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Aliphatic and Chlorinated Alkenes and Epoxides as Inducers of Alkene Monooxygenase and Epoxidase Activities in *Xanthobacter* Strain Py2

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The inducible nature of the alkene oxidation system of *Xanthobacter* strain Py2 has been investigated. Cultures grown with glucose as the carbon source did not contain detectable levels of alkene monooxygenase or epoxidase, two key enzymes of alkene and epoxide metabolism. Upon addition of propylene to glucose-grown cultures, alkene monooxygenase and epoxidase activities increased and after an 11-h induction period reached levels of specific activity comparable to those in propylene-grown cells. Addition of chloramphenicol or rifampin prevented the increase in the enzyme activities. Comparison of the banding patterns of proteins present in cell extracts revealed that polypeptides with molecular masses of 43, 53, and 57 kDa accumulate in propylene-grown but not glucose-grown cells. Pulse-labeling of glucose-grown cells with [^{35}S]methionine and [^{35}S]cysteine revealed that the 43-, 53-, and 57-kDa proteins, as well as two additional polypeptides with molecular masses of 12 and 21 kDa, were newly synthesized upon exposure of cells to propylene or propylene oxide. The addition to glucose-grown cells of a variety of other aliphatic and chlorinated alkenes and epoxides, including ethylene, vinyl chloride (1-chloroethylene), *cis*- and *trans*-1,2-dichloroethylene, 1-chloropropylene, 1,3-dichloropropylene, 1-butylene, *trans*-2-butylene, isobutylene, ethylene oxide, epichlorohydrin (3-chloro-1,2-epoxypropane), 1,2-epoxybutane, *cis*- and *trans*-2,3-epoxybutane, and isobutylene oxide stimulated the synthesis of the five propylene-inducible polypeptides as well as increases in alkene monooxygenase and epoxidase activities. In contrast, acetylene, and a range of aliphatic and chlorinated alkanes, did not stimulate the synthesis of the propylene-inducible polypeptides or the increase in alkene monooxygenase and epoxidase activities.

Xanthobacter strain Py2 is one of a number of bacteria capable of aerobic growth using aliphatic alkenes such as ethylene, propylene, and 1-butylene as the sole carbon and energy source (5). The oxidation of alkenes is initiated by an alkene monooxygenase which catalyzes the O_2- and reductant-dependent oxidation of the alkenes to their corresponding epoxides (16). The epoxide products are further metabolized through the involvement of an enzyme designated an epoxidase. Recently, evidence has been presented that the epoxidase-catalyzed degradation of propylene oxide in *Xanthobacter* strain Py2 proceeds via a novel carboxylation reaction requiring CO_2 as a cosubstrate and yielding acetoacetate or an acetoacetyl derivative as the product (12).

In addition to metabolizing aliphatic alkenes and epoxides, propylene-grown cell suspensions of *Xanthobacter* strain Py2 will degrade a number of chlorinated alkenes of environmental concern, including trichloroethylene (TCE), *cis*- and *trans*-1,2-dichloroethylene, vinyl chloride, 1-chloropropylene, and 1,3- and 2,3-dichloropropylene (2). The degradation of these compounds is initiated by an alkene monooxygenase which apparently transforms these compounds by epoxidation reactions analogous to the epoxidation of physiological growth substrates (2). The ability to cometabolically degrade chlorinated alkenes in this manner is a property which a number of additional, nonspecific bacterial oxygenases possess, including methane monooxygenase (3, 9, 15), ammonia monooxygenase (1, 10), toluene mono- and dioxygenases (20, 21), and propane monooxygenase (18).

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As part of an ongoing interest in the physiology, biochemistry, and biodegradative capabilities of *Xanthobacter* strain Py2, the regulation of expression of the enzymes involved in alkene and epoxide metabolism has been examined in the present work. *Xanthobacter* strain Py2 is a nutritionally versatile organism that is capable of growing with a variety of carbon sources in addition to alkenes and epoxides, including sugars, alcohols, aldehydes, diols, and H_2 plus CO_2 (16). Cultures grown with these other carbon sources do not have detectable alkene monooxygenase or epoxidase activities, indicating that the enzymes are inducible. Since the composition of the medium used to culture the bacteria differs only in the carbon source, propylene, or a molecule derived therefrom, must serve as an activator of gene expression, protein synthesis, or preexisting proteins present within the cell. Recent studies of toluene-oxidizing *Pseudomonas* strains capable of degrading TCE have demonstrated that TCE, as well as several other chlorinated solvents and alkanes, can also serve as an inducer of toluene oxidation activity in these bacteria (6, 8). In light of these studies, an investigation of the range of aliphatic and chlorinated alkenes and related compounds that might serve as inducers of alkene and epoxide oxidation activities in *Xanthobacter* strain Py2 is of particular interest.

