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Pyrogallol-to-Phloroglucinol Conversion and Other Hydroxyl-Transfer Reactions Catalyzed by Cell Extracts of *Pelobacter acidigallici*

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Permeabilized cells and cell extracts of *Pelobacter acidigallici* catalyzed the conversion of pyrogallol (1,2,3-trihydroxybenzene) to phloroglucinol (1,3,5-trihydroxybenzene) in the presence of 1,2,3,5-tetrahydroxybenzene. Pyrogallol consumption by resting cells stopped after lysis by French press or mild detergent (cetyltrimethylammonium bromide [CTAB]) treatment. Addition of 1,2,3,5-tetrahydroxybenzene to the assay mixture restored pyrogallol consumption and led to stoichiometric phloroglucinol accumulation. The stoichiometry of pyrogallol conversion to phloroglucinol was independent of the amount of tetrahydroxybenzene added. The tetrahydroxybenzene concentration limited the velocity of the transhydroxylation reaction, which reached a maximum at 1.5 mM tetrahydroxybenzene (1 U/mg of protein). Transhydroxylation was shown to be reversible. The equilibrium constant of the reaction was determined, and the free-energy change ($\Delta G^{\circ\prime}$) of phloroglucinol formation from pyrogallol was calculated to be −15.5 kJ/mol. Permeabilized cells and cell extracts also catalyzed the transfer of hydroxyl moieties between other hydroxylated benzenes. Tetrahydroxybenzene and hydroxyhydroquinone participated as hydroxyl donors and as hydroxyl acceptors in the reaction, whereas pyrogallol, resorcinol, and phloroglucinol were hydroxylated by both donors. A novel mechanism deduced from these data involves intermolecular transfer of the hydroxyl moiety from the cosubstrate (1,2,3,5-tetrahydroxybenzene) to the substrate (pyrogallol), thus forming the product (phloroglucinol) and regenerating the cosubstrate.

Anaerobic degradation of aromatic compounds proceeds via several independent pathways, which have been reviewed recently (4). In all strains growing anaerobically with trihydroxybenzenoids (8, 14, 17, 19), the breakdown of phloroglucinol (1,3,5-trihydroxybenzene) seems to proceed via a novel phloroglucinol pathway (15). Figure 1 shows the phloroglucinol pathway postulated for the metabolism of trihydroxybenzenoids in *Pelobacter acidigallici*. The trihydroxybenzoic acids are converted by decarboxylation to the respective trihydroxybenzenes (10, 13). Reduction of phloroglucinol, the primary attack on the aromatic nucleus, is relatively easy to demonstrate in cell-free systems (10, 12, 13, 19) and also occurs in some aerobic bacteria (2, 7). Recently the NADPH-dependent phloroglucinol reductase from *Eubacterium oxidoreducens* was purified and characterized (5). Hydrolytic cleavage of the product dihydrophloroglucinol by cell extracts of the same bacterium to 3-hydroxy-5-oxohexanoate had already been demonstrated (10). Further metabolism of the aliphatic products, regeneration of NADPH, and site(s) of ATP formation still have to be elucidated for *P. acidigallici*. The second isomer of trihydroxybenzene, pyrogallol (1,2,3-trihydroxybenzene), is never reduced directly. Transformation of pyrogallol to phloroglucinol was postulated early for *P. acidigallici* on the basis of excretion of phloroglucinol by dense cell suspensions growing on pyrogallol or gallate (13). However, demonstration of this activity in vitro was accomplished only recently with *E. oxidoreducens* (9). The authors reported a restoration of pyrogallol-phloroglucinol isomerase activity in cell extracts upon addition of 1,2,3,5-tetrahydroxybenzene and postulated that the latter is an intermediate of the reaction. Most of their findings are confirmed for *P. acidigallici* in the present paper. Moreover, evidence is provided for participation of 1,2,3,5-tetrahydroxybenzene as the cosubstrate rather than the intermediate of the transhydroxylation reaction, and nonspecific reactions of cell extracts with other di- and trihydroxylated benzenes are reported.

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MATERIALS AND METHODS

Cultivation of *P. acidigallici*. Strain MaGal2 (DSM 2377) was grown in bicarbonate-buffered, sulfide-reduced salt-water mineral medium under a 90% N_2–10% CO_2 atmosphere. The basal medium had the following composition (in millimoles per liter): KH_2PO_4, 1.5; NH_4Cl, 4.7; KCl, 6.7; $CaCl_2 \cdot 2H_2O$, 1.0; NaCl, 342.0; $MgCl_2 \cdot 6H_2O$, 15.0. Sodium bicarbonate (30 mmol/liter), sodium sulfide (1 mmol/liter), trace-element solution SL 10 (20), and vitamin solution (21) were added to the autoclaved medium from sterile stock solutions as described in detail previously (21). The pH was adjusted to between 7.2 and 7.3 with HCl. Aliquots (100-ml) were dispensed into sterile 125-ml infusion bottles, which were sealed with a latex septum and gassed with N_2-CO_2. The substrate sodium gallate (7.5 mmol/liter) was added from a sterile stock solution which was filter sterilized (pore size, 0.2 μm) and stored under an N_2 atmosphere. The cultures were incubated at 30°C in the dark.

