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Reductive *ortho* and *meta* Dechlorination of a Polychlorinated Biphenyl Congener by Anaerobic Microorganisms

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We used gas chromatography-mass spectrometry to study the metabolic fate of 2,3,5,6-tetrachlorobiphenyl (2356-CB) (350 μM) incubated with unacclimated methanogenic pond sediment. The 2356-CB was dechlorinated to 25-CB (21%), 26-CB (63%), and 236-CB (16%) in 37 weeks. This is the first experimental demonstration of *ortho* dechlorination of a polychlorinated biphenyl by anaerobic microorganisms.

The fate of polychlorinated biphenyls (PCBs) in aquatic sediments is a public concern because PCBs are relatively persistent and tend to bioaccumulate. Environmental dechlorination of PCBs via losses of *meta* and *para* chlorines has been reported for freshwater, estuarine, and marine sediments, including those from the Acushnet Estuary, the Hudson River, the Sheboygan River, and Waukegan Harbor (2–5), and via *ortho*, *meta*, and *para* dechlorination in Silver Lake (Pittsfield, Mass.) (5). Reductive dechlorination of PCBs by anaerobic microorganisms from Hudson River and Silver Lake sediments has recently been confirmed in the laboratory, but only losses of *meta* and *para* chlorines were observed (1, 6–8). Here we report the first experimental demonstration of biologically mediated *ortho* dechlorination of a PCB and stoichiometric conversion of a PCB congener to less-chlorinated forms.

Core samples (45 cm) of sediment were collected from the west side of Woods Pond (Lenox, Mass.), a shallow impoundment on the Housatonic River located 10.5 miles (ca. 16.9 km) downstream from Silver Lake. The pond's sediments are a mixture of black humic matter, sand, and silt contaminated with a hydrocarbon oil and Aroclor 1260. The sediment PCBs exhibited slight environmental dechlorination via loss of *meta* and *para* chlorines.

Methanogenic slurries were prepared under nitrogen in an anaerobic chamber by mixing wet sediment (2 volumes) with reduced anaerobic mineral medium (3 volumes) (9) and L-cysteine-HCl (0.1%). The slurries were dispensed into serum bottles, and 2,3,5,6-tetrachlorobiphenyl (2356-CB) (350 μM, 99% purity; AccuStandard, North Haven, Conn.) was added from a concentrated stock solution (70 mM in acetone). The bottles were crimp-sealed with teflon-lined butyl rubber septa (Wheaton). Sterile controls were prepared by sequential pasteurization (75°C, 20 min), incubation (23 to 25°C, 24 h), and autoclaving (121°C, 3 h). Duplicate samples and controls were incubated in the dark at 23 to 25°C. Aliquots (1 ml) of the slurries were sampled weekly and extracted by vigorous shaking (24 h) with anhydrous ether (5 volumes) and elemental mercury (1/4 volume, to remove sulfur) in vials with Teflon-lined foam-backed screw caps. Samples were analyzed by gas chromatography (GC) with an electron capture detector and a DB-1 capillary column (J & W Scientific; 30 m by 0.25-mm [inside diameter] by 0.25 μm) as previously described (5). PCBs that were formed as dechlorination products were initially identified by matching GC retention times with those of authentic standards (99% purity; AccuStandard). The identifications were subsequently confirmed by GC-electron capture detection with a C-87 capillary column (Chrompac; 60 m by 0.32 mm [inside diameter] by 0.2 μm) and by GC-mass spectrometry with a Hewlett-Packard 5890/5971A GC-mass spectrometer. An 18-point calibration curve (third order, not forced through zero) was used to determine the relative molar distribution of 2356-CB and its dechlorination products throughout the experiment.

Autoclaved controls (Fig. 1, top panel) showed no change throughout the experiment. Small amounts of three transformation products, tentatively identified as 25-CB, 235-CB, and 236-CB, were first detected at 21 weeks (Fig. 1, center panel). At later times a fourth product, identified as 26-CB, was also present (Fig. 1, bottom panel).

All transformation products were analyzed by GC-mass spectrometry to confirm their identifications. A sample containing the products tentatively identified as 25-CB, 26-CB, and 236-CB was ionized by electron impact (70 eV) and was scanned from 50 to 550 mass units. The observed molecular ions (*m/z* 222 and *m/z* 256), isotope patterns, and fragments (M^+, −70) were those expected for di- and trichlorobiphenyls, respectively. We confirmed our identification of 235-CB by selective ion monitoring at *m/z* 256. Collectively, these data indicate that the 2356-CB was reductively dechlorinated by anaerobic microorganisms in the sediment.

