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The Redox Cycling of PCB Quinones

by

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Abbreviations

dGp – Deoxyguanosine 3'-monophosphate
HQ^- - Hydroquinone monoanion
H_2Q – Hydroquinone
Q – Quinone
$Q^{\bullet-}$ – Benzosemiquinone
GSH – Glutathione
$NADP^+$ - Oxidized nicotinamide adenine dinucleotide phosphate
NADPH - Reduced nicotinamide adenine dinucleotide phosphate
$O_2^{\bullet-}$ - Superoxide
PCB – Polychlorinated biphenyl
SOD – Superoxide dismutase

Outline

Abstract

Polychlorinated biphenyls (PCBs) are relatively stable compounds that have been used for decades for industrial purposes. Tons of PCBs have been inadvertently dumped into the environment. While they are typically biologically benign, lower chlorinated PCBs may be metabolized into PCB quinones by cytochrome P450. These quinones redox cycle, causing damage to cells by producing reactive oxygen species and forming adducts with nucleophilic substrates. Such adducts in DNA can be detected using ^{32}P labeling. Although PCBs are chemically stable, the have been shown to be a major health risk. Their metabolites, such as PCB quinones, may be the main cause behind the cellular damage.

Introduction

Polychlorinated biphenyls (PCBs) are compounds that exhibit thermal stability [1], are resistance to chemical decomposition [2], and are non-flammable [3]. Because of their stability they were used for many chemical and industrial applications, such as, insulation, hydraulic fluids, and sealants [4,5]. PCBs were discovered in 1881 and commercial production of these compounds began in 1929 [3]. For decades, PCBs were a major component of industrial business. In fact, more than 600 million kg were used in North America between the 1930s and 1970s [1]. A commercial blend of PCBs was trademarked Aroclor in the United States [1]. PCBs are not naturally found and were commercially produced by catalytically chlorinating of biphenyls with anhydrous chlorine [1,3].

In the 1960's people started realizing the environmental consequences of these compounds. Now PCBs can be found in lake and river sediment, surface water, vegetation, animal tissue, and even precipitation [2]. Because of their stability they are not being broken down in the environment. Rather, they can be taken up and metabolized within organisms. It is at this point that the PCBs become toxic. One subclass of PCB metabolites is PCB quinones. These compounds play an important role in the redox environment of the cells that contain them. This paper will focus on the structure of these PCB quinones, as well as, their formation and redox cycling, role in cellular damage, and health risks.

Structure

As their name indicates, polychlorinated biphenyls are two phenylic rings with between 1 and 10 chlorines around the rings (**Figure 1a**) [3]. Their chemical structure is $C_{12}H_{10-n}Cl_n$ [1,3].

Any combination of chlorines around the biphenyl ring is possible, giving rise to 209 different isomers of PCBs [1,2,3]. PCB quinones have a quinone structure on one of the rings in either the *para* or *ortho* form (**Figure 1b**). In crystal form, the dihedral angle between the phenyl rings is 37°. From this it is calculated to be 48° in aqueous solution [4].

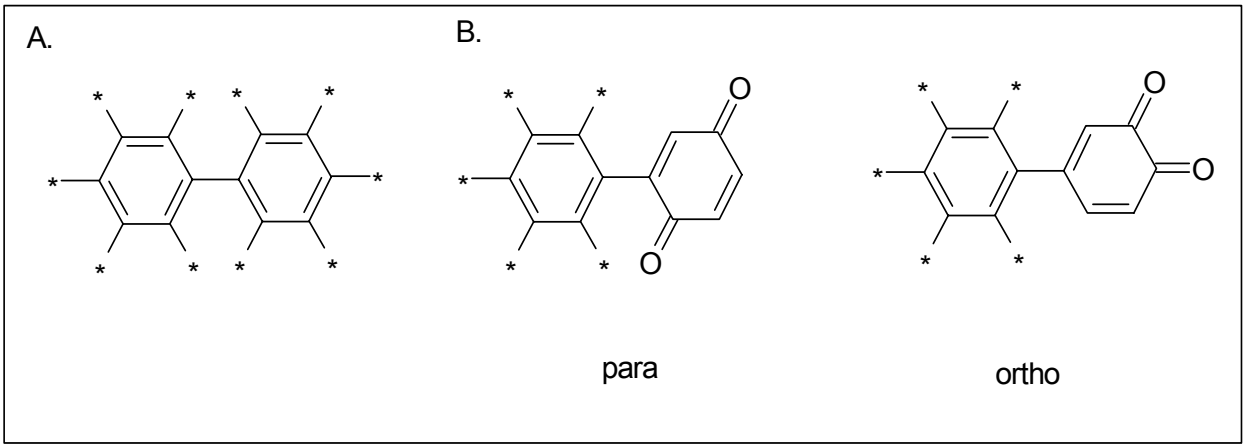

Figure 1. A. General structure of PCBs. * respresents either a chlorine or a hydrogen atom. B. *para* and *ortho* structures of PCB quinones.

