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Dehalogenation of Lindane by a Variety of Porphyrins and Corrins

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The dehalogenation of lindane by a range of hemoproteins, porphyrins, and corrins has been tested under reducing conditions in the presence of dithiothreitol. In addition, a series of porphyrin-metal ion complexes have been prepared and have also been screened for the capacity to dehalogenate lindane. Hemoglobin, hemin, hematin, and chlorophyll *a* all catalyzed the dehalogenation of lindane, as did all of the corrins tested. The porphyrins which did not contain metal centers—coproporphyrin, hematoporphyrin, protoporphyrin, and uroporphyrin—were inactive. However, when these porphyrins were then complexed with Co, Fe, Mg, Mo, Ni, or V, lindane dehalogenation was observed. In all cases, the reaction proceeded by an initial dechlorination to produce tetrachlorocyclohexene, which was further dehalogenated to yield chlorobenzene as the end product.

Halogenated organic compounds may enter soil and aquatic environments by several routes. Such compounds are generated as waste products in large quantities by the chemical industry; disposal of these wastes is by incineration, which is expensive, or more commonly by landfill dumping, which, although inexpensive, introduces locally high concentrations of organochlorides into the environment. Organohalides in industrial wastes may also be released directly into surface waters. The deliberate use of organochlorides as pesticides has also increased the level of these compounds in the environment. Many of the compounds are extremely persistent in the environment by virtue of their halogen substitution. Therefore, systems which can remove halogen substituents may be beneficial in generating compounds which are more amenable to subsequent biodegradation.

Lindane (γ-hexachlorocyclohexane) is a broad-spectrum organochloride insecticide which has been in use since the 1940s. It has been used as a sheep-dip insecticide in many parts of the world (13). Additionally, it has also entered the aquatic environment where sheep fleece is processed. In Yorkshire, United Kingdom, lindane levels in surface waters have been reported in excess of the European Economic Community limits of 100 ng/liter by such routes of contamination (1).

The ability of iron-containing porphyrins, such as hemin, to dehalogenate organochlorides, such as DDT, is known (8, 14). The dehalogenation of the organochloride pesticides dieldrin and lindane by cobalamin has also been reported (2). Porphyrin-mediated dehalogenation appears to display a broad substrate specificity (12), much more so than is observed with enzyme-catalyzed dehalogenation. Although the wide substrate specificity of iron porphyrins has been established, little is known of the optimum metal ion-porphyrin combinations for these dehalogenations. The ability of porphyrins to dehalogenate a wide range of organochloride compounds may have potential for use as detoxification systems, e.g., to dehalogenate highly recalcitrant organochlorides in waste effluents to produce compounds which are more readily biodegradable.

To assess the potential of porphyrin- and corrin-based detoxification systems, a variety of hemoproteins, porphyrins, and corrins were assayed for the ability to catalyze the dehalogenation of lindane. In addition, a series of porphyrin-metal ion complexes were prepared and tested for their ability to degrade lindane as a means of determining which metal ions were most effective in the dehalogenation reaction.

MATERIALS AND METHODS

Chemicals. The chemicals used were obtained from the following sources: lindane, hematin, hemin, protoporphyrin, porphobilinogen, coproporphyrin, and uroporphyrin, Aldrich Chemical Co. Ltd., Gillingham, Dorset, United Kingdom; chlorophylls *a* and *b*, bacteriochlorophyll, cytochrome *c*, hemocyanin, hemoglobin, cyanocobalamin, adenosylcobalamin, and cobinamide dicyanide, Sigma Chemical Co. Ltd., Poole, Dorset, United Kingdom; cobalt phthalocyanine and copper phthalocyanine, Kodak Ltd., Liverpool, United Kingdom; cobalt protoporphyrin and zinc protoporphyrin, Porphyrin Products Inc., Logan, Utah.