In this work, the induction of the alkene-oxidizing enzymes of *Xanthobacter* strain Py2 by the physiological substrates propylene and propylene oxide (1,2-epoxypropane) is characterized. A variety of aliphatic and chlorinated alkenes and epoxides are shown to serve as effective inducers of the system. The relevance of these findings to the degradation of chlorinated alkenes by *Xanthobacter* strain Py2 is demonstrated and discussed.

MATERIALS AND METHODS

Materials. Ethylene (99.9% minimum), propylene (99.0% minimum), *cis*-2-butylene (99.0% minimum), *trans*-2-butylene (99.0% minimum), and vinyl chloride were obtained from Matheson Gas Products, Cucamonga, Calif. Acetylene (99.6%) was obtained from Airco Gases, San Leandro, Calif. Ethylene oxide (>99%) and *trans*-1,2-dichloroethylene (98%) were obtained from Eastman Kodak, Rochester, N.Y. 1,3-Dichloropropylene and isobutylene oxide (2-methyl-1,2-epoxypropane) were obtained from Lancaster, Windham, N.H. Ethane (≥99%), propane (98%), 1-butylene (≥99%), isobutylene (2-methylpropylene) (99%), propylene oxide (≥99%), 1,2-epoxybutane (≥99%), *cis*-2,3-epoxybutane (97%), *trans*-2,3-epoxybutane (96%), chloroethane (99.7%), 1,2-dichloroethane (99.8%), 1-chloropropane (99%), 1,1-dichloroethylene (99%), *cis*-1,2-dichloroethylene (97%), TCE (≥99%), tetrachloroethylene (≥99.9%), 1-chloropropylene (99%), epichlorohydrin (≥99%), and epifluorohydrin (3-fluoro-1,2-epoxypropane) (98%) were obtained from Aldrich Chemical Co., Milwaukee, Wis. Tran-^{35}S-label (70% [^{35}S]Met + 20% [^{35}S]Cys) was obtained from ICN Radiochemicals, Irvine, Calif. All other chemicals were of reagent grade.

Growth and preparation of cells for induction experiments. *Xanthobacter* strain Py2 was cultured at 30°C by using the mineral salts medium described previously (19) with either propylene gas or glucose (10 g/liter) as the carbon source. Cultures were grown either in shake flasks, in sealed shake flasks with propylene (10% [vol/vol] gas phase) added as an overpressure as described previously (13), or in a 1-liter water-jacketed Celstir culture vessel (Wheaton) with forced aeration and, where indicated, with 5% propylene included in the air supply to the culture. Cell growth (optical density) was monitored by removing a portion of cells from the cultures and measuring A_{600}. At the desired time entire cell cultures, or a portion thereof, were harvested by centrifugation, washed, and resuspended in buffer (50 mM potassium phosphate, pH 7.2) to concentrations between 5 and 20 mg of protein per ml as described previously (13). All subsequent studies were performed using 50 mM potassium phosphate, pH 7.2, as the buffer.

Degradation of organic compounds. The degradation of propylene, propylene oxide, and other organic compounds was quantified by monitoring the time-dependent depletion of these compounds in whole-cell suspensions by gas chromatography as described previously (13). Assays were performed in stoppered serum vials (9 ml) which were crimp-sealed with gray butyl stoppers and which contained buffer, substrate, and cells (0.1 to 0.3 mg of protein) in a total volume of 1 ml. The assays were initiated by the addition of substrates, which were added either as a gas (propylene) or from freshly prepared stock solutions in H_2O (propylene oxide) by using gas-tight syringes. The vials were shaken (150 cycles/min) in a water bath at 30°C. Samples of the gas phase (for propylene quantification; 50 μl) or liquid phase (for propylene oxide quantification; 1 μl) were removed periodically with microsyringes and analyzed by gas chromatography.

Induction of alkene- and epoxide-degrading activities in Celstir culture. *Xanthobacter* cells which had been grown for several generations with glucose as the carbon source were used to inoculate 1 liter of glucose-containing medium in a Celstir culture. The cells were grown as described above, initially in the absence of propylene. When an optical density (A_{600}) of 0.93 was reached, propylene (5% vol/vol) was added to the air supply to the culture. At 1-h intervals after the introduction of propylene, portions of the cells were removed, the optical densities were measured, and the cells were harvested and assayed for propylene and propylene oxide degradation activities as described above.

