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PROPOSED MECHANISM FOR MICROBIAL DEGRADATION OF POLYACRYLATE

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ABSTRACT

A possible aerobic degradation mechanism for polyacrylate (PA) was examined with acrylic oligomer-utilizing bacteria (*Microbacterium* sp., *Xanthomonas maltophilia*, and *Acinetobacter* sp.), using a model compound (1,3,5-pentane tricarboxylic acid, PTCA). Acyl-coenzyme A synthetase activities were detected with dialyzed cell-free extracts of PTCA-utilizing bacteria toward PTCA, PA 500, and PA 1000. This result suggested that PA is activated by coenzyme A and metabolized via PA-coenzyme A. Metabolic products formed from PTCA were detected in culture filtrates and reaction mixtures of washed cells. Fraction A was detected as a main metabolite by high-performance liquid chromatography. A small amount of fraction B was concomitant with fraction A. Also, another fraction, C, was detected. These intermediate metabolites were characterized by LC-MS as 1,3,5-(1- or 2-pentene)tricarboxylic acid for fractions A and B and as 1,3,5-(2-oxopentane)tricarboxylic acid for fraction C. Fraction A was metabolized far faster than fraction B. Fraction B was thought to be an artifact formed from fraction A under alkaline conditions. Thus PTCA and also PA seemed to be metabolized by the mechanism similar to β-oxidation of fatty acids. The degradation of PTCA by washed cells was slower than that by growing cells and was inhibited by 5 mM NaN_3. This suggests that the metabolism is linked to a respiratory chain of bacteria.

THE METABOLISM OF PTCA AS A MODEL FOR ACRYLIC OLIGOMERS

PTCA-utilizing bacteria (*Microbacterium* sp., *Xanthomonas maltophilia*, and *Acinetobacter* sp.) [1] grew on 2-methylglutarate (acrylic dimer) and PTCA, but appreciable amounts of metabolic products could not be detected in a culture supernatant or reaction mixture of intact cells with 2-methylglutarate. As accumulation of metabolic products from PTCA was found on HPLC, this compound was used as a model. No detectable amounts of significant artifacts were detected with an autoclaved PTCA medium. Then, culture supernatants of PTCA-utilizing bacteria were analyzed by HPLC. The main product was fraction A with a small amount of fraction B formed, as shown in Fig. 1 [2]. Although a large amount of fraction A was formed in 2–3 days, it was quickly further metabolized and disappeared from the culture supernatant after 4–5 days. Fraction B was only slowly metabolized and remained in the culture supernatant for an extended time (5–7 days). The same elution profiles as those with the culture supernatants of growing cells were obtained with reaction mixtures of washed cells, although the substrate was metabolized more slowly by washed cells than by growing cells. Substantially no difference in

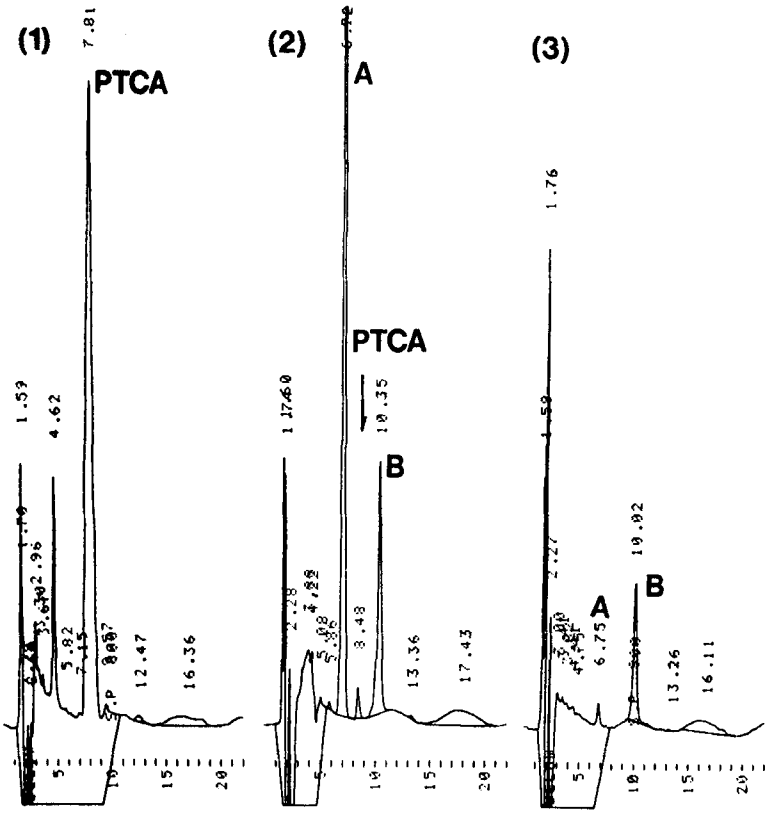

FIG. 1. Time course for the consumption of PTCA and the amounts of Fractions A and B formed by growing cells of *Microbacterium* sp. (1) Autoclaved medium; (2) 3-day culture supernatant; (3) 7-day culture supernatant. Reprinted from Ref. 2.

the formation of metabolic products by the three bacteria was found with either culture supernatants or reaction mixtures. Biodegradation rates and accumulation of intermediate metabolites were affected by aerobic conditions. With increased shaking (120 rpm), approximately 100% of PTCA was consumed in 2 days, but small amounts of metabolites accumulated in the culture supernatant. With moderate shaking (100 rpm), considerable amounts of metabolites accumulated in 2–3 days in the culture supernatant.

