Short Communication

Microbial Reduction of Benzoate to Benzyl Alcohol

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Received April 7, 1988

The microbial degradation of benzoate is known to be initiated by its oxidation to catechol or monohydroxy benzoate under aerobic conditions¹⁾ and through ring hydrogenation on anaerobic metabolism.²⁾ In the course of a screening program for microbes able to convert aromatic compounds, we observed the reduction of benzoate to benzyl alcohol by a bacterium, *Nocardia asteroides* JCM 3016, and several strains of molds. Such reduction of the carboxyl group is thought to be a unique reaction occurring in microbial cells under aerobic conditions.

N. asteroides JCM 3016 was cultured in test tubes (18×180 mm) containing 5 ml of the complex medium (medium GS) reported by Shimizu *et al.*,³⁾ at 30°C for 48 hr with aerobic shaking. To each 48 hr-culture was added sodium benzoate to a final concentration of 0.5% (w/v), and then the shaking was continued for a further 48 hr at 30°C. The pooled clear supernatants of the reaction mixtures in the tubes (45 ml) were extracted with ethyl acetate. The solvent was removed under reduced pressure to give an oil mainly consisting of benzyl alcohol (90 mg), as judged on gas chromatography.⁴⁾ The benzyl alcohol was purified by preparative TLC (SiO₂, 60F₂₅₄, 20×20 cm, Merck, benzene:ether:acetic acid = 45:10:5). The ¹H NMR spectrum (CDCl₃) of the preparation [δ 1.62 (s, 1H), 4.70 (s, 2H), 7.3~7.4 (m, 5H)] agreed precisely with that of authentic

benzyl alcohol.

The microbial conversion was carried out using [α -¹³C] benzoate (10 atom% ¹³C) as the substrate, and the product, benzyl alcohol, was analyzed by ¹³C-NMR. As shown in Fig. 1, the peak intensity ratio of -CH₂- to C-4 of the product of the ¹³C-enriched benzoate (Fig. 1A) was 10 times higher than that of the authentic benzyl alcohol (Fig. 1B). This indicates that the α -carbon of benzyl alcohol is directly derived from the α -carbon of benzoate, and that the reduction of the -COOH group to -CH₂OH occurs in the microbial cells under aerobic conditions.

Aerobic shaking reaction of benzoic acid with *N. asteroides* JCM 3016 afforded benzyl alcohol in a good yield (60%) (Fig. 2). Benzoate was almost completely consumed on 60 hr-incubation, and the pH of the reaction

FIG. 1. 67.9 MHz ¹³{¹H}NMR Spectra of Benzyl Alcohol in CDCl₃.

(A) Benzyl alcohol produced by *N. asteroides* JCM 3016 from [α -¹³C] benzoate (10 atom% ¹³C) under the conditions given in the text. Sodium [α -¹³C] benzoate (99 atom% ¹³C) (MSD Isotopes, Quebec, Canada) was diluted 10 times with naturally abundant (1.1% ¹³C) benzoate and then added to the culture fluid. (B) Authentic benzyl alcohol (1.1 atom% ¹³C, natural abundance).

FIG. 2. Time Course of the Conversion of Benzoate to Benzyl Alcohol by *N. asteroides* JCM 3016.

The bacterium was grown in medium GS for 48 hr at 30°C, and then the reaction was started by the addition of sodium benzoate to a final concentration of 0.5%. The cultivation and the following reaction were carried out in test tubes under reciprocal shaking. After the indicated periods, three tubes were taken, and then benzoic acid (○) and benzyl alcohol (●) were assayed. Trimethylsilyl derivatives of benzoate and benzyl alcohol were determined by gas chromatography.³⁾

mixture ranged from 6.5 to 5.0 throughout the reaction. Different substituted benzoates, such as 3- or 4-hydroxybenzoate, 2- or 3-fluorobenzoate, 2-, 3- or 4-chlorobenzoate, 3- or 4-iodobenzoate, or 2-, 3- or 4-bromobenzoate,

were also converted to the corresponding benzyl alcohols.

The ability to reduce benzoate added to the culture medium to benzyl alcohol was surveyed in a variety of microorganisms including 331 strains of molds belonging to 97 genera and 62 strains of nocardioform bacteria, the molds being grown in medium containing 5% glucose and 5% corn steep liquor. Several molds, including *Rhizopus oryzae* IFO 5440 and IFO 4706, *Mucor fragilis* IFO 6449 and *Mucor javanicus* IFO 4569, were found to exhibit this ability. From the results of the screening, the reduction of the -COOH group substituted in the benzene ring does not seem to be a special reaction in a limited number of microbes. The enzyme system participating in the reduction will be described elsewhere.

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