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STEREOSPECIFIC OXIDATION OF ALIPHATIC ALCOHOLS CATALYZED BY GALACTOSE OXIDASE

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SUMMARY: The stereospecificity of galactose oxidase (EC 1.1.3.9) from Dactylium dendroides in the oxidation of simple three-carbon alcohols has been examined. The enzyme oxidizes glycerol to optically pure S(-)glyceraldehyde. In addition to this prochiral stereospecificity, galactose oxidase also exhibits enantiomeric stereospecificity in the oxidation of 3-halo-1,2-propanediols: the R isomer appears to be a better substrate than its S counterpart. The above stereochemistry of galactose oxidase-catalyzed oxidation of "unnatural" substrates, non-sugar alcohols, can be predicted on the basis of the conformation of the natural substrate of the enzyme, D-galactose.

INTRODUCTION

Galactose oxidase (EC 1.1.3.9) from Dactylium dendroides contains one atom of Cu(II) per molecule as the sole cofactor and catalyzes a two-electron redox reaction which involves oxygen as an electron acceptor (1-4). In addition to the oxidation of C-6 in D-galactose and its derivatives, galactose oxidase can also oxidize to the corresponding aldehydes a number of aliphatic and aromatic alcohols including 1,2-propanediol and glycerol (2), dihydroxyacetone (5), 3,4-dimethoxybenzyl alcohol (6), hydroxypyruvate, hydroxyacetone, glycolaldehyde, 2-methylene-1,3-propanediol and hydroxyacetophenone (7).

While the specificity of the enzyme with respect to its natural substrate, D-galactose, and its derivatives has been thoroughly investigated (8-10), its specificity with respect to the aforementioned unnatural substrates has not been addressed. In particular, it is not known whether galactose oxidase is capable of stereospecific oxidation of simple non-sugar alcohols and whether

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such a specificity, if expressed, can be predicted on the basis of that towards D-galactose. This question is not only of fundamental significance but may also be important for enzyme-catalyzed syntheses of optically active compounds (11).

In this study it has been found that D. dendroides galactose oxidase displays both prochiral and enantiomeric stereospecificity in the oxidation of simple three-carbon alcohols

MATERIALS AND METHODS

Galactose oxidase of D. dendroides was purchased from Sigma and had a specific activity of 500 units/mg protein. Other biochemicals and chemicals used were obtained as follows: from Sigma, horse liver alcohol dehydrogenase (specific activity of 1.6 units/mg protein), beef liver catalase (specific activity of 32,000 units/mg protein), NADH and L- and D- (S- and R-). glyceraldehyde; from Mallinckrodt, glycerol; from Aldrich, 3-chloro-1,2 propanediol and 3-bromo-1,2-propanediol. (2R)-3-Chloro-1,2-propanediol was synthesized by literature methods (12-14) and had an $[\alpha]_D = +3.1$ (c = 2.5, chloroform). All other chemicals were of analytical grade.

Optical rotations were measured at 589 nm (the sodium lamp) using a Perkin-Elmer polarimeter (model #141). Concentrations of aldehydes were determined by spectrophotometric titration with NADH catalyzed by horse liver alcohol dehydrogenase (15).

RESULTS AND DISCUSSION

In accordance with Jones (16), the following discussion shall distinguish between prochiral stereospecificity (the ability to produce chiral, optically active molecules from prochiral ones) and enantiomeric specificity (the ability to react at different rates with the optical antipodes).

The simplest alcohol substrate of galactose oxidase possessing a prochiral carbon is glycerol. For this reason glycerol was chosen to reveal whether the enzyme will oxidize it to glyceraldehyde asymmetrically. Three hundred units of galactose oxidase were added to 6 ml of 2 M glycerol in 0.1 M phosphate buffer (pH 7.0) at 4°C. To prevent inactivation of the enzyme by hydrogen peroxide (which is the second reaction product), the system was supplemented with 1000 units/ml of bovine liver catalase. The reaction was carried out at 4°C and every other day an aliquot was withdrawn and assayed for glyceraldehyde (as described in Materials and Methods). In 3 weeks the concentration of glyceraldehyde in the enzymatic system reached 105 mM, after which

there was no further production of the aldehyde. The glyceraldehyde obtained was positively identified by paper chromatography using a solvent mixture consisting of 70% (v/v) n-propanol + 20% (v/v) water + 10% (v/v) ethyl acetate and alkaline $AgNO_3$ and $KMnO_4$ as a developer (17) (R_f = 0.22).

