D-Lactate Dehydrogenase

Substrate Specificity and Use as a Catalyst in the Synthesis of Homochiral 2-Hydroxy Acids

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ABSTRACT

This note compares the substrate specificity of D-lactate dehydrogenase (D-LDH, EC 1.1.1.28) to that of L-lactate dehydrogenase (L-LDH, EC 1.1.1.27), illustrates three procedures that use D-LDH in synthesis and two methods for recycling NADH, and provides experimental details illustrating the use of D-LDH in organic synthesis.

Index Entries: D-lactate dehydrogenase, substrate specificity; D-lactate dehydrogenase, use in synthesis; reduction of 2-oxoacids; 2-hydroxy acids, synthesis; enzymes, immobilized; enzymes, use in synthesis; (R)-butene oxide, synthesis.

INTRODUCTION

L-LDH is a broadly useful enzyme in organic synthesis; it transforms a wide range of 2-oxoacids, such as pyruvate, to S-2-hydroxy acids, such as L-lactate [Eq. 1] (1). D-LDH converts 2-oxoacids to R-2-hydroxy acids [Eq. 2]. We hoped that L-LDH and D-LDH would provide complementary catalysts for organic synthesis. The objective of the work reported in this paper was to establish the potential of D-LDH as a catalyst in organic synthesis, by examining its substrate specificity, stability, and enantioselectivity. D-LDH does indeed reduce a range of 2-oxo acids, but the range is substantially narrower than that of L-LDH. Thus, although D-LDH is a

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catalyst that is in some sense "enantiomeric" to L-LDH, it is a less useful catalyst than, and is not a true complement to, L-LDH.

$$\text{COOH} + \text{NADH} + \text{H}^+ \xrightarrow{\text{L-LDH}} \text{COOH} + \text{NAD}^+ \quad (1)$$

$$\text{COOH} + \text{NADH} + \text{H}^+ \xrightarrow{\text{D-LDH}} \text{COOH} + \text{NAD}^+ \quad (2)$$

In vivo, D-LDH catalyzes the NADH-dependent interconversion of pyruvate and D-lactate in anabolic and catabolic pathways. Neither the structure nor mechanism of D-LDH, found in lower organisms, has been studied as extensively as in the case of L-LDH, found in higher organisms (2–5). Although the structures of the L- and D-enzymes are thought to be similar, their respective mechanisms of catalysis appear to be different (5,6).

Several properties of D-LDH make it appealing for use in synthesis, in addition to its ability to provide the opposite enantiomer obtained from reductions with L-LDH. In vivo, the reduction of pyruvate to lactate is highly favored (4). The commercial preparation of D-LDH from *Leuconostoc mesenteroides* was examined in this work because it is inexpensive (~$4/1000 U; 1000 U converts ~1 mol of substrate to product per day under assay conditions) and has a high specific activity of 1,000–1,500 U/mg of protein (7,8). Like L-LDH, D-LDH has an air-sensitive thiol group, but it is stable if used in an inert atmosphere in the presence of reducing agents such as dithiothreitol to prevent autooxidation. NADH must be recycled, but several good techniques exist for this purpose (for reviews, see 9 and 10).

Other methods for the enzyme-catalyzed production of R-2-hydroxy acids have been reported using D-LDH (11), D-2-hydroxyisocaproate dehydrogenase (12), 2-oxocarboxylate reductase (13), and L-2-haloacid dehydrogenase (14).

**METHODS**

The preparation of substrates, kinetic measurements, immobilization of enzymes, the determination of enantiomeric excess (ee) of 2-hydroxy acids according to the method of Mosher (15), and general experimental procedures are described elsewhere (1). When enzymes were confined within dialysis membranes (the MEEC technique, 16), the following procedure was followed: a section of dialysis tubing (SpectraPor 2, 12-14,000 MW cut off, 16 mm flat width, Spectrum Medical Industries or VWR) was rinsed with distilled water and a knot was tied at one end. The enzymes