Dehalogenation assay. Lindane dehalogenation was assayed in the following reaction mixture: 1 ml of 100 mM Tris hydrochloride buffer, pH 9.0, containing 5 mM dithiothreitol and 10 mg of lindane per liter. The reaction was initiated by the addition of 10 μl of sample, and the reaction mixture was purged with nitrogen before incubation at 37°C for 30 min. The reaction mixture was then extracted with 2 ml of hexane-diethyl ether (85:15, vol/vol), and 0.5 μl of this extract was analyzed by electron capture gas-liquid chromatography. The chromatograph was equipped with a silanized glass column (1.5 m by 4 mm [inside diameter]) packed with 3% SE30 on a 100/120-mesh Supelcoport with nitrogen (40 ml/min) as the carrier gas. The injector and detector were maintained at 250°C, while the column oven was maintained at 190°C.

To assess the kinetics of dehalogenation, the following reaction mixture was used: 10 ml of 100 mM Tris hydrochloride buffer, pH 9.0, containing 5 mM dithiothreitol and 10 mg of lindane per liter. The reaction mixture was equilibrated at 37°C before the addition of 100 μl of sample and then purged with nitrogen and sealed with a butyl rubber septum and incubated at 37°C. At 5-min intervals, a hypodermic syringe was used to withdraw 0.5-ml samples, which were extracted with 1 ml of hexane-diethyl ether (85:15, vol/vol), and 0.5 μl of this extract was analyzed by gas-liquid chromatography as described above.

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Identification of the products of dehalogenation. The products of dehalogenation were identified by gas chromatography-mass spectrometry performed with a Kratos MS80 RFA mass spectrometer interfaced to a Carlo-Uba 5160 chromatograph. The chromatograph was equipped with a capillary column (12 m by 0.2 mm) packed with BP-1 (SGE, Milton Keynes, United Kingdom). Helium was used as carrier gas at a flow rate of 1 ml/min. The injector and detector ovens were maintained at 250°C, while the column oven was temperature programmed from 50 to 130°C at 20°C/min and from 130 to 250°C at 10°C/min.

Ionization was effected by electron impact at an ionization energy of 70 eV. The trap current was 100 μA, and the magnet was repetitively scanned at 0.3 s per decade.

Preparation of porphyrin-metal ion complexes. Porphyrins (15 μg) not containing metal ions (protoporphyrin, coproporphyrin, uroporphyrin, and hematoporphyrin) were dissolved in 100 mM Tris hydrochloride buffer (150 μl), pH 9.0, before incubation with equimolar solutions of a range of metal ions at 37°C for 30 min in the dark. Samples (10 μl) of the porphyrin-metal ion mixture were then removed and assayed for the ability to dehalogenate lindane.

Addition of ligands onto porphyrins and corrins. Samples (15 μg) of either commercially obtained cobalt-containing protoporphyrin or cyanocobalamin were dissolved in 100 mM Tris hydrochloride buffer (150 μl), pH 9.0, before incubation with equimolar solutions of a variety of ligands at 37°C for 30 min in the dark. Samples (10 μl) of the porphyrin or corrin-ligand complex were removed and assayed for the ability to dehalogenate lindane. The absorbance spectrum of each sample was determined between 250 and 700 nm in a recording spectrophotometer (Shimadzu model UV 240; V. A. Howe Ltd., London, United Kingdom).

Removal of cyanide ligands from cobinamide dicyanide. Cobinamide dicyanide (10 mg) was dissolved in water (10 ml) before addition of 0.1 M HCl to pH 2.0. The absorbance spectrum of the sample before and after acidification was determined between 250 and 700 nm in a recording spectrophotometer. The acidified sample was freeze-dried overnight and subsequently redissolved in 100 mM Tris hydrochloride buffer (10 ml), pH 9.0, and the pH was adjusted to 9.0 by the addition of 0.01 M NaOH. The absorbance spectrum of the sample was then redetermined. Samples (10 μl) were diluted in 100 mM Tris hydrochloride buffer (90 μl), pH 9.0, and 10 μl of this diluted material was assayed for the ability to dehalogenate lindane.

Production of cobalt-chelate complexes. A series of metal-chelating agents (0.04 μmol) were dissolved in 100 mM Tris hydrochloride buffer (150 μl), pH 9.0, before incubation with equimolar solutions of cobalt at 37°C for 30 min in the dark. Samples (10 μl) of the cobalt-chelate complex were then assayed for the ability to dehalogenate lindane.