Preparation of dense cell suspensions and cell extracts. Cells were harvested in the late exponential growth phase by centrifugation at 3,300 × *g* for 20 min in an infusion bottle under N_2 in a rotor equipped with rubber adaptors. Cells were washed in the same volume of 50 mM N_2-sparged sodium phosphate buffer (pH 7.0) containing the same amounts of NaCl and $MgCl_2$ as the medium to prevent lysis. The suspension was centrifuged again and finally suspended in 2 to 3 ml of buffer to yield a stock suspension with a final cell density of 1.7 mg (dry weight) per ml. The dense cell

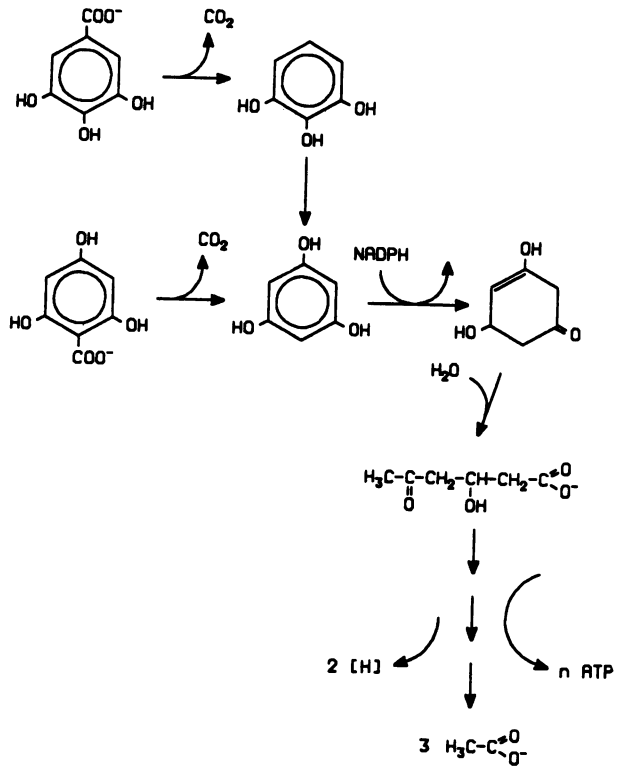

FIG. 1. Proposed phloroglucinol pathway for acetate formation from trihydroxybenzenoids in *P. acidigallici*. For explanations, see the Introduction.

suspensions were transferred into N_2-gassed vials (5 ml) with butyl rubber septa and kept on ice until used. Cell extracts were prepared by disruption at 140 MPa in a French pressure cell, which was gassed with N_2 and equipped to maintain anaerobic conditions throughout the procedure. The crude extract was centrifuged again in N_2-gassed vials at 5,000 × *g* and 4°C to remove cell debris.

Permeabilization of cells in dense suspensions. Ten minutes before the reaction was started, the mild detergent cetyltrimethylammonium bromide (CTAB) was added to the dense cell suspensions. Before this, the amount of CTAB necessary for sufficient permeabilization of the bacterial membranes was determined by measuring the activity of dihydrophloroglucinol reductase by the method of Patel et al. (12) in dense cell suspensions at various CTAB concentrations. Dihydrophloroglucinol-dependent NADPH oxidation is not detectable with intact cells and rises with increasing CTAB concentration in the assay. Maximum activity was reached at 0.5 mg of CTAB per mg (dry weight) per 5 ml of buffer; these amounts were then used in all permeabilization experiments.

Assay of transhydroxylating activity. Assays were performed in N_2-gassed vials with butyl rubber septa at room temperature. The reaction mixture contained cell suspension or cell extracts, substrates, and the N_2-gassed sodium phosphate buffer described above in a final volume of 2 ml. The reaction was started by addition of the substrates. Samples (100 μl) were taken at regular intervals, and the reaction was stopped by injection of the samples into 1-ml high-pressure liquid chromatography (HPLC) vials gassed with N_2 and containing 400 μl of 0.1 M H_3PO_4. Samples were kept on ice and analyzed within 1 h. All additions and samples were handled with gas-tight Unimetrics microliter syringes to prevent the entry of air into the system. In addition, reaction mixtures lacking either cells or cell extract, or heated for 5 min at 100°C before addition of the substrates, were used as controls.

