PCB Genotoxicity: Activation and DNA Binding

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Introduction

Polychlorinated biphenyl (PCB) toxicity and carcinogenicity have been extensively studied, but there is no consensus regarding the mechanism(s) by which these environmental pollutants exert their toxic and carcinogenic activities [1]. Hesse and coworkers [2] suggested that the mechanism of PCB toxicity, analogous to that of *o*-phenylphenol and benzene, is characterized by the formation of dihydroxy metabolites which are further oxidized to semiquinones/quinones. The metabolic conversion of PCBs to catechol and hydroquinone metabolites has been reported [3,4]. The goal of the present paper was to determine if oxidation of catechols and hydroquinones to quinoid species could be responsible for the genotoxicity of PCBs. We report here the covalent binding of lower chlorinated biphenyls to DNA and the detection of these adducts using ^{32}P-postlabeling.

Materials and Methods

Materials

2-Chloro-, 3-chloro-, 3,4-dichlorobiphenyl were purchased from Riedel-deHaën AG (Seelze-Hannover, Germany) and 3,4,5-trichlorobiphenyl was synthesized as described [5]. $NADP^+$ (sodium salt), glucose-6-phosphate, glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and horseradish peroxidase (HRP)(EC 1.11.1.7, Type VI) were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals and PEI-cellulose plates used in the ^{32}P-postlabeling assay have been described elsewhere [6,7]. Microsomes were prepared from livers of male Sprague-Dawley rats [8].

Microsome-Mediated Metabolite Production

PCB congeners (0.2 -1mM) were incubated in 40 mM Tris-HCL buffer, pH 8.0 containing 2 mM $MgCl_2$, 1 mg/mL microsomes from rats treated with phenobarbital and 3-methylcholanthrene and a regenerating system consisting of 5 mM glucose-6-phosphate, 0.5 mM $NADP^+$ and 1.0 unit/mL glucose-6-phosphate dehydrogenase; final volume 5 mL. After incubations at 37° C for 1 h, 12 aliquots of 400 uL were removed and extracted with diethyl ether. The ether extracts were evaporated to dryness. Control reactions were processed in parallel in the absence of a regenerating system or the PCB congener.

Reaction of DNA or Mononucleotides with PCB Metabolites in the Presence of Horseradish Peroxidase

The sample residues were dissolved in 400 uL of 5 mM sodium citrate containing 1% DMSO, 150 ug DNA or 25 ug of dGp or dAp, 0.03 units of HRP and 0.5 mM of H_2O_2. After incubation at 37°C for 4h, the reaction mixtures were extracted with diethyl ether. DNA was further purified by solvent extractions and ethanol precipitation [7]. Control reactions were processed in parallel in the absence of DNA.

DNA Adduct Analysis

DNA adducts were analyzed by the nuclease P1 and butanol enhanced enrichment of the ^{32}P-postlabeling assay as described [7] except for TLC solvents which were as follows: D1 (oppcsite D3), 1.0 M sodium phosphate, pH 5.7; D3, 0.6 M lithium chloride, 0.37 M Tris-HCl and 5.2 M urea pH 8.0; D4, isopropanol, 4 M ammonium hydroxide, (1.4:1); and D5, 2.3 M sodium phosphate, pH 5.7; both D4 and D5 were run 2-3cm onto a Whatman No. 1 paper wick attached to the top (D3 was omitted with the 3,4-dichlorobiphenyl derived adducts). The adduct levels were calculated using a Packard Image Analyzer.

Results and Discussion

Comparison of adduct patterns and recoveries with nuclease P1 and butanol enrichment

Due to the variation of recovery of adducts with different enrichment procedures of the ^{32}P-postlabeling assay [9,10] and the lack of characterization of PCB adducts a comparison of butanol and nuclease P1 enrichment was done to choose a suitable enrichment procedure for continued studies. These procedures showed similar adduct patterns but different adduct recoveries, depending upon the nature of the chlorobiphenyls: adduct levels derived from monochlorobiphenyls were 2-3 fold higher with butanol, indicating partial susceptibility of these compounds to 3'-dephosphorylation activity of nuclease P1. The di- and trichlorobiphenyl adducts however, were recovered 5-7 times more with nuclease P1 than with butanol (Figure 1). All subsequent experiments were optimized with nuclease P1 or butanol depending on the PCB to ensure maximum recovery of adducts.