Chemistry

Metabolism of PCBs to PCB quinones

Cytochrome P450 is able to catalyze the oxidation of lower chlorinated PCBs (three or fewer chlorines) to PCB quinone metabolites [2,4-6]. In general, cytochrome P450 causes hydroxylation of its substrate by binding oxygen and releasing water (reaction 1) [7].

$$RH + O_2 + NADPH \xrightarrow{P450} ROH + H_2O + NADP^+ \quad (1)$$

Two hydroxylations of the PCB by cytochrome P450 puts two hydroxyl groups on the unchlorinated phenyl ring, creating benzohydroquinone. Although either the *para* or *ortho* form can be made, the *para* form is produced 7 to 10 times more often than the *ortho* form [6]. PCBs

with four or more chlorine atoms are not easily metabolized [6]. Highly chlorinated PCBs have been hypothesized to undergo dechlorination to lower chlorinated PCBs in order to be hydroxylated by cytochrome P450 [2]. **Figure 2** illustrates the dechlorination and metabolism of PCBs.

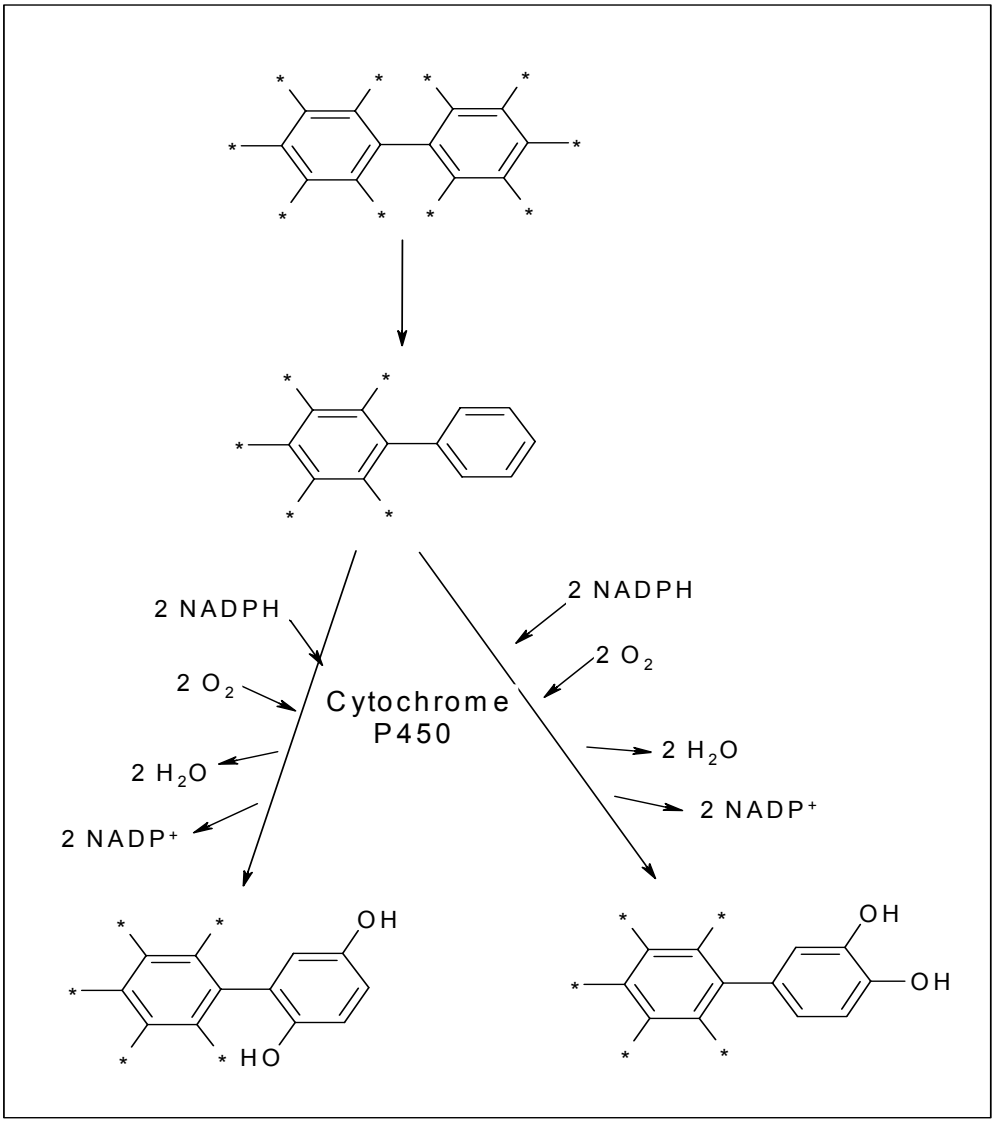

Figure 2. Metabolism of PCBs to PCB hydroquinone. Highly chlorinated PCBs are dechlorinated to lower chlorinated PCBs. The lower chlorinated PCBs are hydroxylated by cytochrome P450 to either *para* or *ortho* benzohydroquinones.