The solution containing enzymatically produced glyceraldehyde was used directly for determination of optical rotation (other components of the system in the concentrations employed did not interfere). It was found that the aldehyde reaction solution rotated light in the negative direction. When the optical rotation obtained was divided by the independently measured concentration of glyceraldehyde (105 mM), a specific optical rotation of -9.3° resulted. The literature data on the specific optical rotation of S-glyceraldehyde vary from -8.5° to -11°, most frequently cited is $[\alpha]_D$ = 8.7° (18). Comparing this with the specific optical rotation obtained in this work, one can conclude that galactose oxidase converts glycerol to nearly 100% pure S-(L)-glyceraldehyde. That is, the enzyme exhibits absolute prochiral specificity.

This result can be rationalized as follows. Oxidation of D-galactose occurs at carbon 6 to produce the aldehyde at that position (Fig. 1A). When carbon 2 of glycerol is superimposed with carbon 5 of D-galactose and the rest of the glycerol molecule assumes the same conformation as the corresponding fragment of the sugar molecule (Fig. 1B), then only the pro-S arm of glycerol is in the proper orientation to be enzymatically oxidized. Therefore, this conformational requirement dictates that only S-glyceraldehyde is produced (Fig. 1B).

In order to reveal the enantiomeric stereospecificity of galactose oxidase, the enzyme-catalyzed oxidation of racemic 3-chloro-1,2-propanediol and 3-bromo 1,2-propanediol was studied. In contrast to glycerol which is prochiral at C2, the C2 atom in these two alcohols is chiral. The reaction conditions were the same as for glycerol: 1 M alcohol, 50 units/ml galactose oxidase, 1000 units/ml catalase, 0.1 M phosphate buffer (pH 7.0), 4°C. The concentration of the aldehydes produced was 19.2 mM after 20 days for the Cl-compound and 19.8

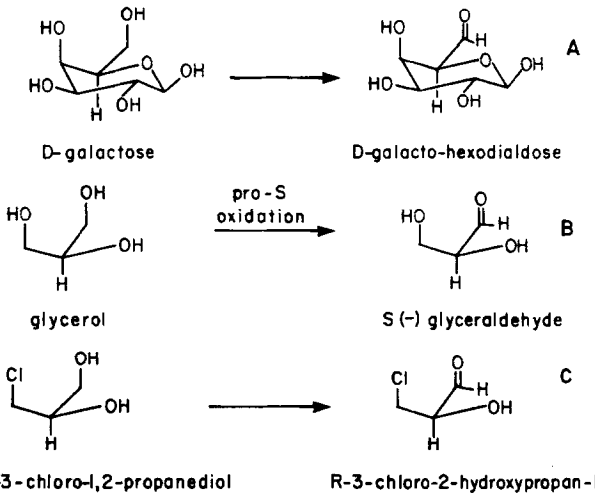

Fig. 1. Conformations of substrates leading to oxidation by galactose oxidase. (A) D-galactose; (B) glycerol; (C) R-3-chloro-1,2-propanediol. Configurations of all chiral crabon atoms are absolute configurations. The conformations depicted for unnatural substrates are superimposable on the corresponding portion of D-galactose. In case (B), pro-R oxidation would preclude glycerol from assuming the "correct" (D-galactose-like) conformation. In case (C), the D-galactose-like conformation of the S-diol would expose the chlorine atom (instead of the hydroxyl group as in the R diol) to the redox site of the enzyme.

mM after 24 days for the Br-compound. After these periods of time, the reactions stopped.

Both mixtures rotated light in the negative direction. The optical rotations obtained divided by the concentrations of the aldehydes (see above) yielded specific optical rotations of -6.1° and -6.8° for the Cl- and Br- aldehydes, respectively. It should be pointed out that these values represent the difference between the specific optical rotations of the corresponding aldehyde and the alcohol from which this aldehyde is enzymatically produced.

The data described above provide no information with respect to which enantiomer(s) is a substrate of galactose oxidase. The stereochemical analysis analogous to that carried out for glycerol indicates that only the R isomer of a 3-halo-1,2-propanediol will have the correct orientation to react with the enzyme (Fig. 1C).

To verify this prediction, we synthesized the optically pure R enantiomer of 3-chloro-1,2-propanediol and carried out its reaction with the enzyme under the same conditions as those for the racemic alcohols. It has been found that

this isomer is oxidized by galactose oxidase at the same rate as the racemic mixture. Hence this result confirms the stereochemical prediction (Fig. 1C). Unfortunately, a chemical synthesis of the S isomer of 3-chloro-1,2-propanediol is not known so it was not possible to directly measure its interaction with the enzyme.

It has been demonstrated in this work that stereochemistry of galactose oxidase-catalyzed oxidation of "unnatural" substrates, non-sugar alcohols, can be predicted on the basis of the conformation of the natural substrate of the enzyme, D-galactose.

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