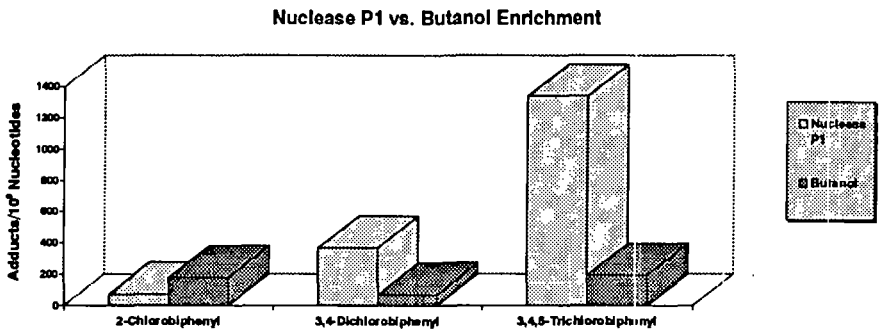

Figure 1

Microsome/peroxidase catalyzed DNA adducts of lower chlorinated biphenyls

Analysis of adducts derived from reacting the microsome mediated metabolites of 2-, and 3-monochloro-, 3,4-dichloro-, and 3,4,5-trichlorobiphenyls with DNA in the presence of HRP produced 2 to 3 major and several minor adducts with DNA. The binding seemed to increase with increasing chlorine content: 2-chloro- (150-250 adducts/10^9N), 3,4-dichloro- (250-450 adducts/10^9N) and 3,4,5-trichlorobiphenyl (1200-1500 adducts/10^9N) (Fig 2)

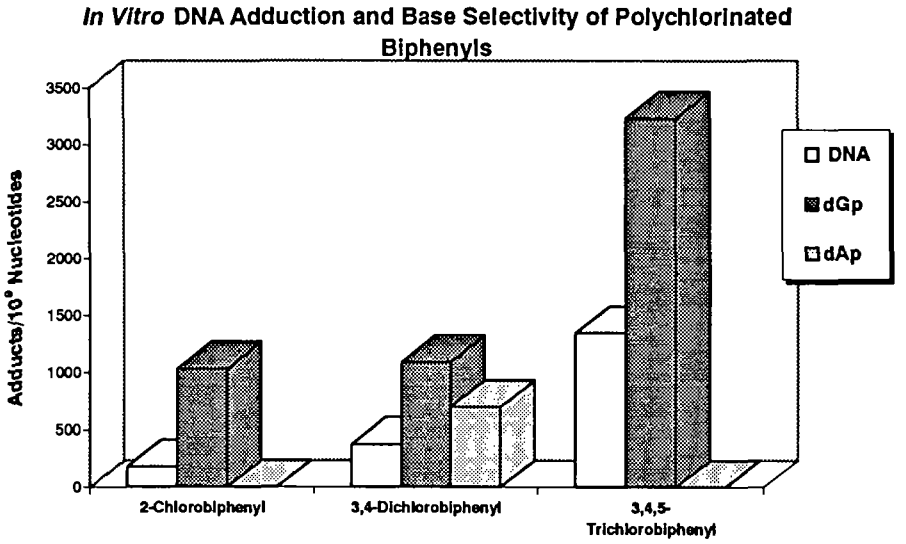

Figure 2

We also examined the formation of adducts in DNA reacted with lower chlorinated biphenyls in the presence of a NADPH-dependent microsomal activation system . No unique adducts were produced that could be attributed to epoxide formation (data not shown). Although adducts of weak intensity were formed, presumably due to auto-oxidation of the hydroquinone-like metabolites, they were identical to the adducts produced with the peroxidase activating system. In the absence of either the PCBs, the regenerating system or DNA, formation of adducts was not observed (Figure 3A).

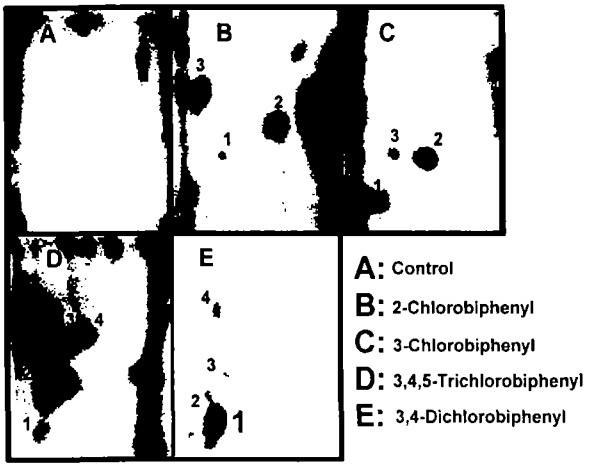

Figure 3

These data have important implications for understanding the mechanism of metabolic activation of PCBs to genotoxins. PCBs are first metabolized to phenolic compounds which can be further oxidized to dihydroxy metabolites by microsomal cytochromes P-450. The previous paper showed the formation of the 3',4' diol and the 2',5' diol as dihydroxy metabolites of the 4-chlorobiphenyl. We have also, identified the analogous metabolites for the 3,4-dichlorobiphenyl by GC-MS analysis. The hydroquinone like products are oxidized by peroxidases to quinones via semiquinone intermediates. These reactive metabolites bind to DNA as detected by ^{32}P-postlabeling (Figure 3). We propose the genotoxicity and potential mutagenicity of PCB-quinone-DNA adducts of the lower chlorinated biphenyls in conjunction with the promoting activity of higher chlorinated biphenyls may explain PCBs as complete carcinogens.

Acknowledgements

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