RESULTS

A range of porphyrins and hemoproteins were tested for their ability to dehalogenate lindane. Both of the iron-containing porphyrins (hemin and hematin) and one of the iron-containing hemoproteins (hemoglobin) dehalogenated lindane at substantial rates (Table 1), although the other iron-containing hemoprotein, cytochrome *c*, did not. Similarly, the corrins also catalyzed dehalogenation. Chlorophyll *a* dehalogenated lindane, albeit at a relatively low rate and only when illuminated. Copper-containing hemoproteins (hemocyanin) and those without metal ions (protoporphyrin, coproporphyrin, uroporphyrin, and hematoporphyrin) were inactive against lindane.

TABLE 1. Dehalogenation of lindane by a variety of hemoproteins, porphyrins, corrins, and analogs

Porphyrin	Activity[a]
Bacteriochlorophyll *a* (dark)	0
Chlorophyll *a* (dark)	0
Chlorophyll *b* (dark)	0
Bacteriochlorophyll *a* (light)	0
Chlorophyll *a* (light)	0.1
Chlorophyll *b* (light)	0
Hemocyanin	0
Cytochrome *c*	0
Hemoglobin	10
Hemin	380
Hematin	710
Hematoporphyrin	0
Uroporphyrin	0
Coproporphyrin	0
Protoporphyrin	0
Porphobilinogen	0
Cyanocobalamin	800
Adenosylcobalamin	750
Cobinamide dicyanide	6,750
Cobinamide	6,825
Cobalt phthalocyanine	560
Copper phthalocyanine	0
Cobalt protoporphyrin	1,300
Zinc protoporphyrin	0

[a] Expressed as nanomoles of lindane dehalogenated per minute per milligram of porphyrin. All incubations were carried out in the dark (unless otherwise stated) at 37°C.

Two commercial preparations of protoporphyrins containing metal ions were assessed for lindane dehalogenation. Protoporphyrin containing cobalt dehalogenated lindane, whereas protoporphyrin containing zinc did not. Additionally, the commercially prepared phthalocyanine containing cobalt dehalogenated lindane, while the phthalocyanine containing copper did not.

A series of porphyrins containing different metal ions were prepared and screened for the ability to dehalogenate lindane (Table 2). When preincubated at pH 9 with certain metal

TABLE 2. Dehalogenation of lindane by porphyrin-metal ion complexes

Preincubation metal ion	Rate of lindane removal with[a]:			
	Protoporphyrin (pH 5.5)[b]	Uroporphyrin (pH 9.0)	Coproporphyrin (pH 9.0)	Hematoporphyrin (pH 9.0)
None	0	0	0	0
Calcium(II)	0	0	0	0
Cobalt(II)	320	160	280	350
Copper(II)	0	0	0	0
Iron(II)	240	56	110	250
Iron(III)	250	110	0	280
Magnesium(II)	350	140	300	350
Manganese(II)	0	0	0	0
Molybdenum(III)	260	120	240	250
Nickel(II)	220	130	210	200
Vanadium(V)	20	24	16	30
Zinc(II)	0	0	0	0

[a] Results are expressed as nanomoles of lindane removed per minute per milligram of porphyrin. Porphyrins and metal ions were preincubated together for 30 min at the indicated pH in the dark before assay unless otherwise stated.

[b] Values for protoporphyrin at pH 9.0 were zero with every metal ion tested.

