APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1990, p. 1169–1171
0099-2240/90/041169-03$02.00/0

Vol. 56, No. 4

Degradation of Halogenated Aliphatic Compounds by the Ammonia-Oxidizing Bacterium *Nitrosomonas europaea*

TODD VANNELLI, MYKE LOGAN, DAVID M. ARCIERO, AND ALAN B. HOOPER*

Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108

Received 3 October 1989/Accepted 12 January 1990

Suspensions of *Nitrosomonas europaea* catalyzed the ammonia-stimulated aerobic transformation of the halogenated aliphatic compounds dichloromethane, dibromomethane, trichloromethane (chloroform), bromoethane, 1,2-dibromoethane (ethylene dibromide), 1,1,2-trichloroethane, 1,1,1-trichloroethane, monochloroethylene (vinyl chloride), *gem*-dichloroethylene, *cis*- and *trans*-dichloroethylene, *cis*-dibromoethylene, trichloroethylene, and 1,2,3-trichloropropane. Tetrachloromethane (carbon tetrachloride), tetrachloroethylene (perchloroethylene), and *trans*-dibromoethylene were not degraded.

The ubiquitous soil- and water-dwelling nitrifying bacteria, exemplified by *Nitrosomonas europaea*, are obligate autotrophic aerobes which depend for growth on the oxidation of ammonia by ammonia monoxygenase, as shown by the following equation: $2H^+ + 2e^- + O_2 + NH_3 \rightarrow NH_2OH + H_2O$. Two electrons for the reaction originate in the subsequent reaction of hydroxylamine oxidoreductase, as shown by the following equation: $H_2O + NH_2OH \rightarrow 4e^- + 5H^+ + NO_2^-$ (3, 11). Trichloroethylene is degraded by bacteria in a process which requires toluene (9, 10) or methane (8). *N. europaea* catalyzes the oxidation of CO, methane, methanol, ethylene, propylene, and bromoethane (5, 11) and trichloroethylene (1). The reactions are probably catalyzed by ammonia monoxygenase. We report here that *N. europaea* is able to degrade many halogenated aliphatic compounds.

Sources were as follows: vinyl chloride, Fluka, Ronkonkoma, N.Y.; other substrates (>97% pure), Aldrich Chemical Co., Inc., Milwaukee, Wis.; calcium carbide, Fisher Chemical, Fairlawn, N.J.; nitrapyrin (2-chloro-6-trichloromethylpyridine), Dow Chemical Co., Midland, Mich.

Growth of *N. europaea*, assay of utilization of oxygen or halogenated substrates, production of nitrite, and treatment of cells with acetylene were as previously described (1). Substrates, except for vinyl chloride, were at a concentration of 1 ppm (10 to 15 μM). Correction was not made in the data for the ±5% change in concentration of the halogenated compound in 24 h in the absence of cells. Vinyl chloride (200 μM), introduced as a gas into the headspace, was analyzed at 80°C in a Hach-Carl AGC-100 gas chromatograph with an Alltech (Ann Arbor, Mich.) 6-foot-long, 1/8-in.-diameter stainless steel packed column (1 in. = 2.54 cm) of 60/80 mesh VZ-10; the nitrogen carrier gas was introduced at 60 ml/min. Analysis was by a flame ionization detector which used hydrogen and air (the flow was at 30 and 300 ml/min, respectively). The peaks for *cis*- and *trans*-dibromoethylene, which were utilized only as a mixture (total 12 μM), were identified after enrichment of each form by distillation.

The time course of degradation of six of the halogenated aliphatic compounds (Fig. 1) is representative of the shape of the curve observed with the 16 compounds. All the compounds, except tetrachloromethane, tetrachloroethylene, and *trans*-dibromoethylene, were degraded (Table 1). The change in substrate during the first 30 or 60 min of incubation indicates a substantial rate of degradation at the concentration of cells, ammonia, and halogenated hydrocarbon utilized. The conditions for optimum activity have not yet been determined; therefore, the rates given in Table 1 are minimum values. The rates are comparable to values for trichloroethylene catalyzed by the ammonia-oxidizing (1), toluene-oxidizing (9, 10), and methane-oxidizing (8) bacteria. Specificities for the systems may also be similar; soluble methane monoxygenase of *Methylococcus capsulatus* oxidizes dichloro- and trichloromethane but not tetrachloromethane (2).