Figure 2 shows the relative molar distribution of 2356-CB and its dechlorination products over the course of the experiment. Small amounts of 25-CB, 235-CB, and 236-CB were first detected at 21 weeks. Both 25-CB and 236-CB increased with time, concomitant with a decrease in 2356-CB, but 235-CB never increased beyond 0.2 mol%. The 236-CB peaked at 64 mol% at 28 weeks, then steadily declined, and was replaced by 26-CB, which eventually increased to 58 mol%. Hence, the major route of dechlorination was 2356-CB → 236-CB → 26-CB (Fig. 3, pathway 1). There are several possible routes of formation of 25-CB. The simultaneous loss of both chlorines is theoretically possible, but there is no biological precedent for this type of dechlorination. It is more likely that the chlorines were removed sequentially by one or both of two possible routes: 2356-CB → 235-CB → 25-CB (Fig. 3, pathway 2) or 2356-CB → 236-CB (identical to 256-CB) → 25-CB (pathway 1A). To

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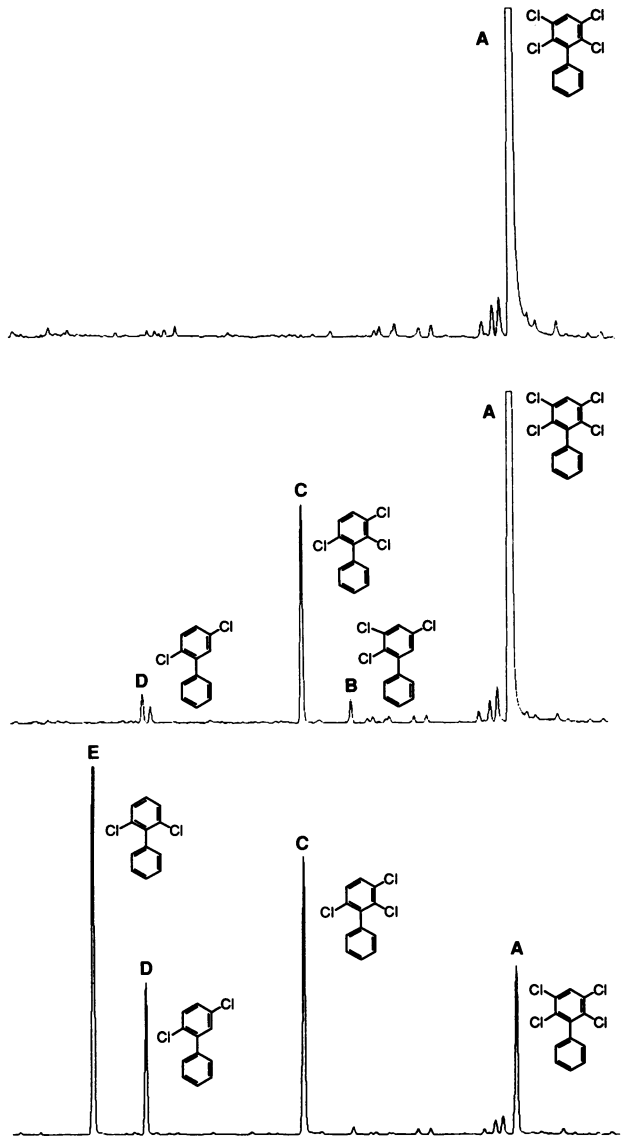

FIG. 1. GC-electron capture detector profile (DB-1 column) of 2356-CB and dechlorination products. (Top) Autoclaved control (37 weeks) showing 2356-CB. (Center) Live sample (21 weeks) showing 2356-CB (A), 235-CB (B), 236-CB (identical to 256-CB) (C), and 25-CB (D). (Bottom) Live sample (37 weeks) showing 2356-CB (A), 236-CB (C), 25-CB (D), and 26-CB (E). The vertical scale was 120 Hz for the top and center panels and 480 Hz for the bottom panel.

gain a better understanding of how the 25-CB was formed, the duplicate cultures were combined at 37 weeks and were then used to inoculate autoclaved sediment slurries. The slurries were amended with 2356-CB, 236-CB, or 235-CB (350 μM, 99% purity). Dechlorination of 236-CB to 26-CB was detected at 3 weeks and was complete at 9 weeks of incubation. No dechlorination of 2356-CB or 235-CB occurred despite incubation for 21 weeks.