Analysis of substrate conversion rates and identification of products. Samples were analyzed with a Beckman System Gold high-pressure liquid chromatograph equipped with an Ultrasphere-ODS column (4.6 by 150 mm), with a 100 mM ammonium phosphate buffer–methanol solvent system (pH 2.6) of varying composition. Samples (20 μl) were injected with a Spark Promise I autosampler and eluted at a flow rate of 1 ml/min, and aromatic compounds were detected in a Beckman 167 scanning variable-wavelength detector at the appropriate wavelengths. Data were analyzed by a computer program and quantitated by comparison with external and internal standards of known composition. Peak identification was performed by on-line spectral scans of respective peaks and comparison of retention time and UV spectrum with those of standard samples. Rates were expressed in units; 1 U was defined as turnover of 1 μmol of substrate per min.

Protein assay. Protein was determined by a microbiuret method modified from the method of Zamenhoff (23), with bovine serum albumin as the standard.

Chemicals. 1,2,3,5-Tetrahydroxybenzene was synthesized as described by Baxter and Brown (1) and further purified by chromatography on an XAD-2 column. The compound was applied and eluted with water, while several contaminations were bound to the matrix and could be eluted only with methanol. Purity was confirmed by HPLC. All other chemicals used were of analytical grade.

RESULTS

Restoration of transhydroxylase activity in cell extracts. Suspensions of resting cells of *P. acidigallici* readily degraded pyrogallol at a rate comparable to that of substrate consumption by growing cells (1 U/mg of protein). With cell extracts of the same preparation, or after permeabilization of the cell suspension with CTAB, no pyrogallol-degrading activity was detectable.

Krumholz and Bryant (9) reported a qualitative restoration of transhydroxylase activity in crude extracts of *E. oxidoreducens* upon addition of 1,2,3,5-tetrahydroxybenzene. We confirmed this for *P. acidigallici*. Consumption of pyrogallol and formation of phloroglucinol in the presence of tetrahydroxybenzene were stoichiometric (Fig. 2). Similar results were obtained with permeabilized cells in dense cell suspensions (data not shown).

Transhydroxylase activity was linearly dependent on the amount of cell extract added. A saturating tetrahydroxybenzene concentration was reached at 1.5 mM tetrahydroxybenzene with 1 U/mg of protein in the extract (Fig. 3); at higher tetrahydroxybenzene concentrations, activity decreased again. The maximum velocity of the in vitro reaction corresponds to the activity found in resting as well as in growing cells. The K_m for tetrahydroxybenzene was calculated to be 0.71 mM by double-reciprocal plot; V_{max} was 1.54 U/mg of protein.

Addition of reducing agents to assays and preparations did not increase the transhydroxylase activity, nor did exposure to air destroy activity. However, all experiments had to be performed under a nitrogen atmosphere owing to the oxygen sensitivity of tetrahydroxybenzene and other hydroxybenzenes tested in the assay (see below). Control experiments showed the stability of substrates in the absence of cell suspension or cell extract under the assay conditions. Heat-

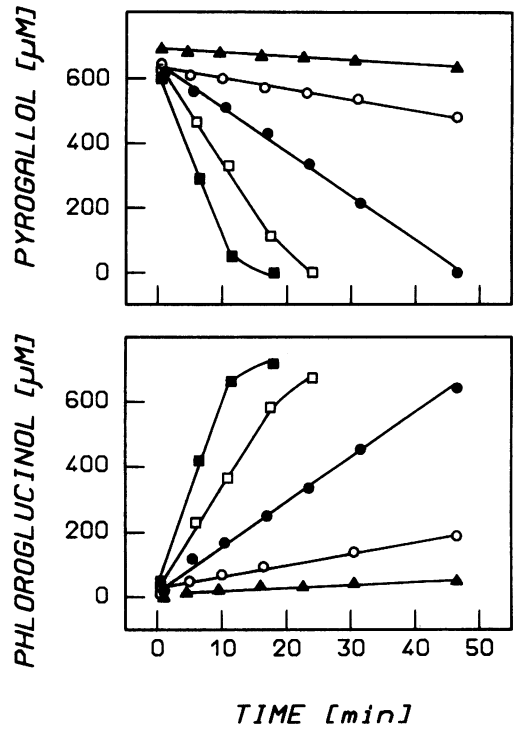

FIG. 2. Conversion of pyrogallol to phloroglucinol by cell extracts of *P. acidigallici*. Assays contained cell extract (0.091 mg of protein per ml), pyrogallol, and increasing amounts of tetrahydroxybenzene (35 μM [▲], 88 μM [○]; 175 μM [●], 350 μM [□], and 700 μM [■]) and were performed as described in Materials and Methods.

inactivated extracts completely lost transhydroxylating activity.

