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Metabolism of Polychlorinated Biphenyls by Marine Bacteria[1]

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Due to the depth of the oceans and their dominance of the earth's surface, the oceans should be the final repository in the global cycling of polychlorinated biphenyls (PCBs). Since 1970 the marine organic geochemistry of PCBs has been studied at Woods Hole Oceanographic Institution (HARVEY and STEINHAUER 1976). During that time, analyses have shown the decline of PCB levels in North Atlantic Ocean water. This observation can be attributed to various causes, of which the most important is probably the decrease in commercial usage of PCBs (HARVEY et al. 1974). However, biological uptake of PCBs and their conversion to polar metabolites may also account for diminishing concentrations in Atlantic Ocean water (HARVEY and STEINHAUER 1976).

Previous laboratory studies of PCB metabolism include work on such diverse systems as rats (GOTO et al. 1974; SAFE et al. 1974; GREB et al. 1975a; JENSEN and SUNDSTROM 1974), rabbits (SAFE et al. 1975a; GARDNER et al. 1973; BLOCK and CORNISH 1959), birds (HUTZINGER et al. 1972), pigs (SAFE et al. 1975b), primates (GREB et al. 1975b; HSU et al. 1975), fish (MELANCON and LECH 1976), plants (MOZA et al. 1974), and microorganisms (MAAS et al. 1976; WALLNÖFER et al. 1973; AHMED and FOCHT 1973). Most of these studies report metabolic production of hydroxylated derivatives of various chlorobiphenyl isomers. Rats converted 4-chloro and 4,4'-dichlorobiphenyl to 4-chloro- and 4,4'-dichloro, 3-hydroxybiphenyl (SAFE et al. 1974). GREB et al. (1975a) found mono- and dihydroxyderivatives of 2,4'-dichlorobiphenyl and 2,5,2'-trichlorobiphenyl and trihydroxyderivatives of 2,5,2' trichlorobiphenyl in rats. A summary of the various laboratory studies of PCB metabolism is given by HUTZINGER et al. (1974).

There have been no reports of laboratory studies of PCB metabolism by marine organisms. A few workers have analyzed marine animals for products of PCB metabolism. A search for hydroxylated PCBs in marine fish proved inconclusive (ZITKO et al. 1974). Phenolic metabolites of PCBs have been identified in seals and guillemot (JANSSEN et al. 1975). PCBs that had been hydroxylated and excreted by marine organisms would most likely be found in the sediments, so in our laboratory we conducted a search for these compounds in marine sediments. Two kilograms of organic-rich surface sediment from Buzzards Bay, Massachusetts, were extracted.

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The phenolic fraction was isolated and analyzed by gas chromatography-mass spectrometry (GC-MS). Neither wide mass scans nor selected mass searches produced any evidence of hydroxylated PCB derivatives.

We felt that if any marine organisms were capable of metabolism of PCBs, some marine bacteria should have that capability. Thus we conducted a series of laboratory experiments to test this possibility. Reported here is our finding of PCB metabolism by marine bacteria in batch culture.

MATERIALS AND METHODS

2,5,2',5'-tetrachlorobiphenyl (ring-UL-^{14}C) specific activity 9.87 mCi/mM, and 2,5,2'-trichlorobiphenyl (2,5-ring-UL-^{14}C) specific activity 9.91 mCi/mM, were purchased from Mallinckrodt Radiochemical Department and purified by thin-layer chromatography on silica gel plates before use. 2,5,2',5'-tetrachlorobiphenyl and 2,5,2'-trichlorobiphenyl were purchased from Analabs (North Haven, Connecticut). ^{3}H-Aroclor 1254 (3.21 mCi/36.1 mg) was purchased from New England Nuclear (Boston, Massachusetts). Commercially distilled in glass solvents (Burdick and Jackson or Mallinckrodt) were used for all experiments. Thin layer chromatography plates used were plastic-backed J. T. Baker Silica Gel 1B or aluminum foil-backed Merck Silica Gel F-254.

Glassware for all experiments was washed with detergent, rinsed with tap and distilled water, then rinsed with ethanol and baked in a 100^{o}C oven for at least 8 hours.