Redox cycling of PCB quinones

Redox cycling of the PCB quinones leads to the consumption of oxygen and NADPH, as well as, the formation of superoxide and hydrogen peroxide. This redox cyling of the PCB quinones produces much of the damage caused by PCBs.

The hydroquinone (H_2Q) can autoxidize with oxygen to generate a quinone (Q) at a rate of 750 M^{-1} s^{-1}, or a semiquinone ($Q^{\bullet -}$) (reaction 2 and 3) [6].

$$H_2Q + 2O_2 \leftrightarrow Q + 2O_2^{\bullet -} + H^+ \qquad (2)$$

$$H_2Q + O_2 \leftrightarrow Q^{\bullet -} + O_2^{\bullet -} + H^+ \qquad (3)$$

Benzosemiquinone will also autoxidize with O_2 to form superoxide and Q (reaction 4) [5].

$$2Q^{\bullet -} + O_2 \leftrightarrow Q + O_2^{\bullet -} + H^+ \qquad (4)$$

This reaction is reversible and the reduction potential of Q/ $Q^{\bullet -}$ is +99 mV. In saturated air, the reduction potential of $O_2/O_2^{\bullet -}$ is -214 mV [7]. Studies have shown that the increase in superoxide due to autoxidations of these quinones leads to increases in superoxide dismutase (SOD) levels [5,7]. Amaro *et al.* found that 2-(3',4'-dichlorophenyl)-1,4-benzohydoquinone was oxidized more in the presence of SOD [5]. This is because the removal of superoxide by SOD will cause a change in the equilibrium of H_2Q's autoxidation, shifting the balance of reactions 2 and 3 to the right [6]. Because the autoxidation of H_2Q is relatively slow, H_2Q may react with Q to form $Q^{\bullet -}$ (reaction 5) [5].

$$Q + H_2Q \leftrightarrow 2\ Q^{\bullet -} \qquad (5)$$

In this reaction, one Q is sacrificed to make 2 $Q^{\bullet -}$s. However, the autoxidation of $Q^{\bullet -}$ is much higher than that of H_2Q, therefore, quinone pools will be replenished quickly, *via* comproportionation [6].

The monoanion form (HQ^-) is also involved in the quinone redox cycling and will react with superoxide to form hydrogen peroxide and $Q^{\bullet-}$ or with O_2 to form Q and $O_2^{\bullet-}$ (reaction 6 and 7) [6].

$$HQ^- + O_2^{\bullet-} + H^+ \rightarrow Q^{\bullet-} + H_2O_2 \qquad (6)$$

$$HQ^- + O_2 \leftrightarrow Q + 2\ O_2^{\bullet-} + H^+ \qquad (7)$$

Like H_2Q, HQ^- will also comproportionate with Q, however, there is an extra proton in the products (reaction 8) [6].

$$HQ- + Q \leftrightarrow 2Q^{\bullet-} + H^+ \qquad (8)$$

NADPH is able to directly reduce Q to H_2Q by two-electron reduction. This process is thermodynamically favorable, as the two-electron reduction potential of Q/H_2Q is +280 mV, as opposed to -320 mV for NADP+/NADPH [6]. If the reactant stoichiometry has a molar ratio of 10:1 (NADPH:Q), then Q will be completely reduced within 20 min [6].

Damage due to PCB quinones

PCBs contribute to cellular damage by injuring cellular components through oxidative stress. As previously discussed, redox cycling of PCB quinones lead to the formation of superoxide and hydrogen peroxide. The PCB quinones typically attack nucleophilic targets and covalently bind to them [2]. Lower chlorinated PCBs that have been enzymatically oxidized can bind to DNA, preferentially guanine [5,8,9]. PCB quinones also bind to glutathione (GSH) within the cell. This lowers available GSH levels to detoxify the cell. Also, glutathionyl-hydroquinone conjugates have been shown to redox cycle and form ROS [5]. Although PCB quinones have an affinity for nucleophiles, they react very slowly with nitrogen rich amino acids, k is between 7.5×10^{-3} and 1.25×10^{-2} M^{-1} s^{-1} [5]. At physiological pH, the amino acids

containing nitrogens in their side chains are mostly in their protonated form and are not very nucleophilic. However, PCB quinones react instantaneously with sulfur containing amino acids forming an adduct [5].