Induction of alkene- and epoxide-degrading activities in batch cultures. *Xanthobacter* cells which had been grown for several generations with glucose as the carbon source were used to inoculate 4- and 1-liter shake flasks containing 800 and 200 ml, respectively, of glucose-containing medium. When an A_{600} of 0.5 to 1.0 was reached, 25-ml portions of the cells (for subsequent propylene and propylene oxide degradation assays) or 15-ml portions of cells (for subsequent ^{35}S-labeling studies) were transferred to sterile 125-ml serum bottles. The serum bottles were crimp-sealed with gray butyl stoppers (for induction studies with organic compounds) or Teflon-lined silicone septa (for induction studies with halogenated compounds). Compounds to be tested for their abilities to stimulate propylene and propylene oxide degradation activities were added to the sealed serum bottles either as gases, as liquids, or from aqueous stock solutions by using gas-tight syringes. The cells were incubated with shaking (200 cycles/min) in a water bath at 30°C. The serum bottles sealed with Teflon-lined septa were incubated in the inverted position to prevent loss of the halogenated compounds from the bottles. After a 4-h incubation period the cells to be used for specific activity determinations were harvested and assayed as described above. After a 1.5-h incubation period, the cells to be used for ^{35}S-labeling experiments were manipulated as described below.

Preparation of ^{35}S-labeled cell extracts. Thirty microcuries of a mixture of [^{35}S]methionine and [^{35}S]cysteine were added to the serum bottles containing cells which had been exposed to organic and halogenated organic compounds for 1.5 h. The cells were incubated with shaking at 30°C for an additional 5 min. The cells were then sedimented by centrifugation, resuspended in 0.5 ml of lysis buffer (50 mM Tris-Cl, 10 mM EDTA, 2% sodium dodecyl sulfate [SDS], 3% 2-mercaptoethanol [pH 8.0]), frozen in liquid nitrogen, and stored at −80°C. For analysis, the cell suspensions were thawed and lysed by 3 cycles (30 s each) of sonication. Insoluble material was sedimented by centrifugation in microcentrifuge tubes, and the supernatant was diluted with SDS-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer, boiled for 1 min, and used for SDS-PAGE analyses.

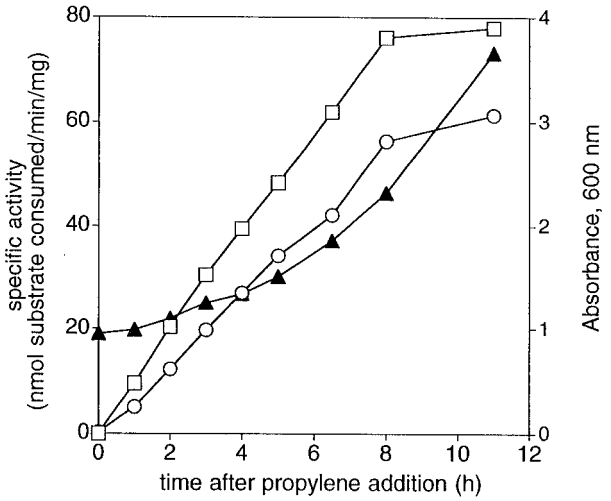

FIG. 1. Increase in alkene monooxygenase and epoxidase activities after addition of propylene to glucose-grown *Xanthobacter* strain Py2. Cells were grown in a 1-liter Wheaton Celstir culture vessel, sampled, and assayed as described in Materials and Methods. For the assays, 2 μmol of propylene or propylene oxide was added as a substrate. Activities are reported as nanomoles of substrate consumed per minute per milligram of whole-cell protein. Symbols: ○, propylene monooxygenase activity; □, epoxidase activity; ▲, optical density (A_{600}) of culture.

SDS-PAGE and autoradiography. SDS-PAGE (12% total gel; 2.7% cross-linker running gel) was performed in a Mini-Protean II gel apparatus (Bio-Rad) following the Laemmli procedure (7). For autoradiographic analysis of ^{35}S-labeled proteins, gels were stained with Coomassie blue, dried onto filter paper backing, and exposed to X-ray film at −80°C for various times. Apparent molecular weights of polypeptides were determined by comparison with the R_f values for molecular weight standard proteins. The standards were bovine serum albumin (68 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), and cytochrome *c* (12.3 kDa).

Protein determination. Protein concentrations of whole-cell suspensions were determined by the biuret assay (4) after the cells were solubilized in 3 M NaOH for 30 min at 65°C. Bovine serum albumin was used as the standard.