Seawater was collected from local beaches in a sterile stainless steel bucket, and sediment was collected in a sterile glass beaker. Cultures were incubated in a 2 liter reaction vessel for anaerobic experiments and in 1 to 4 liter Erlenmeyer flasks or 5 gallon Pyrex carboys for aerobic experiments. Cultures for aerobic experiments were shaken on a gyratory shaker or stirred with a magnetic stirrer. Cultures were enriched with glucose, yeast extract or phosphate at various concentrations in sterile distilled water. When there was visible turbidity in the flasks (usually after 12 hours incubation), PCBs were added in ethanolic solution.

Samples were aseptically removed from cultures by pipetting, acidified to pH 2, and extracted with diethyl ether or methylene chloride. Water samples were extracted in separatory funnels and sediment samples extracted in Soxhlet extractors (Kontes K-292000). Extracts were concentrated to 0.1 ml in a Kuderna-Danish concentrator (Kontes K-570000) in a 80^{o}C water bath or under vacuum at 25^{o}C. Some organic extracts were then extracted with saturated aqueous $NaHCO_3$, the aqueous fraction acidified to pH 1 with HCl, and then extracted with diethyl ether or hexane. The carboxylic and lactone acids in this organic fraction were esterified by stirring for 24 hours with 6 ml methanol, 1 drop

concentrated HCl and 25 ml diethyl ether over Na_2SO_4. The ether was then extracted with 10 ml saturated aqueous $NaHCO_3$, and aliquots of both were counted in a Packard Tri-Carb liquid scintillation spectrometer.

Radiochromatograms of extracts were prepared by thin layer chromatography on silica gel plates developed in benzene, in which PCBs had an Rf of 0.75 and 2-chloro, 4-phenylphenol had an Rf of 0.3 (visualized by staining with iodine vapors). Starting at the origin, developed plates were cut into 1 cm sections; each section was dropped into a scintillation vial containing 10 ml Aquasol (New England Nuclear) and counted in a Packard Tri-Carb liquid scintillation spectrometer.

RESULTS

Anaerobic incubation. 770 grams (wet weight) of black marsh mud with a strong H_2S smell were incubated with 1300 ml raw seawater for 45 days in a two liter reaction kettle with 0.01 mCi labeled tetrachlorobiphenyl. At weekly intervals throughout the incubation, aliquots of water and sediment were withdrawn, extracted, and radiochromatograms prepared. None of the extracts showed radioactivity with Rf different from that of the tetrachlorobiphenyl. A representative chromatogram is shown in Fig. 1A.

At the end of the 45 day period, the entire enrichment was extracted. No radioactive compounds other than the parent molecule were detected.

Aerobic incubation. Two liters of raw seawater and 100 g sandy beach sediment were incubated in a flask into which a stream of sterile air was bubbled (the air was passed through a sterile cotton trap). Air leaving the flask was bubbled through an alkali trap followed by an ethylene glycol trap. The contents of the flask were stirred periodically with a magnetic stirrer. The flask was enriched with 150 mg yeast extract and 0.68 µCi labeled tetrachlorobiphenyl. Periodically aliquots of the water were removed, extracted and radiochromatograms prepared. The first indication of metabolism was seen after 10 days when there was some radioactivity remaining at the origin of the TLC plate.

The incubation was concluded after 25 days. The entire contents of the enrichment were acidified and extracted. One per cent of the recovered radioactivity was extracted into saturated aqueous $NaHCO_3$, recovered from the aqueous fraction and esterified, indicating that the metabolite was a carboxylic or lactone acid. The unesterified compound could not be extracted from aqueous NaOH, indicating that the compound was probably a lactone acid, rather than a carboxylic acid. No significant amounts of radioactivity were found in the alkali or ethylene glycol traps.