Acetylene or 2-chloro-6-trichloromethylpyridine (1 mM), which both inhibit the ammonia-oxidizing system in preference to the hydroxylamine system in *N. europaea* (4, 6), inhibited degradation of halogenated aliphatics by at least 70% (data not shown). Thus, the reaction is at least dependent on and probably catalyzed by the ammonia oxygenase. In all cases in which the compound was degraded, the presence of the compound also decreased the rate of nitrite production from ammonia (Table 1), consistent with competition for an active site.

Under the conditions shown in Table 1, degradation was not accompanied by inactivation of the enzyme; within 24 h most or all of the test compound had disappeared. Degradation of slower-reacting compounds was incomplete only because all ammonia had been consumed after 4 h of incubation. Degradation of substrate was observed in the absence of added ammonia, although the rates and extent were always greater with ammonia (Table 1).

The ratio of moles of nitrite produced (ammonia oxidized) to moles of compound degraded ranged from 45 to 330. With vinyl chloride, which was used at a higher concentration, the ratio was 4.

Carbon tetrachloride was not degraded by *N. europaea* (Table 1), nor was the compound a good inhibitor of nitrite production (at a concentration of 1 mM, ammonia-dependent oxygen utilization was inhibited by only 30%). Tetrachloroethylene was not degraded by *N. europaea* but was an effective inhibitor of nitrite production or oxygen utilization (Table 1). Oxidation of hydroxylamine to nitrite was not

* Corresponding author.

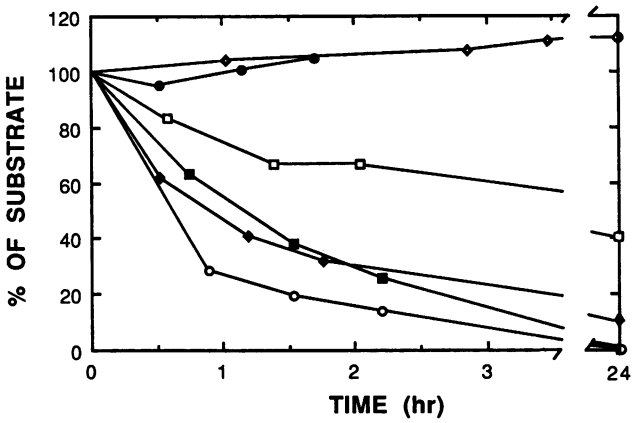

FIG. 1. Time course of disappearance of halogenated aliphatic compounds catalyzed by *N. europaea*. The reaction mixture contained cells, NH_3, and added compounds as follows: tetrachloromethane (◇), tetrachloroethylene (●), trichloromethane (□), dichloromethane (■), 1,2 dibromoethane (◆), and *cis*-dibromoethylene (○). Conditions are described in Materials and Methods and Table 1.

inhibited by carbon tetrachloride (1 mM) or tetrachloroethylene (10 μM).

