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CHORISMATE MUTASE-CATALYZED REACTION OF (±)-CHORISMIC ACID

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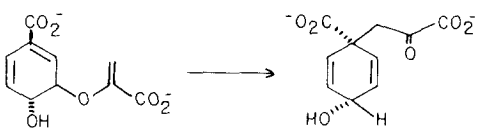
SUMMARY. Chorismate mutase-prephenate dehydrogenase from E. coli and chorismate mutase from S. aureofaciens do not catalyze the conversion of (+)-chorismate (unnatural enantiomer) to prephenate. (+)-Chorismate does not inhibit the isomerization of (-)-chorismate to prephenate with chorismate mutase-prephenate dehydrogenase from E. coli.

Chorismate is the last common intermediate in the biosynthesis of aromatic amino acids and growth factors via the shikimate pathway in bacteria, fungi, and higher plants. The first step in the biosynthesis of tyrosine and phenylalanine from chorismate is the isomerization to prephenate (Scheme I).



CHORISMATE PREPHENATE

Scheme I

The reaction is catalyzed by chorismate mutase (1). Recently we have completed a total synthesis of (±)-chorismic acid (2), and it was of interest to establish whether the unnatural enantiomer [(+)-chorismate] was metabolized by chorismate mutase and whether it inhibited the metabolism of (-)-chorismate.

MATERIALS AND METHODS

Enzymes. Chorismate mutase-prephenate dehydrogenase from E. coli K12 was a gift from Dr. John F. Morrison, the John Curtin School of Medical Research, Canberra City, Australia.

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Chorismate mutase from S. aureofaciens Tü 24 was isolated from culture growth of the organism (3) kindly supplied by Dr. Helmut Görisch, Institut für Mikrobiologie der Universitat Hohenheim, Stuttgart, West Germany.

Chorismic Acid. (-)-Chorismic acid was isolated from culture growth of A. aerogenes 62-1 (4) kindly supplied by Professor Frank Gibson, the John Curtin School of Medical Research, Canberra City, Australia.

Determination of Prephenate. Prephenate formed was measured by literature procedures (5,6). To a 1 mL-aliquot of incubation mixture was added 0.5 mL of 2 N HCl. After 10 min, 1 mL of 3 N NaOH was added; and the absorbance at 320 nm was measured. A molar extinction coefficient of 17,000 M^{-1} cm^{-1} was used. Results were corrected for nonenzymatic conversion of chorismate to prephenate.

Enzymatic Reactions. A. Chorismate mutase-prephenate dehydrogenase from E. coli K12. (a) To a solution (5 mL) of either (-)-chorismic acid (0.0962 mM) or (±)-chorismic acid (0.112 mM) in phosphate buffer (pH 7.0) at 30°C was added 10 µL of enzyme solution (specific activity 13.5 U/mg, 0.2 mg of protein). Aliquots were removed at specific time intervals and assayed for prephenate. (b) To a solution (6 mL) of either (-)-chorismic acid (0.061 mM) or (±)-chorismic acid (0.122 mM) in phosphate buffer (pH 7.0) at 30°C was added 7.5 µL of enzyme solution (1.5 µg of protein). Aliquots were removed at specific time intervals and assayed for prephenate.

B. Chorismate mutase from S. aureofaciens Tü 24. To a solution (5 mL) of either (-)-chorismic acid (0.0962 mM) or (±)-chorismic acid (0.112 mM) in phosphate buffer (pH 7.0) at 30°C was added 1 mL of enzyme solution (specific activity 1.3 U/mg, 0.4 mg of protein). Aliquots were removed at specific time intervals and assayed for prephenate.

RESULTS

In incubations with a high concentration of chorismate mutase-prephenate dehydrogenase from E. coli (40 µg/mL of incubation mixture), (-)-chorismate was quantitatively converted to prephenate by the time of the first reading (2 min). Under the same conditions 49 ± 3% of (±)-chorismate was converted to prephenate within 2 min, and no further isomerization was observed. Similar results were observed from incubation mixtures containing a high concentration of chorismate mutase from S. aureofaciens (67 µg/mL of incubation mixture).

In incubations with a low concentration of chorismate mutase-prephenate dehydrogenase (0.25 µg/mL of incubation mixture), the rate of production of prephenate from (-)-chorismate and from (±)-chorismate was linear over a period

of 10 min [14% reaction of (-)-chorismate]. The rates of prephenate formation from (-)-chorismate (8.17×10^{-4} μmol mL^{-1} min^{-1}) and from (±)-chorismate (8.32×10^{-4} μmol mL^{-1} min^{-1}) were identical when the concentration of (-)-chorismate used was one-half that of (±)-chorismate.

DISCUSSION

Since the conversion of (±)-chorismate to prephenate stops at 50% conversion under conditions where (-)-chorismate is rapidly utilized (100%), it is concluded that neither chorismate mutase-prephenate dehydrogenase from *E. coli* nor chorismate mutase from *S. aureofaciens* catalyzes the rearrangement of (+)-chorismate to prephenate. The observation that prephenate is formed at the same rate from (-)-chorismate and from (±)-chorismate, when the concentration of the latter is twice the concentration of the former, establishes that (+)-chorismate does not inhibit the conversion of (-)-chorismate to prephenate under the conditions investigated with chorismate mutase-prephenate dehydrogenase from *E. coli*.

ACKNOWLEDGMENT

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