Seawater collected throughout the year was incubated with labeled tetrachlorobiphenyl. From 2 to 4% metabolism was always

detected by radiochromatography within three days after commencement of the experiment. Levels of metabolism did not increase after periods of incubation up to several weeks. Incubation of seawater samples without any sediment or without forceful aeration

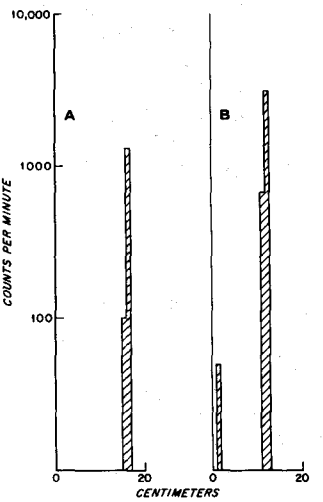

Fig. 1 A. Radiochromatogram prepared by thin layer chromatography on silica gel plate of a methylene chloride extract of seawater from anaerobic incubation of marsh sediment with 2,5,2',5'-tetrachlorobiphenyl (ring-UL-^{14}C) after 7 days incubation. Origin 1.5 cm, front 18 cm, benzene developer.
B. Radiochromatogram prepared by thin layer chromatography on silica gel plate of a diethyl ether extract of seawater from aerobic incubation of seawater with 2,5,2'-trichlorobiphenyl (2,5-ring-UL-^{14}C) after 5 days incubation. Origin 1.5 cm, front 15 cm, benzene developer.

(by bubbling of air) produced as much metabolism as samples with sediment or vigorous aeration. Thus in later experiments, samples were incubated in flasks with aluminum foil-covered rubber stoppers for closures eliminating extraneous apparatus. Attempts to enhance the amount of metabolism by addition of other nutrients, such as phosphate and glucose or by maintaining the pH at 7.8 were not successful. Autoclaved, uninoculated seawater samples incubated with labeled tetrachlorobiphenyl did not produce any new labeled compounds.

Attempts were made to identify the metabolite isolated from large scale incubations of 16 liters of seawater with 3.6 mg tetrachlorobiphenyl and 0.1 μCi labeled tetrachlorobiphenyl. Analysis by GC-MS proved unsuccessful because the compound decomposed during gas chromatography, as one might expect of a lactone acid.

Incubation of seawater samples with ^{3}H-Aroclor 1254 produced metabolism to the same extent as was seen with ^{14}C-labeled tetrachlorobiphenyl, determined by the recovery of labeled compounds in the extraction procedure. However, no changes were detected in gas chromatograms of the ^{3}H-Aroclor 1254, neither in number nor in the relative heights of peaks. If each of the isomers present in the Aroclor were metabolized to the small extent (1-4%) that the tetrachlorobiphenyl was metabolized, electron capture gas chromatography would not detect the small changes in absolute or relative amounts of the isomers, because it is not a sufficiently sensitive detection system.

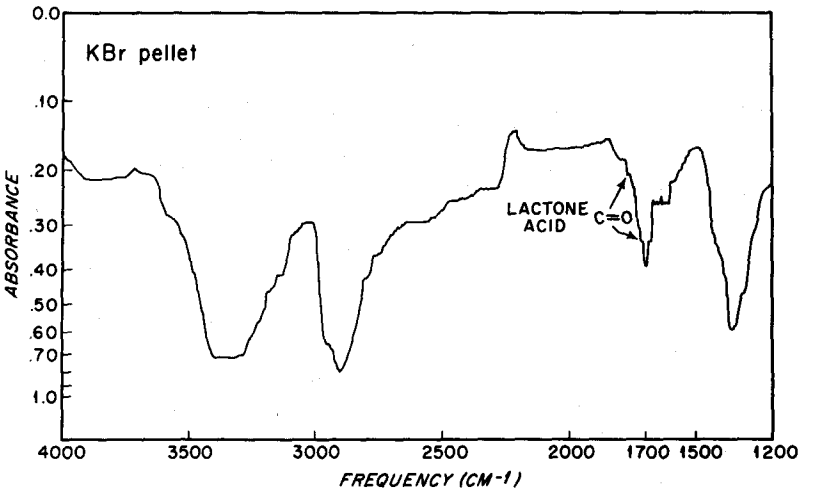

Fig. 2. Infrared spectrum (KBr pellet) of the diethyl ether extract of seawater from aerobic incubation of seawater with 2,5,2'-trichlorobiphenyl (2,5 ring-UL-^{14}C). Absorption bands at 1780 and 1730 cm^{-1} are typical features of γ-lactones.