Culture supernatants were analyzed by LC-MS on Waters or Cosmosil $5C_8$. By positive and negative ion detection, the molecular weights of fractions A and B were suggested to be the same, 202, which is 2 less than the molecular weight of the substrate, 204. Fractions A and B were eluted later than the substrate, suggesting that these compounds are less hydrophilic. Therefore, fractions A and B were consistent with 1,3,5-(1- or 2-pentene)tricarboxylic acid. Fraction B was found to be an artifact formed from fraction A at an alkaline site. Also, another peak was eluted on Cosmosil 5CN-R. The molecular weight of fraction C was considered to be 217 by negative ion detection. Considering the β-oxidation pathway, 1,3,5-(2-oxopentane) tricarboxylic acid is consistent with fraction C.

From these results, the metabolic pathway for PTCA is proposed, as shown in Fig. 2. Considering a β-oxidation mechanism is working for PTCA, fraction A as the main metabolite might correspond to 1,3,5-(1-pentene)tricarboxylic acid. Two possibilities still remain for the metabolic pathway of PTCA: 1) decarboxylation of a depolymerized compound by one acrylic unit followed by the ordinary β-oxidation process to liberate acetic acid and 2) repeated liberation of malonic acid from the depolymerized compound. Malonic acid is known as a potent inhibitor for succinate dehydrogenase in eucaryotic cells. As three kinds of PTCA-utilizing bacteria utilized malonic acid as a growth substrate, malonic acid is nontoxic to these procaryotic cells.

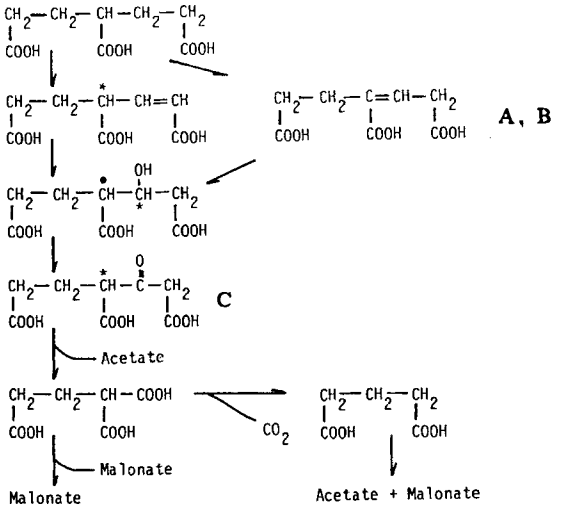

FIG. 2. The proposed metabolic pathway for PTCA. Reprinted from Ref. 2.

PROPOSED METABOLIC PATHWAY FOR PA

As PTCA and PA 500 and PA 1000 can be activated by acyl-coenzyme A synthetase which is a key enzyme for starting the β-oxidation of saturated fatty acids and organic acids [1]. As described above, the characterization of metabolic products also suggested that enzymes similar to those for β-oxidation are involved in the metabolism of PTCA. PA 1000-3000 was metabolized by washed cells of PTCA-utilizing bacteria. As the oxidation site is known to be located on bacterial membranes, a polymer has to contact the metabolizing enzymes on a cytoplasmic membrane. Actually the degradation of PTCA by washed cells was completely inhibited by 5 mM NaN_3, an inhibitor for a respiratory chain, suggesting that the metabolism of PTCA proceeds on the cytoplasmic membrane. When considering a polymer, a short length of the chain might penetrate through the outer cell membrane or cell wall, although long chain polycarboxylic acids could hardly reach the cytoplasmic membrane. Therefore, it is quite reasonable to think that the metabolism of PA is exogenous and not randomly endogenous.

CONCLUSION

1. PTCA, PA 500, and PA 1000 were activated by coenzyme A.
2. The intermediate metabolites from PTCA, a model for the metabolism of PA, were identified as 1,3,5-(1- or 2-pentene)tricarboxylic acid and 1,3,5-(2-oxopentane)tricarboxylic acid.
3. From these results, PA seemed to be metabolized by the mechanism similar to β-oxidation of fatty acids.
4. The metabolism seemed to be linked with the respiratory chain of bacteria.
5. The degradation mechanism is possibly exogenous.

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