RESULTS

Propylene-dependent induction of alkene monooxygenase and epoxidase in *Xanthobacter* strain Py2. Cultures of *Xanthobacter* strain Py2 grown with glucose as the carbon source do not have detectable alkene monooxygenase or epoxidase activities. As shown in Fig. 1, the addition of propylene to the air supply to a Celstir culture with glucose as the carbon source resulted in time-dependent increases in alkene monooxygenase and epoxidase activities. After an 11-h period, the specific activities for alkene monooxygenase and epoxidase in whole-cell suspensions were 61 and 78 nmol of propylene or propylene oxide consumed per min per mg of protein, respectively. These activities are comparable to the specific activities of alkene monooxygenase and epoxidase in whole-cell suspensions prepared from cultures grown with propylene as the sole carbon source, which are both typically in the range of 70 to 110 nmol of substrate consumed per min per mg of protein.

The propylene-dependent increase in alkene monooxygenase activity was further studied by growing the bacteria in batch cultures with glucose or propylene as the carbon source and then transferring the bacteria to serum bottles to which propylene was added. The time courses of propylene disappearance were used as a measure for the presence and/or induction of alkene monooxygenase activity in the cells. After a lag period of approximately 1 h, the propylene added to glucose-

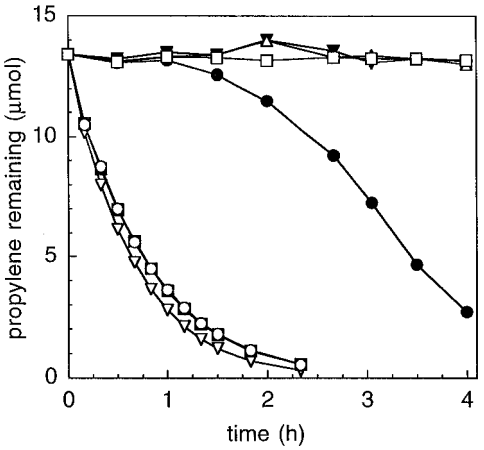

FIG. 2. Effect of RNA and protein synthesis inhibitors on the induction of alkene monooxygenase in glucose-grown *Xanthobacter* cells. Cells were grown in batch cultures with glucose as the carbon source to an optical density (A_{600}) of 0.85 or with propylene as the carbon source to an optical density of 0.91. Portions (25 ml) of the cells were then transferred to 125-ml serum bottles as described in Materials and Methods. At 0 h, propylene (13.4 μmol) was added to each bottle. The amount of propylene remaining was analyzed at the indicated times by gas chromatography. Symbols: □, boiled, glucose-grown cells; △, glucose-grown cells plus 10 mg of chloramphenicol; ▼, glucose-grown cells plus 5 mg of rifampin; ●, glucose-grown cells; ○, propylene-grown cells; ■, propylene-grown cells plus 10 mg of chloramphenicol; ▽, propylene-grown cells plus 5 mg of rifampin.

grown cells began to disappear, and the rate of propylene disappearance increased over time (Fig. 2). In contrast, no significant propylene degradation was observed when either rifampin or chloramphenicol was added to the glucose-grown cells (Fig. 2). Propylene-grown cells treated identically to the glucose-grown cells degraded propylene from the outset of the incubations, and the presence of rifampin or chloramphenicol had no effect on the rate or extent of propylene degradation (Fig. 2).

Identification of polypeptides synthesized upon induction of alkene monooxygenase and epoxidase. The results presented in Fig. 1 and 2 indicate that the propylene-dependent increases in alkene monooxygenase and epoxidase activities in glucose-grown cells result from new protein synthesis. It was therefore of interest to determine whether the induction of these activities could be correlated with the synthesis and accumulation of specific polypeptides. The gel banding patterns of proteins present in cell extracts prepared from glucose- and propylene-grown cells are presented in Fig. 3A. Three polypeptides with apparent molecular masses of 43, 53, and 57 kDa are clearly visible in the cell extract prepared from propylene-grown cells but not from glucose-grown cells. In order to further correlate the presence of these polypeptides with the induction of enzymes involved in alkene and epoxide metabolism, cells which had been grown with glucose as the carbon source were exposed to either propylene or propylene oxide for 1.5 h and then labeled for a brief period (5 min) with [^{35}S]methionine and [^{35}S]cysteine. The patterns of proteins synthesized by the cells during this labeling period are presented in the autoradiogram shown in Fig. 3B. Five polypeptides whose synthesis was dependent upon the addition of propylene or propylene oxide are apparent in this autoradiogram (Fig. 3B). Three of these polypeptides have the same apparent molecular masses (43, 53, and 57 kDa) as the polypeptides which accumulate in propylene-grown cells, while the two additional polypeptides, with apparent molecular masses of 12 and 21 kDa, are readily apparent in the autoradiogram but not in the gel showing accumulated proteins.

