

## Production of Epoxides from $\alpha,\beta$ -Halohydrins by *Flavobacterium* sp.

JOHN GEIGERT,\* SAUL L. NEIDLEMAN, TE-NING E. LIU, SUSANNE K. DEWITT, BARBARA M. PANSCHAR, DEMETRIOS J. DALIETOS, AND ERIC R. SIEGEL

*Cetus Corp., Berkeley, California 94710*

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The relative activity of *Flavobacterium* whole cells on the enzymatic synthesis of epoxides from  $\alpha,\beta$ -chlorohydrins, -bromohydrins, and -iodohydrins is described.

Castro and Bartnicki (1, 2) observed an enzymatic route to epoxides that involved  $\alpha,\beta$ -halohydrins (Fig. 1). They isolated an organism, a *Flavobacterium* sp., that converted the chlorohydrin of allyl chloride and the bromohydrins of allyl alcohol, allyl bromide, propylene, and 2-butene to their respective epoxides. The enzyme responsible for this reaction was named halohydrin epoxidase. We have continued studies with this microorganism and have found that not only chlorohydrins and bromohydrins but also iodohydrins are converted to epoxides.

*Flavobacterium* slants were obtained from C. E. Castro of the Department of Nematology, University of California, Riverside. From one slant, a culture was grown under sterile conditions for 48 h at 25°C and 200 rpm in a 1-liter Erlenmeyer flask containing the following: ammonium nitrate (800 mg), potassium hydrogen phosphate (800 mg), potassium dihydrogen phosphate (320 mg), magnesium sulfate (40 mg), sodium sulfate (40 mg), ferrous sulfate (4 mg), calcium nitrate (0.4 mg), manganese sulfate (0.04 mg), zinc sulfate (0.4 mg), and yeast extract (40 mg) in 400 ml of distilled water containing 1% glycerol. This culture was used to inoculate other Erlenmeyer flasks (20-ml inoculum). The culture in each flask was then harvested by centrifuging for 10 min at 5,500 rpm. The cell pellet was suspended in 100 mM potassium phosphate buffer (pH 6.0) and then recentrifuged. The washed cell pellet weighed approximately 400 mg (wet weight).

$\alpha,\beta$ -Halohydrins and epoxide standards were obtained either from Aldrich Chemical Co. (Milwaukee, Wis.) or Pfaltz and Bauer, Inc. (Stamford, Conn.). 2-Bromo-1-propanol and 1-iodo-2-propanol were synthesized as previously described (3).

*Flavobacterium* whole cells (200 mg, wet weight) and  $\alpha,\beta$ -halohydrin (20 mM final concentration) were mixed in a 10-ml vial containing 5 ml of 300 mM potassium phosphate buffer at

pH 6.0. The vial was sealed with a cap containing a Teflon-lined septum and then strapped to an Erlenmeyer flask sitting on a rotary shaker. The enzymatic reaction proceeded at 30°C and 200 rpm for 7 h.

For each halohydrin tested, a control vial was run. This vial contained halohydrin (20 mM final concentration) and 5 ml of 300 mM potassium phosphate buffer at pH 6.0, but no *Flavobacterium* whole cells were added. The control vial was run alongside the reaction vial. This control permitted the subtraction of the nonenzymatic contribution to the conversion of an  $\alpha,\beta$ -halohydrin to epoxide under the experimental conditions tested.

Samples of the reaction mixtures were analyzed by gas chromatography-mass spectrometry as previously described (3). Confirmation of product identity was made by comparing gas chromatography retention times and mass spectra of reaction products with authentic standards.

Table 1 summarizes the relative activity of cells on the various substrates. The enzyme reacted best with the bromohydrin when a chlorohydrin, a bromohydrin, and an iodohydrin were compared in a series. This was first indicated in the study by Bartnicki and Castro (1) with the mixed halogen substrate, 1-bromo-3-chloro-2-hydroxypropane. Some of the alkene halohydrins have positional isomers, for example, the two positional isomers for propylene bromohydrin. The enzyme reacted more efficiently with a terminal halo-containing compound (e.g., 1-bromo-2-propanol) than with an internal halo-containing compound (e.g., 2-bromo-1-propanol).

For the most reactive substrate tested, 1-bromo-2-propanol, 100% conversion to propylene oxide was obtained in 7 h in the nonoptimized system. The control for this substrate (i.e., substrate but no microorganism) showed less than 3% nonenzymatic conversion of substrate to epoxide product under the same experi-

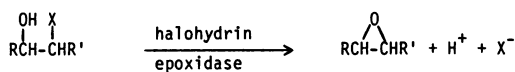


FIG. 1. Enzymatic route from  $\alpha,\beta$ -halohydrins to epoxides.

mental conditions. An additional, more rigorous, control is boiled whole cells plus substrate. Lack of conversion in this case would indicate two things: (i) the activity is heat labile, consistent with the catalyst being protein in nature, and (ii) the addition of whole cells is not contributing a base which could chemically cause the conversion. When such a control was run (the whole cells were boiled for 5 min), less than 3% conversion of 1-bromo-2-propanol to propylene oxide occurred in 7 h.

Since the enzymatic reactions were run with whole cells, cell wall permeability to the sub-

strate or product (or to both) may have affected the activity observed. Furthermore, the reactions were examined at only one substrate concentration and one pH (pH 6 was chosen to minimize nonenzymatic conversion), and there was no attempt to measure initial reaction velocity. As a consequence, the activities reported on the various substrates should be viewed only as an indication of the potential activity of the enzyme.

Gas chromatography-mass spectrometry provided a rapid analysis for both the alkene halohydrin substrate and the epoxide product. With the mass spectra obtained, the identity of each product could be confirmed. This was important since reactions other than that due to halohydrin epoxidase could have occurred (e.g., dehalogenation to yield alcohols or hydrolysis to yield diols). In all reactions run with gas chromatography-mass spectrometry, only epoxide products were detected.

This microbial reaction coupled with another enzyme, haloperoxidase, allows for the biological conversion of an alkene to an epoxide via an  $\alpha,\beta$ -halohydrin (3).

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#### LITERATURE CITED

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TABLE 1. Relative activity of *Flavobacterium* whole cells on the enzymatic synthesis of epoxides from  $\alpha,\beta$ -halohydrins

| Substrate             | R <sup>a</sup>      | R' <sup>a</sup> | X <sup>a</sup> | Relative activity (%) <sup>b</sup> |
|-----------------------|---------------------|-----------------|----------------|------------------------------------|
| 2-Chloroethanol       | H                   | H               | Cl             | 18                                 |
| 2-Bromoethanol        | H                   | H               | Br             | 60                                 |
| 2-Iodoethanol         | H                   | H               | I              | 24                                 |
| 1-Chloro-2-propanol   | CH <sub>3</sub>     | H               | Cl             | 26                                 |
| 1-Bromo-2-propanol    | CH <sub>3</sub>     | H               | Br             | 100                                |
| 2-Bromo-1-propanol    | H                   | CH <sub>3</sub> | Br             | 5                                  |
| 1-Iodo-2-propanol     | CH <sub>3</sub>     | H               | I              | 34                                 |
| 1-Bromo-3-butene-2-ol | CH <sub>2</sub> =CH | H               | Br             | 65                                 |

<sup>a</sup> See Fig. 1.

<sup>b</sup> Relative to activity on 1-bromo-2-propanol (taken as 100%) at 7 h into the reaction.