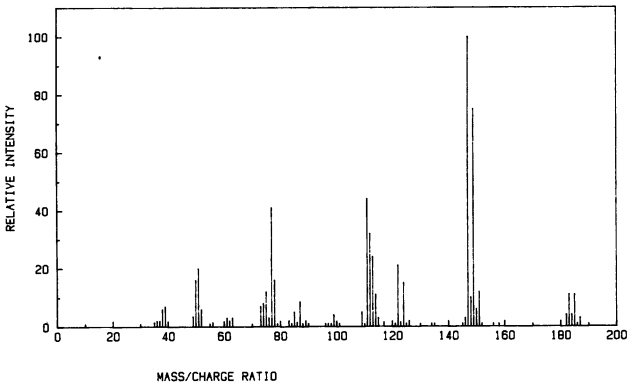

FIG. 1. Mass spectrum of tetrachlorocyclohexene produced as an intermediate in the dehalogenation of lindane.

ions, three of the four porphyrins (coproporphyrin, uroporphyrin, and hematoporphyrin) dehalogenated lindane. In contrast, protoporphyrin did not form active complexes with any of the metal ions under these conditions. However, by preincubation of protoporphyrin in 100 mM sodium acetate buffer, pH 5.5, with specified metal ions, active complexes could be formed. Of the metal ions, cobalt, iron, magnesium, molybdenum, nickel, and vanadium all dehalogenated lindane when complexed with coproporphyrin, uroporphyrin, and hematoporphyrin at pH 9 and when complexed with protoporphyrin at pH 5.5.

As with the hemoproteins, corrins, and commercially available porphyrins containing metal ions, the porphyrin-metal ion complexes were observed to catalyze an initial didechlorination of lindane, producing tetrachlorocyclohexene (Fig. 1). This was further dehalogenated in the system, yielding monochlorobenzene as the end product (Fig. 2). First-order rate kinetics were observed for both the didechlorination of lindane and the dechlorination of tetrachlorocyclohexene (Fig. 3).

The addition of cyanide, perchlorate, thiocyanate, thiosulfate, and sulfite as ligands to both cobalt-containing protoporphyrin and cyanocobalamin produced no significant increase in the rate of lindane degradation. Spectrophotometric analysis of each of the ligand-porphyrin complexes indicated a spectral shift only when cyanide was used as the ligand with cyanocobalamin (to produce dicyanocobalamin) but not with protoporphyrin containing cobalt. No appreciable increase in the rate of dehalogenation was observed with

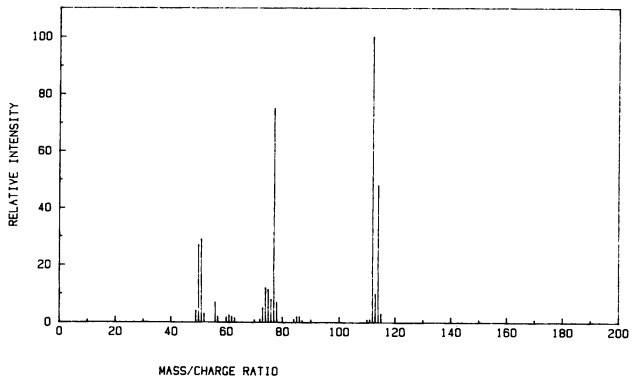

FIG. 2. Mass spectrum of monochlorobenzene produced by dehalogenation of lindane.

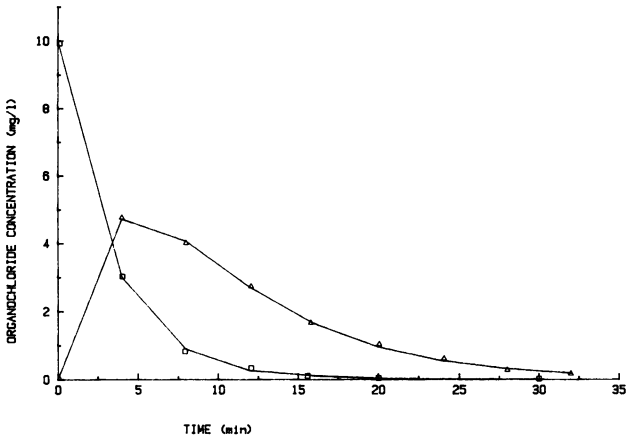

FIG. 3. Dehalogenation of lindane (□) and transient formation of tetrachlorocyclohexene (△) in the presence of 1 μg of cobinamide dicyanide per ml and 5 mM dithiothreitol.

any of these complexes. Similarly, removal of the cyanide ligand from dicyanocobinamide did not result in an increase in the rate of dehalogenation.