Induction of alkene monooxygenase and epoxidase by aliphatic alkenes and epoxides. Other alkenes and epoxides besides propylene and propylene oxide which are capable of supporting the growth of *Xanthobacter* strain Py2 include ethylene, 1-butylene, and 1,2-epoxybutane (16). It was therefore of interest to determine whether the addition of these alkenes and epoxides, as well as a number of other potential substrates for alkene monooxygenase and epoxidase, could stimulate increases in alkene monooxygenase and epoxidase activities and the synthesis of the five propylene-induced polypeptides in glucose-grown cells. The specific activities of alkene monooxygenase and epoxidase in glucose-grown cells after a 4-h exposure to some terminal, internal, and branched-chain aliphatic alkenes, epoxides, and other potential inducers are presented in Table 1. Each of the aliphatic alkenes and epoxides tested was capable of stimulating increases in alkene monooxygenase and epoxidase activities. The aliphatic alkenes and epoxides used for this experiment were all substrates for either alkene monooxygenase or epoxidase in propylene-grown cells, with the exception of isobutylene oxide. Ethane and propane, the alkane analogs of ethylene and propylene, and acetylene, the alkyne analog of ethylene, were ineffective at stimulating increases in either alkene monooxygenase or epoxidase activities (Table 1). Other organic substrates, including propionaldehyde, acetone, propylene glycol, isopropanol, *n*-propanol, and propionic acid, were also ineffective at stimulating increases in either alkene monooxygenase or epoxidase activities (data not shown).

Several of the compounds discussed above were tested to see if they would stimulate the synthesis of the five polypeptides induced upon exposure of glucose-grown cells to propylene and propylene oxide. As shown in the autoradiogram presented in Fig. 4, the addition to glucose-grown cells of ethylene, 1-butylene, *cis*-2,3-epoxybutane, and isobutylene oxide resulted in the synthesis of these five polypeptides. In contrast,

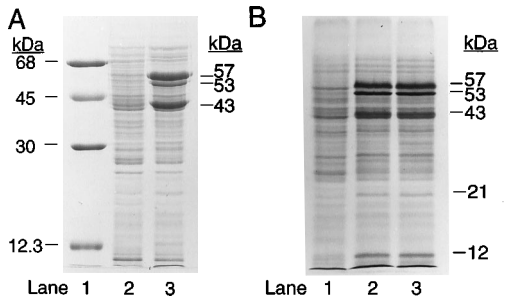

FIG. 3. Gel electrophoretic analysis of propylene- and propylene oxide-induced polypeptides. (A) Profiles of proteins present in cells grown with glucose compared with those present in cells grown with propylene. Cells were grown in batch cultures with either propylene or glucose as the carbon source, harvested at an optical density of 2.0, lysed, and electrophoresed as described in Materials and Methods. The gel was stained with Coomassie blue. Lane 1, molecular weight standards (2 μg each); lane 2, glucose-grown cell extract (20 μg of protein); lane 3, propylene-grown cell extract (20 μg of protein). The apparent molecular masses of the unique polypeptides present in propylene-grown cells are on the right. (B) Autoradiogram of ^{35}S-labeled, newly synthesized proteins. Cells were grown in batch cultures with glucose as the carbon source, labeled with ^{35}S-amino acids, and analyzed by autoradiography as described in Materials and Methods. Each gel lane contains cell extract equal to 20 μg of protein. Lane 1, cell extract prepared from cells labeled in the absence of propylene or propylene oxide; lane 2, cell extract prepared from cells labeled in the presence of propylene (13.4 μmol); lane 3, cell extract prepared from cells labeled in the presence of propylene oxide (14 μmol). The apparent molecular masses of the unique polypeptides synthesized in cells incubated with propylene and propylene oxide are on the right.

TABLE 1. Alkene monooxygenase and epoxidase activities after exposure of glucose-grown *Xanthobacter* strain Py2 to aliphatic alkenes and epoxides[a]

Compound	Enzyme[b]	Sp act[c]	
		Alkene monooxygenase	Epoxidase
No inducer	NA[d]	ND[e]	ND
Ethylene	Alkene monooxygenase	22.6	24.5
Propylene	Alkene monooxygenase	26.6	29.8
1-Butylene	Alkene monooxygenase	26.6	26.8
trans-2-Butylene	Alkene monooxygenase	12.9	9.18
Isobutylene	Alkene monooxygenase	19.4	16.6
Ethylene oxide	Alkene monooxygenase	23.8	30.2
Propylene oxide	Epoxidase	27.5	31.6
1,2-Epoxybutane	Epoxidase	24.5	25.2
cis-2,3-Epoxybutane	Epoxidase	17.4	21.3
trans-2,3-Epoxybutane	Epoxidase	16.8	18.6
Isobutylene oxide	Neither	23.8	18.7
Ethane	Neither	ND	ND
Propane	Neither	ND	ND
Acetylene	Neither	ND	ND

[a] Cells were incubated with the indicated compounds (10 μmol each) for 4 h and then harvested and assayed for alkene monooxygenase and epoxidase activities as described in Materials and Methods.
[b] The ability of compounds to serve as substrates for alkene monooxygenase or epoxidase was determined by monitoring the depletion of 2 μmol of the indicated compounds by using whole-cell suspensions of propylene-grown cells.
[c] Activities are reported as nanomoles of substrate consumed per minute per milligram of protein.
[d] NA, not applicable.
[e] ND, no detectable activity.

acetylene and propane were ineffective at stimulating the synthesis of these polypeptides.