High concentrations of dimethyl sulfoxide (DMSO) could replace tetrahydroxybenzene as a cosubstrate, as already reported by Krumholz and Bryant for *E. oxidoreducens* (9). We could confirm their observation of an odor of dimethyl sulfide (DMS) appearing along with the nonstoichiometric conversion of pyrogallol to phloroglucinol. In *P. acidigallici* cell extracts, an activity of 0.23 U/mg of protein was reached with 640 mM DMSO in the assay. Less than 0.25 mM tetrahydroxybenzene was necessary to give the same activity; obviously, tetrahydroxybenzene was more than 2,500 times as effective as DMSO. Furthermore, the maximum activity in the presence of tetrahydroxybenzene was roughly four times higher.

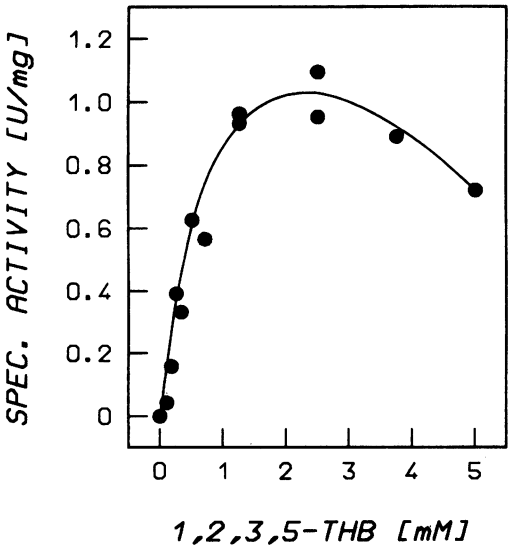

FIG. 3. Dependence of pyrogallol conversion rate on tetrahydroxybenzene concentration. Experimental conditions were as described in the legend to Fig. 2.

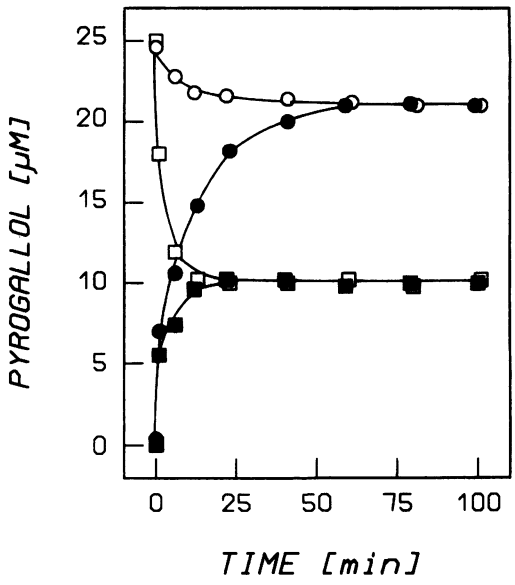

FIG. 4. Reversibility of transhydroxylation in the presence of tetrahydroxybenzene and determination of the equilibrium of the reaction. The assay contained cells (0.151 mg [dry weight] per ml) permeabilized with CTAB, either no (■, ●) or 25 μM (□, ○) pyrogallol, and either 5 mM (■, □) or 10 mM (●, ○) phloroglucinol. The reaction was started by the addition of 0.35 mM tetrahydroxybenzene.

Reverse reaction of transhydroxylase and determination of the reaction equilibrium. No data on thermodynamics or reversibility of the transhydroxylation reaction are available in the literature so far. Figure 4 shows an experiment in which the pyrogallol concentration was monitored over time in the presence of tetrahydroxybenzene (0.35 mM) and a background of 5 or 10 mM phloroglucinol until equilibrium was reached. With either no pyrogallol or a small amount just above the equilibrium concentration present at the start of the reaction, the pyrogallol concentration decreased or increased to a final equilibrium. The equilibrium concentration was independent of the initial pyrogallol concentration, but depended on the background of phloroglucinol present (5 or 10 mM). The equilibrium constant of the transhydroxylase reaction was calculated as 475 and 500 for the two phloroglucinol concentrations, which corresponds to a $\Delta G^{\circ\prime}$ of −15.4 or −15.5 kJ/mol, respectively.

Transformation of other hydroxylated benzenes by permeabilized cells of *P. acidigallici*. Controls showed that cell extracts or permeabilized cells incubated with tetrahydroxybenzene alone transformed the latter to phloroglucinol (stoichiometry, 2:1) and a very polar product detectable by HPLC, which was probably pentahydroxybenzene (see Fig. 6B). The same reaction was noticed when pyrogallol was present in the assay, but it proceeded much more slowly and depended on the tetrahydroxybenzene concentration (Fig.