Although our results do not unequivocally establish the route of formation of 25-CB, we favor pathway 2 for several reasons. (i) Low levels of 235-CB were detected as soon as dechlorination of 2356-CB began. (ii) The formation of

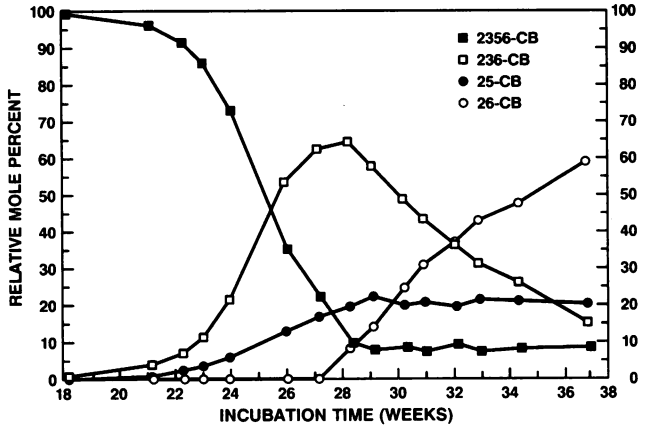

FIG. 2. Relative molar distribution of 2356-CB and its dechlorination products as a function of time. The data shown are from one of duplicate samples. The second sample was essentially identical except for a 1-week shift in the time frame.

25-CB appeared to be coupled to the depletion of 2356-CB (both ceased simultaneously at 29 weeks) but not to the presence of 236-CB. No 25-CB was formed during the last 8 weeks of incubation despite the nearly linear dechlorination of 236-CB to 26-CB. (iii) The transfer culture amended with 236-CB was dechlorinated exclusively to 26-CB. We have consistently seen the same transformation in unacclimated slurries of this sediment after only 2 to 3 weeks of incubation but have never observed the formation of 25-CB from

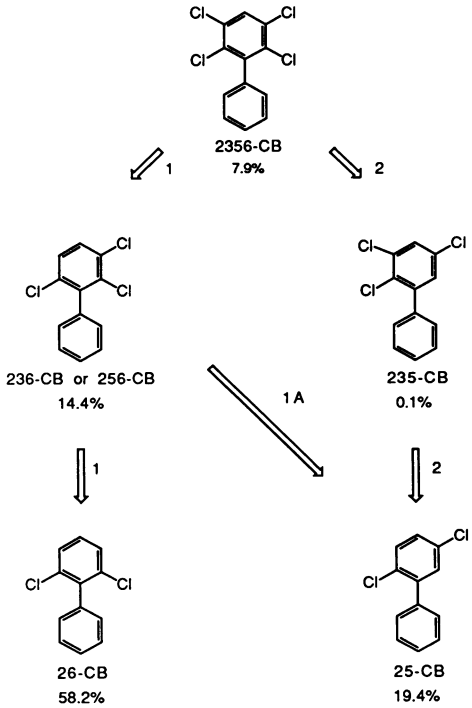

FIG. 3. Proposed routes of dechlorination of 2356-CB, showing all dechlorination products and giving their molar distribution at 37 weeks.

236-CB. (iv) In other experiments, we have observed the dechlorination of 235-CB to 25-CB in unacclimated slurries of this sediment after a long incubation (14 weeks).

Collectively, our observations support the proposal that the dechlorination of 2356-CB occurred in two separate stages. In the first stage (between 21 and 29 weeks of incubation), approximately 92% of the 2356-CB was dechlorinated; of this total, 79% was converted to 236-CB by loss of a *meta* chlorine and 21% was converted to 25-CB, via 235-CB, by sequential loss of an *ortho* chlorine and then a *meta* chlorine (Fig. 3). In the second stage, which began at 28 weeks, the 236-CB was rapidly dechlorinated to 26-CB. We speculate that these two stages may reflect a shift in the microbial population. We propose that the first population has a long acclimation time and can dechlorinate 2356-CB and 235-CB but not 236-CB. The second population has a shorter acclimation time and can dechlorinate 236-CB to 26-CB, but it has no activity against 2356-CB or 235-CB. This would explain why transfers of inocula collected at 37 weeks (stage 2) were able to dechlorinate 236-CB but not 2356-CB or 235-CB.

It has already been demonstrated that anaerobes from the Hudson River remove virtually all *meta* and *para* chlorines from Aroclors 1242 and 1248, leaving predominantly *ortho*-substituted mono- and dichlorobiphenyls (7). Our data clearly establish that anaerobic microorganisms from Woods Pond are capable of removing chlorine from the *ortho* position of at least one PCB congener. If this capability can be extended to 2-CB, 2,2′-CB, and 26-CB, the major products of PCB dechlorination by Hudson River microorganisms (2, 5, 7), then it may be possible to totally dechlorinate PCBs to biphenyl. Experiments with these congeners are in progress.

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