Induction of alkene monooxygenase and epoxidase by chlorinated alkenes and epoxides. A number of chlorinated alkenes and epoxides that are substrates for alkene monooxygenase and epoxidase in whole-cell suspensions of propylene-grown cells (2, 13) were investigated as possible inducers for the expression of alkene monooxygenase and epoxidase in glucose-grown cells. As shown in Table 2, vinyl chloride, *cis*- and *trans*-1,2-dichloroethylene, 1-chloropropylene, 1,3-dichloropropylene, epichlorohydrin, and epifluorohydrin were all capable of stimulating alkene monooxygenase and epoxidase activities after a 4-h incubation period. TCE, tetrachloroethylene, and 1,1-dichloroethylene did not stimulate detectable increases in alkene monooxygenase or epoxidase activities. A number of chlorinated alkanes, including chloroethane, 1,2-dichloroethane, and 1-chloropropane, were also ineffective at stimulating increases in the two enzyme activities. As shown in Fig. 5, each of the chlorinated alkenes and epoxides listed in Table 2 which stimulated alkene monooxygenase and epoxidase activities also induced the synthesis of the five diagnostic polypeptides. Tetrachloroethylene, TCE, 1,1-dichloroethylene, chloroethane, and 1,2-dichloroethane did not induce the synthesis of these polypeptides.

A consideration concerning the induction experiments de-

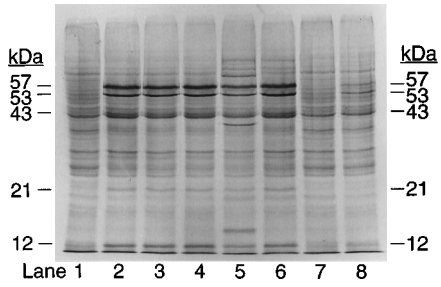

FIG. 4. Gel electrophoretic profiles of proteins synthesized after exposure of glucose-grown *Xanthobacter* strain Py2 to aliphatic alkenes and epoxides. Cells were grown in batch cultures with glucose as the carbon source, labeled with ^{35}S-amino acids, and analyzed by autoradiography as described in Materials and Methods. The autoradiogram of an SDS–12% polyacrylamide gel is shown. Each gel lane contains cell extract equal to 20 μg of protein. The compounds added as potential inducers (10 μmol each) are as follows: none (lane 1), propylene (lane 2), ethylene (lane 3), 1-butylene (lane 4), *cis*-2,3-epoxybutane (lane 5), isobutylene oxide (lane 6), acetylene (lane 7), and propane (lane 8).

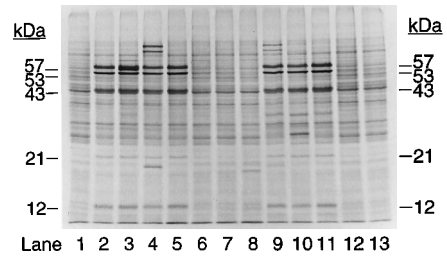

FIG. 5. Gel electrophoretic profiles of proteins synthesized after exposure of glucose-grown *Xanthobacter* strain Py2 cultures to chlorinated alkenes and epoxides. Cells were grown in batch cultures with glucose as the carbon source, labeled with ^{35}S-amino acids, and analyzed by autoradiography as described in Materials and Methods. The autoradiogram of an SDS–12% polyacrylamide gel is shown. Each gel lane contains cell extract equal to 12 μg of protein. The compounds added as potential inducers (4.5 μmol each) are as follows: none (lane 1), propylene (lane 2), vinyl chloride (lane 3), *cis*-1,2-dichloroethylene (lane 4), *trans*-1,2-dichloroethylene (lane 5), 1,1-dichloroethylene (lane 6), trichloroethylene (lane 7), tetrachloroethylene (lane 8), 1-chloropropylene (lane 9), 1,3-dichloropropylene (lane 10), epichlorohydrin (lane 11), chloroethane (lane 12), and 1,2-dichloroethane (lane 13).