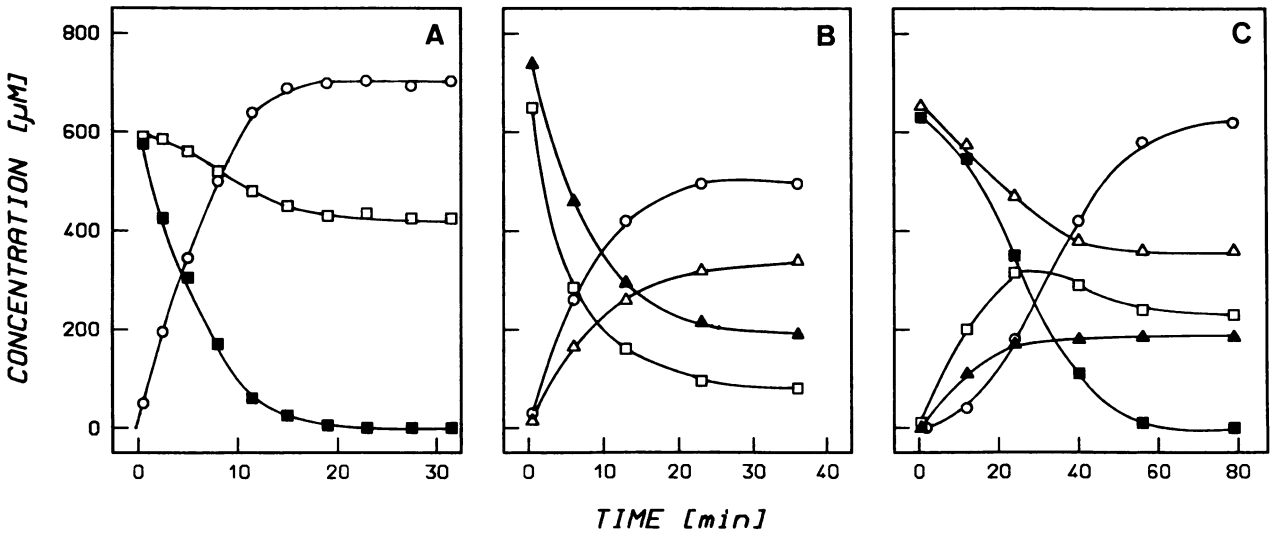

FIG. 5. Reaction of permeabilized cells (0.164 mg [dry weight] per ml) with equimolar amounts of (A) pyrogallol (■) and tetrahydroxybenzene (□), (B) resorcinol (▲) and tetrahydroxybenzene (□), or (C) pyrogallol (■) and hydroxyhydroquinone (△). (A) Formation of phloroglucinol (○); (B) formation of phloroglucinol (○) and hydroxyhydroquinone (△); (C) formation of resorcinol (▲), tetrahydroxybenzene (□), and phloroglucinol (○).

5A). This observation suggested a possible transfer of a hydroxyl group from the 2-position of tetrahydroxybenzene to the 5-position of pyrogallol, which would produce phloroglucinol by dehydroxylation from tetrahydroxybenzene and regenerate tetrahydroxybenzene by hydroxylation of pyrogallol (Fig. 6A). We tested this hypothesis by substituting either the putative hydroxyl donor or the hydroxyl acceptor with other hydroxylated benzenes, thus avoiding the problem of identical cosubstrate and coproduct of the reaction.

Figure 5B shows an experiment in which pyrogallol was replaced by resorcinol. Tetrahydroxybenzene and resorcinol both disappeared rapidly, with phloroglucinol and hydroxyhydroquinone being formed (Fig. 6D). Hydroxyhydroquinone accumulation was not completely stoichiometric with phloroglucinol production because it was also readily hydroxylated by tetrahydroxybenzene (Fig. 6C), as demonstrated in control experiments.

Figure 5C gives the result of a similar experiment in which the putative hydroxyl donor, tetrahydroxybenzene, was substituted with hydroxyhydroquinone. At first, hydroxyhydroquinone and pyrogallol disappeared, with concomitant formation of resorcinol and tetrahydroxybenzene (Fig. 6E). This reaction came to equilibrium quickly, but the pool of tetrahydroxybenzene formed was sufficient to transform the remaining pyrogallol to the final product, phloroglucinol (Fig. 6A), which is the physiological function of the enzyme.