The use of 8-hydroxyquinoline, 8-hydroxyquinoline sulfate, 1,10-phenanthroline, EDTA, 2,2-dipyridyl, indole, imidazole, indole 3-acetic acid, and histidine in combination with cobalt did not result in the formation of complexes which were able to dehalogenate lindane.

DISCUSSION

Previous reports on the ability of porphyrins and corrins to dehalogenate organohalides have concentrated principally on iron-containing porphyrins and the cobalt-containing cobalamin (2, 6, 14). The dehalogenation of DDT by chromium and zinc metal centers, albeit not in combination with either a porphyrin or a corrin, has also been reviewed (11). The wide variety of organohalides susceptible to dehalogenation has been demonstrated by Wade and Castro (12), who observed the dehalogenation of 14 organic halides by iron-containing deuteroporphyrin IX.

In this investigation we have demonstrated that a range of metal ions, when complexed with a series of porphyrins, can catalyze lindane dehalogenation. Three of the four porphyrins containing no metal ion tested, namely, coproporphyrin, hematoporphyrin, and uroporphyrin, were shown to dehalogenate lindane when complexed with the metal ions cobalt, iron, magnesium, molybdenum, nickel, and vanadium. At alkaline pH, preincubation of protoporphyrin with any metal ion did not lead to a product capable of dehalogenating lindane. If protoporphyrin was preincubated under weakly acidic conditions (pH 5.5), active porphyrin-metal ion complexes were formed with all of the metal ions which are capable of complexing with other porphyrins. Protoporphyrin is hydrated to form hematoporphyrin in aqueous mineral acid solutions (3); thus, it is possible that during preincubation of protoporphyrin with metal ions at pH 5.5, hydration of the protoporphyrin to hematoporphyrin occurred and the metal ions complexed with hematoporphyrin rather than protoporphyrin. An enzyme obtained from extracts of *Clostridium tetanomorphum*, cobalt porphyrin synthase, has been shown to catalyze the insertion of cobalt ions into protoporphyrin IX; however, the rate of formation of cobalt protoporphyrin in the absence of the enzyme was low (10). In contrast, the rate of incorporation of cobalt into both

coproporphyrin III and uroporphyrin III in the absence of this enzyme was high. Commercially obtained samples of cobalt-containing protoporphyrin were demonstrated to dehalogenate lindane; it is probable that under alkaline conditions the lack of dehalogenating activity is due to the low rate of formation of protoporphyrin-metal ion complexes rather than formation of complexes which are inactive.

Of the possible ligands which were tested with both cobalt-containing protoporphyrin and cyanocobalamin, only cyanide showed evidence of ligating to the metal center, as judged by spectral analysis. The removal of the cyanide ligands from cobinamide resulted in a slight increase in activity. In the absence of the ribazole moiety of cobalamin (cobinamide), there is a substantial eightfold increase in activity over that observed in its presence. This may be because the ribazole moiety sterically hindered the approach of the lindane molecule to the cobalt ion at the center of the corrin ring or because it caused a deleterious electron shift in the molecule.

The wide range of metal ion-porphyrin complexes which have been demonstrated to dehalogenate lindane together with the relatively broad spectrum of organohalides which are reported to be susceptible to dehalogenation by porphyrins may form the basis for the development of economical detoxification systems for industrial effluents (T. S. Marks and A. Maule, U.K. patent 8810944, May, 1988). The persistence of organohalides in soils can be related in part to the degree of halogen substitution; therefore, dehalogenation of organohalides in industrial effluents to produce compounds with fewer or no halogen substituents would result in waste discharges which are potentially less damaging to the environment.

The dehalogenation of lindane by a variety of anaerobic bacterial isolates has been reported (4, 5, 7, 9). Although the mechanism of dehalogenation has not been fully characterized, it is possible, on the basis of results presented here, that a porphyrin- or corrin-mediated reaction may be involved. Consequently, microbially produced porphyrins and corrins may provide an economic basis for a detoxification process.

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