TABLE 2. Alkene monooxygenase and epoxidase activities after exposure of glucose-grown *Xanthobacter* strain Py2 to halogenated alkenes and epoxides[a]

Compound	Enzyme[b]	Sp act[c]	
		Alkene monooxygenase	Epoxidase
No inducer	NA[d]	ND[e]	ND
Propylene	Alkene monooxygenase	24.9	28.2
Vinyl chloride	Alkene monooxygenase	22.5	25.4
cis-1,2-Dichloroethylene	Alkene monooxygenase	13.7	15.6
trans-1,2-Dichloroethylene	Alkene monooxygenase	17.6	20.7
1,1-Dichloroethylene	Alkene monooxygenase	ND	ND
Trichloroethylene	Alkene monooxygenase	ND	ND
Tetrachloroethylene	Neither	ND	ND
1-Chloropropylene	Alkene monooxygenase	18.9	19.1
1,3-Dichloropropylene	Alkene monooxygenase	10.4	3.39
Epichlorohydrin	Epoxidase	19.5	19.6
Epifluorohydrin	Epoxidase	16.7	23.7
Chloroethane	Neither	ND	ND
1,2-Dichloroethane	Neither	ND	ND
1-Chloropropane	Neither	ND	ND

[a] Cells were incubated with the indicated compounds (4.5 μmol each) for 4 h and then harvested and assayed for alkene monooxygenase and epoxidase activities as described in Materials and Methods.
[b] The ability of compounds to serve as substrates for alkene monooxygenase or epoxidase was determined previously (2, 13).
[c] Activities are reported as nanomoles of substrate consumed per minute per milligram of protein.
[d] NA, not applicable.
[e] ND, no detectable activity.

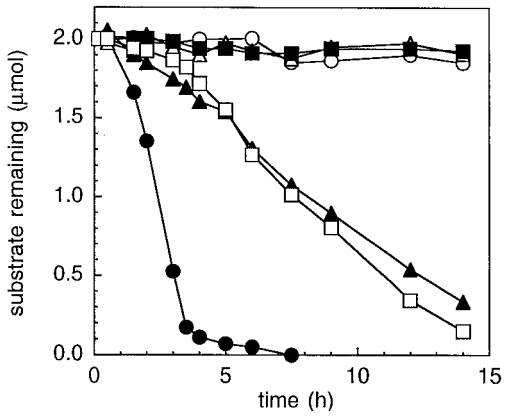

FIG. 6. Chlorinated alkenes as simultaneous inducers of, and substrates for, alkene monooxygenase. Cells were grown in batch cultures with glucose as the carbon source to an optical density (A_{600}) of 0.53. Portions (25 ml) of the cells were then transferred to 125-ml serum bottles, with or without rifampin (5 mg) added, as described in Materials and Methods. At 0 h, vinyl chloride, *trans*-1,2-dichloroethylene, and TCE (2 μmol each) were added to each bottle. The amount of substrates remaining in the bottles is plotted versus time. Symbols: ●, vinyl chloride; ○, vinyl chloride with rifampin present; □, *trans*-1,2-dichloroethylene; ■, *trans*-1,2-dichloroethylene with rifampin present; ▲, TCE; △, TCE with rifampin present.

scribed thus far that needs to be addressed is the purity of the aliphatic and chlorinated alkenes used for these experiments. It is conceivable that some of these compounds might be contaminated with low levels of impurities that might themselves contribute to, or be wholly responsible for, the inductions observed. For example, in initial experiments characterizing TCE as a possible inducer, a stock solution of TCE obtained from the supplier of the TCE used in the experiments presented in Fig. 5 and Table 2 gave positive results as an inducer. Upon analysis of this stock solution by gas chromatography, it was determined that it contained low amounts of an impurity which chromatographed with a retention time identical to that of 1,2-dichloroethylene. The stock solution was distilled to remove this impurity and was then unable to stimulate induction of the system.

In light of these findings and considerations, the compounds tested as inducers in these experiments were analyzed for possible impurities which might contribute to the induction of alkene monooxygenase and epoxidase. Both the gas and liquid phases of assay vials containing buffer and compounds at the concentrations used for the induction experiments were analyzed by gas chromatography. In the gases tested as inducers (1-butylene, ethylene, and vinyl chloride) no detectable impurities were present. Minor impurities (<0.01%) of unknown identity were detected in isobutylene oxide and epifluorohydrin. Epichlorohydrin and the other aliphatic epoxides and chlorinated alkenes were free of detectable impurities.