We tested all available hydroxylated benzenes for their ability to substitute for either pyrogallol or tetrahydroxybenzene or both in the transhydroxylation reaction. Figure 6 summarizes the positive combinations exhibiting transhydroxylating activity with permeabilized cell suspensions in experiments similar to those in Fig. 5, which were assayed for a minimum period of 1 h. Unfortunately, the other isomers of tetrahydroxybenzene and pentahydroxybenzene could not be identified owing to the lack of commercially available reference substances (Fig. 6B, C, and F). However, all the respective HPLC product peaks were in the expected hydrophobicity range. Hexahydroxybenzene, although commercially available, was not tested because of instability in aqueous solution. When the product compounds were commercially available, all reactions shown in Fig. 6 were also measured in the reverse direction. Furthermore, traces of hydroxyhydroquinone were formed in similar assays with tetrahydroxybenzene as the donor and hydroquinone as the acceptor. Hydroxylation of resorcinol by hydroxyhydroquinone could not be detected, probably because the reaction led to products identical to the substrates; however, the reaction of hydroxyhydroquinone with itself (Fig. 6F) was completely inhibited by the presence of equimolar amounts of resorcinol (data not shown). No reaction could be detected with combinations of tetrahydroxybenzene with catechol or phenol or with combinations of pyrogallol with catechol, resorcinol, hydroquinone or phenol.

DISCUSSION

The present paper gives the first report on stoichiometric conversion of pyrogallol to phloroglucinol in the presence of 1,2,3,5-tetrahydroxybenzene, catalyzed by permeabilized cells and cell extracts of *P. acidigallici*. The enzyme activity in vitro is sufficient to account for pyrogallol metabolism of growing cells. On the basis of side reactions with other hydroxybenzenes observed in vitro, a new reaction mechanism for pyrogallol conversion to phloroglucinol is discussed.

Krumholz and Bryant were the first to observe a transformation of pyrogallol to phloroglucinol in cell extracts when DMSO was added to the assay. This reaction seems to be very nonspecific, since DMSO had to be added in the molar range (9). The same observation was made with *P. acidigallici*. However, the amount of DMSO necessary to achieve the same specific activity was 2,500 times higher than with tetrahydroxybenzene.

From growth rates of *P. acidigallici* on pyrogallol and the corresponding molar growth yield (14), an in vivo transhydroxylase activity of about 1 U/mg of protein must be postulated. This corresponds to in vitro activities in cell extracts at a tetrahydroxybenzene concentration of 1.5 mM.