Health Risk

PCB exposure has been related to numerous health problems. Animals were shown to have an LD_{50} for PCBs that ranged between 0.5 g kg^{-1} and 11.3 g kg^{-1} of body weight [1]. Because PCBs are highly fat soluble, they can accumulate in adipose tissue [1]. PCBs can also build up in the liver, the organ used for detoxification. PCB levels in the liver are two times higher that the rest of the body [3]. This leads to hepatotoxicity and an increased risk of hepatitis [1,6]. The DNA damage caused by the PCB quinones can lead to carcinogenesis [6]. PCB also cause damage to the nervous system and immune system [1,6], and cause disruption in hormones [6]. Lesser health risks include skin lesions, weight loss, and fatigue [1].

Detection

Several common methods of detection can be used to detect PCBs, such as NMR, gas chromatography/mass spectrometry [2,5], and UV spectrophotometry at 380 nm [5,10]. The use of ^{32}P labeling allows researchers to detects the formation of PCB-DNA adducts. In this method, PCBs are incubated with deoxyguanosine 3'monophosphate (dGp) for 1 h. Then, half of the samples are incubated with horseradish peroxidase and H_2O_2 for 30 min. The adducts are then labeled with ^{32}P, separated using chromatography, and visualized with autoradiography (**Figure 3**) [10]. This technique can be used to compare the abilities of different PCBs to form DNA adducts.

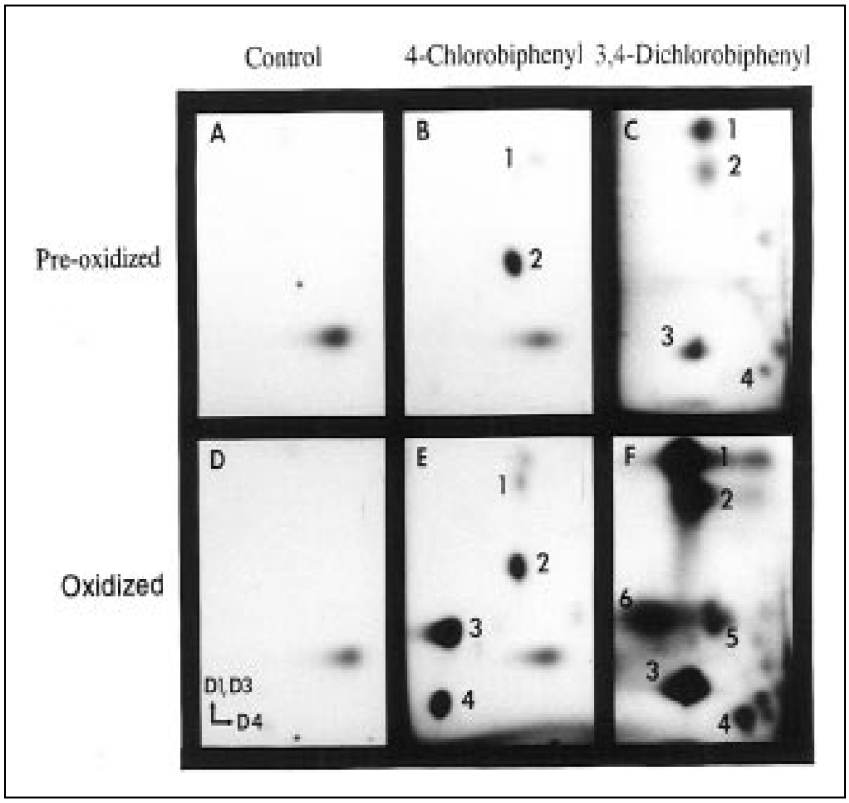

Figure 3. Chromatograms of ^{32}P postlabeled PCB adducts of dGp. A and D. DMSO control. B and E. 4-Chlorobiphenyl. C and F. 3, 4 - Dichlorobiphenyl. Oxidized samples were incubate with 0.5 U of horseradish peroxidase and 1 mM H_2O_2 for 30 min. The chromatogram was resolved in 2-propanol/4 M ammonium hydroxide (1:1). The ^{32}P adducts are shown as an autoradiograph [10].

Conclusion

PCBs are relatively benign compounds until they are metabolized into PCB quinones by cytochrome P450. They are then able to redox cycle within the cell, using reducing equivalents such as NADPH and forming reactive oxygen species. PCB quinones cause damage to the cells directly by binding to nucleophilic substrates such as, proteins or nucleic acids. Their redox cycling also causes oxidative stress within the cells. One of the qualities that made the industrial use of PCBs so attractive was their chemical inertness. However, PCBs have been linked with numerous health defects. This injury is mainly caused by PCB metabolites, namely, PCB quinones.

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