77 and PCB 105 (2,3,3',4,4'-pentaCB) found in the blood samples of experimental animals,[5]show a strong affinity for serum proteins.[6,7]

The present study was undertaken to investigate if PCBs that persist in the body are poor substrates for the phase II enzyme-UDPGT and whether the chlorine substitution pattern of hydroxylated PCBs is a factor affecting their rate of glucuronidation. Compounds synthesized for this purpose included some of the PCB metabolites found in the blood as well as some metabolites with a lower chlorine substitution pattern which are expected to be excreted with relative ease. Enzyme kinetics V_{max} and K_m were determined for each of the substrate and the efficiency with which they undergo glucuronidation (V_{max}/K_m) was calculated.

Materials and Methods

Hyroxylated PCBs were synthesized and characterized as described[3,8,9,10]. Male Wistar rats from Harlan Sprague Dawley Inc. (Indianapolis, IN), 6 to 7 weeks old, were housed in controlled environment with free access to food and water. Animals were treated intraperitoneally with sodium phenobarbital in saline (400 μmol/kg) each day for 3 days to increase the specific activity of microsomal UDPGT. 24 hours after the last injection, animals were euthanized, livers were excised, and microsomes prepared. Microsomal pellets were washed twice with cold sucrose/EDTA solution and resuspended in that solution to a final protein concentration of 10 mg/ml after pooling the microsomes from 6 rats.

Enzyme assay

Enzyme activity was measured using the method of Bansal and Gessner[11] with modifications. Briefly, microsomes (200 μg protein) were treated with Brij 58 (Brij : protein, 0.25 w/w) for 20 minutes at 0 °C. Incubation mixtures were prepared containing 100 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$

, 4 mM non-radiolabeled UDPGA, 0.1 µCi [^{14}C]UDPGA, 5 mM saccharolactone and the aglycone (final concentrations ranging from 0.04 mM to 4 mM) in 2 µl ethyl acetate. The mixtures and the activated microsomes were incubated for 2 minutes at 37 °C and the reaction was started by addition of the activated microsomes to the incubation mixture. Final volume of the reaction mixture was 100 µl. After incubation for 10 minutes at 37 °C in a shaking water bath, the reaction was stopped by adding 200 µl of absolute alcohol at –20 °C. Tubes were centrifuged, protein pellet discarded, and the supernatant was further analyzed. Zero time blanks were conducted by adding 200 µl cold alcohol to the reaction mixture prior to addition of microsomes. Control blanks were also carried out to account for background activity due to glucuronidation of endogenous compounds in the microsomal preparation by substituting the aglycone in the incubation mixture with 2 µl of ethyl acetate.

Statistical calculations

Analysis of Variance (ANOVA) was conducted to determine statistical significance. Comparisons among treatment groups, wherever necessary, were carried out using Bonferroni post-hoc procedures. All statistical tests were performed using SYSTAT software (version 8.0) for Windows (SPSS Inc., Chicago, IL.) and the level of significance a was 0.05 for all statistical testing. Regression analysis was performed using SAS program (PROC General Linear Model).

Results and Discussion

Reaction conditions were determined for optimal product formation in the assay. Incubation conditions were not optimized to linearity for every PCB tested, but 4-OH-biphenyl was used as a surrogate for this purpose. Plots of glucuronide formed vs time and protein were linear under the assay conditions. 5 mM saccharolactone was used to inhibit the breakdown of the glucuronide. For evaluation of optimal concentration of the cofactor UDPGA, enzyme activity as a function of UDPGA concentration at a constant aglycone concentration was measured. The aglycone used was 4-OH-biphenyl. Enzyme activity remained almost constant at concentrations of UDPGA 2 mM and above. No statistically significant difference in activity was observed at UDPGA concentrations greater than 1mM. 4 mM UDPGA was used in all incubation mixtures to ensure a saturated concentration of the cofactor.

Activation of UDPGT by two non-ionic detergents (Triton X-100 and Brij 58) was investigated also using 4-OH-biphenyl as the aglycone. Brij 58 was deemed to be better of the two detergents and therefore used in the assay at an optimal ratio of 0.25 Brij : protein (w/w). After hydrolysis with b-glucuronidase, a decrease in radioactivity was observed in the region corresponding to the glucuronides, while hydrolysis with buffer alone did not indicate a similar decrease, confirming that the reaction product was sensitive to b-glucuronidase activity, and was indeed a glucuronate.