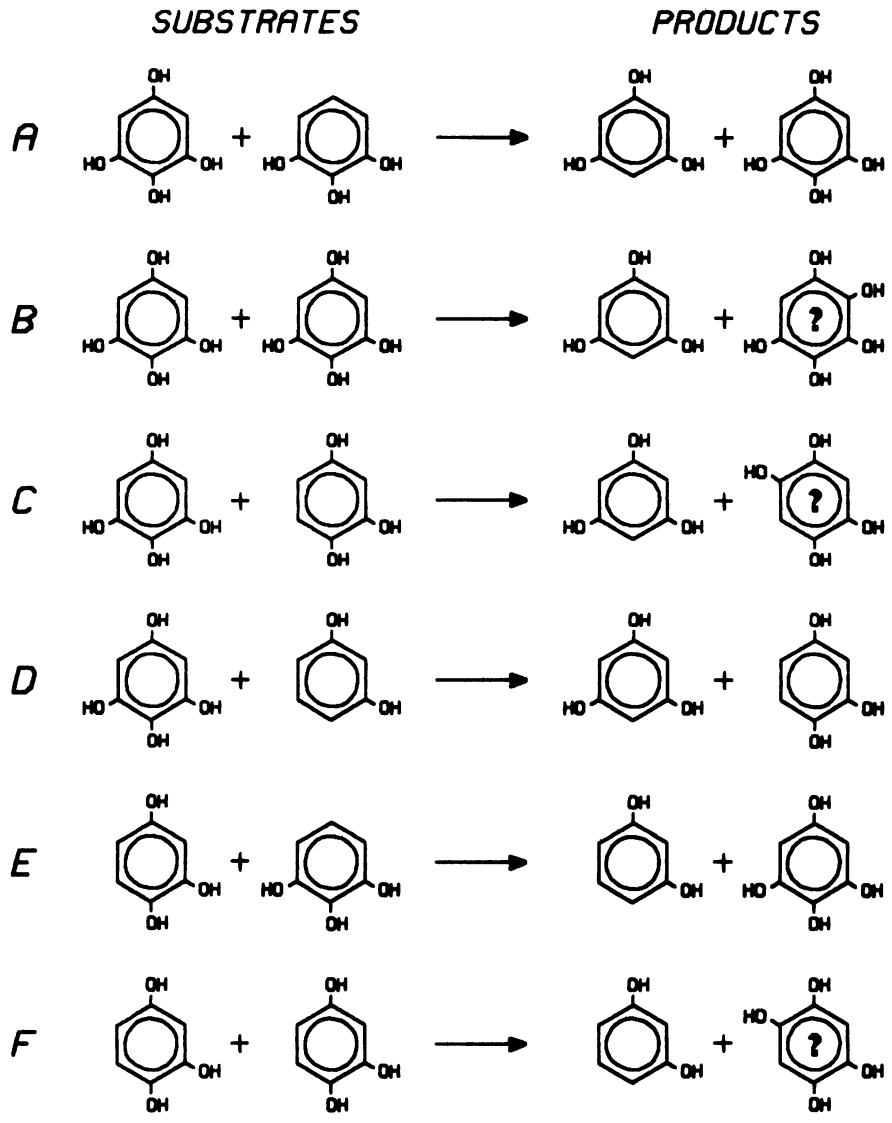

FIG. 6. Substrate combinations reacting in the presence of permeabilized cells, and the corresponding products formed. The structural formulas containing a question mark could only tentatively be identified by HPLC owing to the lack of commercially available reference compounds, but were represented as peaks in the chromatogram at the expected retention times. For further explanations, see the Results.

This transhydroxylase activity exceeds the value of 0.75 U/mg of the 5.2-fold-enriched enzyme in the presence of 1 mM tetrahydroxybenzene that can be calculated from the data of Krumholz and Bryant obtained with *E. oxidoreducens* (see Fig. 4 in reference 9). Furthermore, neither formation of other isomers of tetrahydroxybenzene in the reaction with pyrogallol nor successive disappearance and reaccumulation of tetrahydroxybenzene during the reaction was observed in our experiments. These observations could be due to side reactions in the derivatization procedure necessary for gas chromatography analysis as used by Krumholz and Bryant (9); our analyses with direct injection of the samples on a HPLC column provided highly reproducible chromatograms.

Krumholz and Bryant (9) were the first to report the conversion of pyrogallol to phloroglucinol in the presence of 1,2,3,5-tetrahydroxybenzene and cell extracts of *E. oxidoreducens*. They postulated tetrahydroxybenzene as an intermediate in a reaction sequence involving oxidative hydroxylation of pyrogallol to tetrahydroxybenzene and subsequent reductive dehydroxylation of the intermediate to phloroglucinol. Instead, we suggest a different mechanism for transhydroxylation based on our results with permeabilized cells and cell extracts of *P. acidigallici*, which showed some very interesting side reactions. With certain combinations of hydroxybenzenes, cell extracts exhibited a transhydroxylating activity such that one substrate was reductively dehydroxylated and the other was oxidatively hydroxylated. When applied to the reaction of tetrahydroxybenzene with pyrogallol, this model calls for tetrahydroxybenzene in addition to phloroglucinol as a product of the reaction (Fig. 6A). Consequently, no net turnover of tetrahydroxybenzene could be measured. Nevertheless, if one of the substrates was exchanged for other hydroxybenzenes, the formation of the predicted products could be measured. This was true for the putative hydroxyl donors tetrahydroxybenzene and hydroxyhydroquinone, as well as for a much wider range of putative hydroxyl acceptors (Fig. 6B to F). The same mechanism seems to act in *E. oxidoreducens*, for the authors report a conversion of hydroxyhydroquinone to resorcinol

and isomers of dihydroxyquinone, possible oxidation products of tetrahydroxybenzenes. They also observed a formation of phloroglucinol from tetrahydroxybenzene alone. The fact that these activities copurify with the transhydroxylase suggests that all hydroxyl transfer reactions occur with the same enzyme system.

The given hypothesis demands a pool of 1,2,3,5-tetrahydroxybenzene in the cells; a pool size larger than 1 mM for in vivo activities can be deduced from the in vitro data. Attempts to show the presence of tetrahydroxybenzene in resting cells have failed so far, probably owing to insufficient sensitivity of the tetrahydroxybenzene assay. Theoretical estimation of the tetrahydroxybenzene concentration expected in the assay upon lysis of the cells yields a maximal concentration of 0.5 μM, assuming a hypothetical concentration of 1 mM inside intact cells and a 2,000-fold dilution of cell contents upon lysis.

The proposed reaction mechanism of transhydroxylation should be supported by studies with pure enzyme preparations and by labeling experiments. No incorporation of $H_2{}^{18}O$ into phloroglucinol would be expected, owing to the transfer of the hydroxyl group between the aromatic reaction partners. Unfortunately, the experiment is hampered by several problems, and Krumholz and Bryant, who have already tried the experiment with *E. oxidoreducens*, report problems with a free label exchange in the controls (9). Further evidence of transhydroxylation that is more reliable than a negative result for ^{18}O incorporation could be given by the use of [*ring*-^{14}C]pyrogallol. The label in the product phloroglucinol should be lower than in the substrate, owing to dilution of the label in the unlabeled tetrahydroxybenzene pool, through which every molecule must pass. Specific radioactivity of phloroglucinol should depend on the pool size. The experiment is hampered by the lack of commercially available [^{14}C]pyrogallol and the cost of custom synthesis.