trans-Dibromoethylene, which was incubated only in a mixture with the *cis* isomer, was not degraded even after the *cis* isomer had been fully degraded; i.e., cells still actively oxidized ammonia, trichloroethylene, or additional *cis* isomer in a second addition of the mixture of *cis*- and *trans*-dibromoethylene. Thus, the lack of reactivity of the *trans* isomer was probably not due to competition with the *cis* isomer. Interestingly, *N. europea* will oxidize both *cis*- and *trans*-2-butene (5), which are structural analogs of the dibromoethylenes; 2-butene-1-ol and lesser amounts of an epoxide are produced from *trans*-2-butene, whereas *cis*-2-butene is converted to an even mixture of the two. We rationalize these observations with an active site (Fig. 2) containing an oxygen-activating region, A, and a hydrophobic pocket, H, as suggested by Leak and Dalton (7) for methane monoxygenase. The *cis*-butene may be positioned by the hydrophobic pocket for easy reaction of the double bond with the oxygen-activating site (Fig. 2A), whereas *trans*-butene would be better positioned for hydroxylation at the methyl group (Fig. 2B). The unreactive Br of *trans*-dibromoethylene would be positioned at the oxygen-activating site and block reaction with the double bond (Fig. 2D), whereas the double bond of *cis*-dibromoethylene would be positioned for easy reaction (Fig. 2C). Unlike the brominated compounds, both *cis*- and *trans*-dichloroethylene are actively oxidized, suggesting that chlorine may not be large enough to sterically hinder oxidation. Other compounds such as *gem*-dichloroethylene and trichloroethylene can be easily imagined to orient themselves so that a reactive portion would be at the oxygenating site.

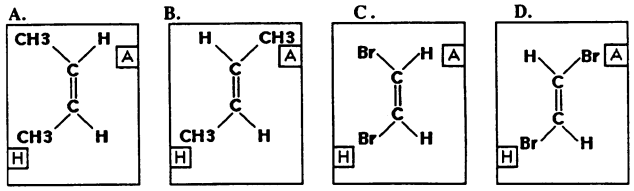

FIG. 2. Aspects of the proposed active site of ammonia monoxygenase of *N. europea*. H, hydrophobic region; A, oxygen-activating site.

TABLE 1. Oxidation of halogenated aliphatic compounds by *N. europaea*

Substrate[a]	Cells		Cells + NH_3			
	ΔSubstrate/Δtime[b]	Substrate remaining[c]	ΔSubstrate/Δtime[b]	ΔNitrite/Δtime[b]	Ratio of Δnitrite:Δsubstrate	Substrate remaining[c]
Ammonia				970		
Dichloromethane	6.5	33	9.8	650	66	0.0
Dibromomethane	5.3	68	7.2	590	82	4.2
Trichloromethane	3.3	71	4.5	600	130	41
Tetrachloromethane	0.0	110	0.0	620		110
Bromoethane	5.5	50	7.3	880	120	25
1,2-Dibromoethane	1.0	92	11	820	75	16
1,1,2-Trichloroethane	1.9	56	4.2	660	160	35
1,1,1-Trichloroethane	1.6	91	2.2	600	270	80
Chloroethylene	15	45	57	230	4.0	23
gem-Dichloroethylene	1.1	80	3.9	780	200	53
cis-Dichloroethylene	0.7	43	8.8	400	45	9.1
trans-Dichloroethylene	2.2	56	3.9	850	220	25
cis-Dibromoethylene	0.4	53	12	690	58	3.0
trans-Dibromoethylene	0.0	110	0.0	690		100
Trichloroethylene	2.8	52	6.7	790	120	6.4
Tetrachloroethylene	0.0	110	0.0	140		120
1,2,3-Trichloropropane	0.9	91	2.0	660	330	77

[a] Common names: dichloromethane, methylene chloride; dibromomethane, methylene bromide; trichloromethane, chloroform; tetrachloromethane, carbon tetrachloride; bromoethane, ethylbromide; 1,2,dibromoethane, ethylene dibromide; chloroethylene, vinyl chloride; *gem*-dichloroethylene, vinylidene chloride; tetrachloroethylene, perchloroethylene.

[b] Initial rates (micromoles per hour per gram [wet weight] of cells).

[c] Value at 24 h (percent of initial value).

We thank Peter Chapman for informative discussions, Tom Krick for use of the gas chromatograph, Larry Wackett for advice and use of the chromatograph for assay of vinyl chloride, and Candace Pilon and Margaret Roddy for growth of bacteria.

This research was supported by grant 88-37120-3957 from the U.S. Department of Agriculture and cooperative agreement CR816157-01 with the U.S. Environmental Protection Agency.

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