UDPGT activity for each PCB was determined in triplicate at all concentrations. Background enzyme activity calculated from the assay blanks for control incubations performed without the aglycone in the incubation mixture was subtracted from the total enzyme activity at each concentration. V_{max} and apparent K_m were calculated from the Michaelis-Menten plots using Prism Graphpad (version 3.0) software, and the efficiency of the enzyme for glucuronidation was compared after determining the V_{max}/K_m ratios of each of the PCBs. For the higher chlorinated compounds (4-7 chlorine atoms) that have been detected in mammalian tissues, the V_{max} ranged from 0.3 – 6 nmols/min/mg. Lower chlorinated compounds (1-3 chlorine atoms) exhibited a V_{max} in the range of 6 - 31 nmols/min/mg. The K_m for these compounds did not vary to the same extent as their V_{max},, so their efficiency for glucuronidation (i.e. V_{max}/Km) followed the same pattern as their V_{max}.

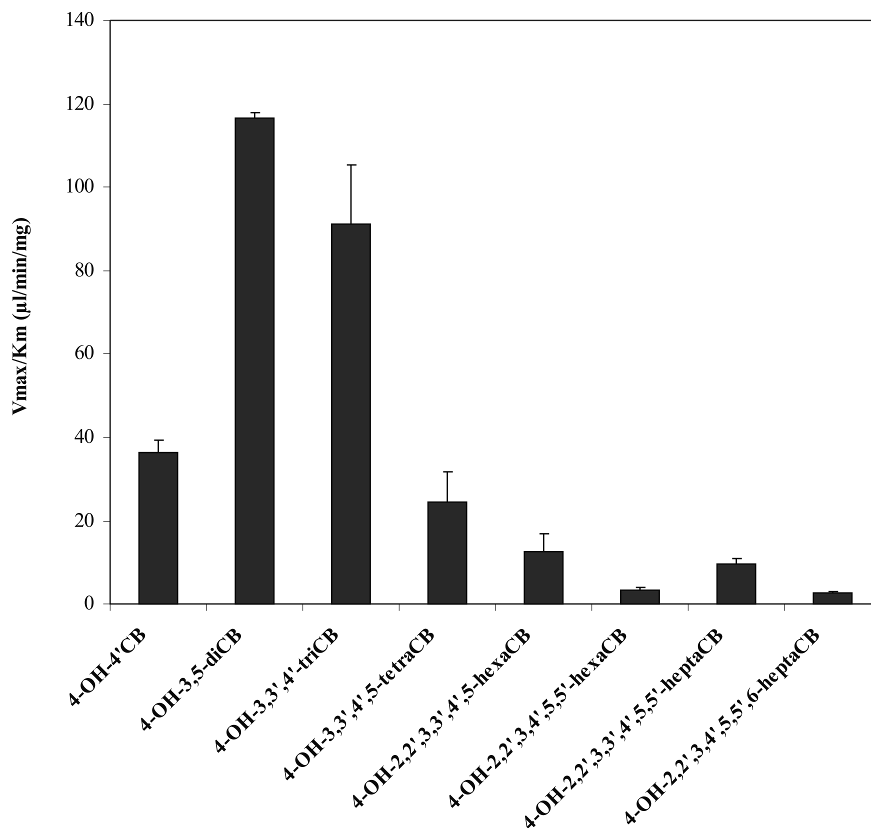

Figure. Efficiency of glucuronidation of selected hydroxylated PCBs. Experiments were performed in triplicate as described under materials and methods, using increasing concentrations of the aglycone in presence of 4 mM UDPGA. Results are means ± standard deviations.

We found that PCB metabolites with 6-7 chlorine atoms, having higher log P and log D values, are poorly conjugated. Hence lipophilicity may not be a crucial factor influencing the rate of glucuronidation. This study reveals that some PCB metabolites, especially those with substitution in positions meta- and para- to the dihedral bond are poor substrates for UDPGT and resist conjugation. This may explain in part why some PCB metabolites persist in the body.

Acknowledgements

This research was made possible by a grant (P42 ES 07380) from NIEHS. Contents reflect the views of the authors and do not represent any official view(s) of NIEHS or NIH.

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