Chlorinated alkenes as simultaneous inducers of, and substrates for, alkene monooxygenase. The application of the findings described above to the simultaneous use of chlorinated alkenes as inducers of, and substrates for, alkene monooxygenase was investigated. Cells were grown with glucose as the carbon source and then transferred to serum bottles as described for the experiment shown in Fig. 2. Vinyl chloride, *trans*-1,2-dichloroethylene, and TCE were then added to the cells. As shown in Fig. 6, the degradation of each of the three compounds was stimulated in a time-dependent fashion analogous to that shown for propylene in Fig. 2. No degradation occurred in the presence of rifampin, demonstrating that new protein synthesis is required. In the assay bottle without rifampin, vinyl chloride was degraded at the highest rate, while TCE and *trans*-1-2-dichloroethylene were degraded at approximately equal rates. After a 7-h incubation, all of the vinyl chloride had been consumed, while the majority of the TCE and *trans*-1,2-dichloroethylene were consumed over an additional 7-h time course.

DISCUSSION

The results of the studies presented in this paper demonstrate that the enzymes involved in the metabolism of aliphatic alkenes and epoxides in *Xanthobacter* strain Py2 are regulated at the level of gene expression. A number of features of this inducible enzyme system are noteworthy. First, the presence of glucose, an alternate carbon source which supports the growth of *Xanthobacter* strain Py2, did not repress the induction of alkene monooxygenase or epoxidase. Second, a range of aliphatic and chlorinated alkenes and epoxides were able to serve as inducers of alkene monooxygenase and epoxidase. Third, the induction of the system is apparently highly specific for compounds with structural similarities to the natural substrates propylene and propylene oxide, since a range of aliphatic and chlorinated alkanes, as well as acetylene and other organic compounds, were ineffective at stimulating alkene monooxygenase and epoxidase activities or inducing protein synthesis.

The present studies add a new dimension to previous studies detailing cometabolic processes for the aerobic degradation of chlorinated hydrocarbons. Previously, the presence of glucose was shown to stimulate the degradation of chlorinated alkenes in propylene-grown suspensions of *Xanthobacter* strain Py2 by serving as a source of energy to sustain chlorinated alkene oxidations (2). The results of the present study extend this observation to demonstrate that glucose can serve as a source of carbon and energy for the synthesis of alkene monooxygenase and epoxidase with chlorinated alkenes and epoxides present as inducers. The induction and function of alkene monooxygenase is independent of the addition of the growth-supporting alkene monooxygenase substrate (propylene).

From an applied biodegradation standpoint, it is unfortunate that TCE was not itself capable of serving as an inducer of alkene monooxygenase and epoxidase in glucose-grown cells. Of the chlorinated alkenes surveyed in this study, TCE is the most prevalent groundwater pollutant, and its biodegradation has received considerable attention. It is likely that sites containing significant TCE contamination would also have quantities of dichloroethylenes and vinyl chloride which could serve as inducers of the alkene oxidation system. These compounds are most likely present as minor contaminants in commercial TCE preparations, and they are also formed as products of TCE dechlorination under anaerobic conditions (17). Figure 6 illustrates that TCE degradation does occur in a chlorinated alkene mixture with glucose-grown cells if suitable inducers are present in the mixture.

Aside from the environmental interest in chlorinated alkene degradation, the studies in this paper raise the interesting question of how alkenes and epoxides might regulate bacterial gene expression and protein synthesis. It is unclear whether both alkenes and epoxides are capable of serving as primary regulators of gene expression or whether an additional transformation of either or both classes of compounds is involved. Possibly, the epoxides are the actual molecules which activate expression of the alkene monooxygenase and epoxidase genes. Although glucose-grown cells do not have levels of alkene monooxygenase activity that are detectable by the assays described in this paper, it is possible that there is sufficient alkene

monooxygenase activity in glucose-grown cells to allow the formation of some epoxide product, which can then serve as the inducer. This possibility is supported by the observations that tetrachloroethylene, TCE, and 1,1-dichloroethylene were incapable of inducing expression. Tetrachloroethylene is not a substrate for alkene monooxygenase and therefore cannot be transformed to an epoxide or other molecule which might serve as the actual inducer. 1,1-Dichloroethylene and TCE are substrates for alkene monooxygenase, but the epoxide products of these compounds are not stable and rapidly decompose (3). Interestingly, isobutylene oxide, which was capable of inducing alkene monooxygenase and epoxidase activities nearly as efficiently as propylene or propylene oxide (Table 1), was not degraded by the epoxidase in propylene-grown cells to any detectable level. This observation supports the idea that the epoxides may be capable of serving directly as activators of gene expression. It will be of great interest in the future to determine the molecular basis of alkene and epoxide regulation of gene expression. Recent studies identifying and characterizing genes involved in microbial alkene (11) and epoxide (14) metabolism have been reported. The further characterization of these genes and related sequences should facilitate the identification of regulatory sequences, proteins, and accessory factors involved in regulating gene expression.

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