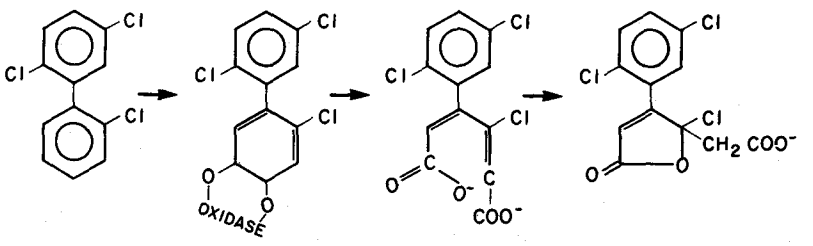

Fig. 3. Proposed pathway for metabolism of 2,5,2'-trichlorobiphenyl by mixed cultures of marine bacteria.

Incubation of 0.39 μCi labeled trichlorobiphenyl and 1.6 mg unlabeled trichlorobiphenyl with one liter of seawater in two separate experiments produced 1.4% metabolism. A radiochromatogram of an aliquot of the ether extract from one of these experiments is shown in Fig. 1B. The ether extract was mixed with finely ground KBr and the ether removed with a stream of nitrogen gas. An infrared spectrum (KBr pellet) of the concentrated extract shows (Fig. 2) absorption bands at 1780 and 1730 cm^{-1}, consistent with the C=O stretching of a lactone acid type of structure. In Fig. 3 is shown a proposed pathway for metabolism of 2,5,2'-trichlorobiphenyl to a lactone acid structure consistent with these findings.

DISCUSSION

Our results demonstrate that mixed cultures of nearshore marine bacteria are capable of metabolizing PCBs. We have isolated and partially characterized an acid lactone metabolite whose structure is unlike any thus far reported in PCB metabolism studies.

We found no metabolism in an anaerobic marine mud incubated with a chloribiphenyl isomer for 6 weeks. Consistent with this is the work of FRIES (1972) who found that chromatograms of Aroclor 1254, that had been incubated in silage that underwent normal anaerobic fermentation for several months were identical to chromatograms of standard Aroclor 1254. These results imply that in the large areas of anoxic sediments found in marshes, estuaries and basins like the Black Sea, PCBs will remain unaltered biologically or chemically. These anaerobic environments may then serve as long term sinks for PCBs.

Most reports of bacterial metabolism of PCBs have been conducted with organisms from sewage sludge (AHMED and FOCHT 1973; TUCKER et al. 1975) which were incubated aerobically. Positive results so obtained may not be relevant to the more usual anoxic conditions of sewage fermentation.

NISSEN (1973) found no evidence of microbial metabolism of Aroclor 1254 in soil samples incubated aerobically, by analyzing for CO_2 production and change in peak height ratios in gas chromatograms of extracts of the soil. We found no evidence of complete mineralization of PCB isomers to CO_2 in our work, and no detectable changes in chromatograms of ^3H-Aroclor 1254.

VOCKEL and KORTE (1974) incubated pure cultures of a variety of soil microorganisms with radiolabeled 2,2'-dichlorobiphenyl. They chose gross loss of label from the area of TLC plates where the chlorobiphenyl migrated as the criterion of metabolism. Their experimental design was not precise enough to detect 2 to 4% metabolism of the chlorobiphenyl. They reported no metabolism among the organisms tested. In addition, their use of pure

cultures minimized the chance of finding organisms capable of the initial ring opening, and did not allow for the possibility that a suite of organisms might be required for metabolism. More careful experimental design by future workers may substantiate our findings and lead to discovery of PCB metabolism by a wide variety of microorganisms.

The low level of metabolism and the failure to increase the total amount of metabolism after extended incubation in our studies may be due to specific inhibition of metabolism by the accumulated lactone metabolite. This phenomenon may be less important in the marine environment because the total amounts of metabolite produced might exist in low, steady state concentrations that would allow for turnover of a larger percentage of the PCBs present in the environment. Concentrations of up to 3.2 mg per liter PCBs were used in our studies to provide sufficient material for chemical analysis. These concentrations are several orders of magnitude higher than those now found in the oceans. Studies of PCB metabolism by mixed cultures of marine bacteria in a continuous culture system that supplied low, constant levels of PCBs might provide insight into how effectively this process is occuring in the marine environment.

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