The products of the nonspecific reaction of cell extracts with hydroxyhydroquinone offer an answer to the question why *P. acidigallici*, which is able to grow on pyrogallol and phloroglucinol, cannot thrive on this third isomer of trihydroxybenzene. Apart from addressing whether the cells are capable of taking up hydroxyhydroquinone, this isomer would act as a metabolic poison in the organism. For every hydroxyhydroquinone molecule hydroxylated, one molecule of tetrahydroxybenzene would be depleted from the tetrahydroxybenzene pool (Fig. 6C). No regeneration of the cosubstrate would occur, because instead of 1,2,3,5-tetrahydroxybenzene, a different isomer of tetrahydroxybenzene would be the hydroxylation product.

On the basis of the postulated reaction mechanism, the enzyme should not be named pyrogallol-phloroglucinol isomerase (9) but, rather, tetrahydroxybenzene:pyrogallol hydroxyltransferase (transhydroxylase), analogous to the transaminases. This group of enzymes is also not classified among the oxidoreductases, but among the transferases, although they catalyze the oxidative deamination of the donor and the reductive amination of the acceptor (6). An analogous argument would apply to the transhydroxylase described here.

During metabolism of trihydroxybenzenes in *P. acidigallici*, no net consumption or production of redox equivalents occurs (14). However, in growing cultures of *P. acidigallici*, a net demand for oxidizing power or release of redox equivalents must be postulated, since an increase in cell material creates a demand for increasing the tetrahydroxybenzene pool in the growing cells. This "anaplerotic reaction" could be a net hydroxylation of pyrogallol. The nature of the physiological electron acceptor and of a possible redox carrier in the transhydroxylation reaction is unknown. To estimate the redox potential (E_0') of the reaction, the E_0' of the oxidative hydroxylation of benzoic acid to 3-hydroxybenzoic acid was calculated with −84 mV from $G_f°$ data (16). Apparently, DMSO can serve as an oxidant, as the E_0' of the DMSO|DMS half-cell has been determined as +160 mV (22). DMSO was reported to serve as the physiological electron acceptor in several anaerobic (24) and phototrophic (11) bacteria, but the tremendous concentrations necessary to initiate transhydroxylation in *E. oxidoreducens*, as well as in *P. acidigallici*, excludes a physiological role in this reaction. Other redox carriers with the appropriately high redox potential are not easily visualized in a fermentative bacterium, especially with a metabolism providing no surplus electrons, as in *P. acidigallici* (Fig. 1).

An interesting but not yet understood observation is the effect of the presence of tetrahydroxybenzene or DMSO in the assay on the utilization of pyrogallol by whole cells in dense suspensions. Cells incubated with pyrogallol in the absence of tetrahydroxybenzene or DMSO express a distinct lag phase before pyrogallol consumption reaches the maximum activity. This lag phase virtually disappears in the presence of tetrahydroxybenzene or DMSO. Apparently, resting cells are lacking oxidation potential for expressing transhydroxylase activity. Although DMSO may pass the cytoplasmic membrane freely to serve as an oxidant acting on the tetrahydroxybenzene pool in the cell, tetrahydroxybenzene itself may not necessarily enter intact cells and presumably reacts somewhere at the outer face of the cytoplasmic membrane.

Transformation of pyrogallol to phloroglucinol was reversible, even though the equilibrium was far on the side of phloroglucinol, as shown in the Results. The only data available concerning the free energy change possibly involved in the transhydroxylation reaction are the $G_f°$ values, which can be calculated by the increment procedure in a reference book (3). This procedure is very general, and the calculated $\Delta G°'$ (−7.35 kJ/mol) is much lower than the experimentally determined $\Delta G°'$ (−15.5 kJ/mol) reported in this paper. The reason for this discrepancy probably lies in the excellent resonance stabilization of the highly symmetrical phloroglucinol as compared with pyrogallol, which is not taken into account by the generalized data in the reference book. The free energy of the transformation reaction is not exploited by *P. acidigallici*, as indicated by identical growth yields with pyrogallol or phloroglucinol as the substrate (14).

Even though the equilibrium of transhydroxylation lies far on the side of phloroglucinol, there is evidence in the literature of an aerobic organism that makes use of the reverse reaction. A strain of *Fusarium solani* is reported to convert phloroglucinol to pyrogallol prior to aerobic degradation of the aromatic nucleus via the *meta* fission pathway (18). The authors postulate an intermolecular arrangement of the hydroxyl groups, but show an intramolecular mechanism by formation of a resorcinol epoxide in the figure to which they refer. Because of the unfavorable equilibrium, this organism must have a high affinity for the product pyrogallol, probably provided by an oxygenase reaction. Aerobic bacteria seem to favor a reductive attack on phloroglucinol (2, 7), as anaerobic (10, 12, 13) and phototrophic (19) bacteria do.

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