DOGFISH LACTATE DEHYDROGENASE THE STEREOCHEMISTRY OF HYDROGEN TRANSFER

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1. Introduction

All the L- and D-lactate dehydrogenase (EC 1.1.1.27) and 1.1.1.28) isolated so far and tested for the stereochemistry of the hydrogen transfer to the coenzyme have been shown to be of class A [1]. The three dimensional X-ray structural determination of the lactate dehydrogenase isolated from dogfish raised the question of the stereochemistry of the hydrogen transfer for this enzyme [2]. In this paper we show that this dehydrogenase belongs to class A and that the hydrogen transfer with 3-acetyl $PyAD^+$† has the same stereochemistry as NAD^+.

2. Methods and materials

The lactate dehydrogenase from dogfish was provided by Dr. M. Rossmann. The sodium [2-^{3}H]DL-lactate (5 mCi/2.4 mg) was obtained from New England Nuclear as an aqueous solution, and the yeast alcohol dehydrogenase (320 U/mg) from Sigma. Radioactivity was determined with an Intertechnique SL 30 scintillation counter.

2.1. *Oxidation of lactate*

2.1.1. With NAD^+

To 0.02 M glycine buffer pH 9.55 (100 ml), 0.04 M in semicarbazide hydrochloride containing NAD^+ (100 mg) and labelled sodium DL-lactate (67 mg) (10^9 cpm/mM) is added dogfish lactate dehydrogenase (400 IU). After 24 hr, approx. 20% of the NAD^+ is converted to NADH which is isolated by two successive chromatographies on DEAE cellulose (washed with 0.5 N NaOH, H_2O, M $KHCO_3$, H_2O (column 2 × 20 cm) with ammonium bicarbonate gradient from 0 to 0.4 M. The tubes showing the correct absorption are concentrated to small volume and yield [4-^{3}H]NADH (6.9 mg) (2.8×10^8 cpm/mM) which is immediately oxidised.

2.1.2. With 3-acetyl $PyAD^+$

As for NAD^+, except that the volume of buffer was 60 ml, sodium DL-lactate (16.8 mg) (2.1×10^9 cpm/mM) and enzyme (80 IU) were used. After 6.5 hr, 50% of 3-acetyl $PyAD^+$ was reduced. The 3-acetyl [4-^{3}H]PyADH (14.5 mg) (1.1×10^9 cpm/mM) was isolated in the same manner as the [4-^{3}H]NADH.

2.2. *Oxidation of [4-^{3}H] NADH*

To 0.01 M phosphate buffer pH 7.6 (5 ml) containing [4-^{3}H]NADH (6 mg) prepared above and freshly distilled acetaldehyde (200 µl), a suspension of yeast alcohol dehydrogenase (2 mg) is added. After 45 min, the reaction is complete. The solution is lyophilised and 65% of the initial radioactivity is in the lyophilisate. The NAD^+ is isolated by chromatography on Dowex 1 × 2 (washed with 3 N HCl, H_2O, HCOONa, H_2O (column 2 × 20 cm) using a gradient of formic acid 0 to 0.4 N. The isolated NAD^+ had a radioactivity: 8.4×10^6 cpm/mM (3% of the initial radioactivity).

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† *Abbreviations:*
3-acetyl $PyAD^+$: 3-acetyl pyridine adenine dinucleotide;
3-acetyl PyADH: its reduced form.

2.3. *Oxidation of 3-acetyl PyADH*

As for [4-^{3}H]NADH, 82% of the initial radioactivity is in the lyophilisate and the 3-acetyl PyAD$^+$ had an activity of 9.5×10^7 cpm/mM (6.5% of the initial radioactivity).

3. Discussion

The lactate dehydrogenase was used to oxidise labelled DL-lactic acid and the tritium was found in the reduced form of the coenzyme: NADH or 3-acetyl PyADH. The difference in the specific activity between the lactate and the reduced coenzyme is probably due to the existence of a primary isotope effect. The reduced form is oxidised as rapidly as possible in order to avoid its destruction through radiolysis. This explains the limited transfer of the radioactivity to the ethanol in the reduction of acetaldehyde by yeast alcohol dehydrogenase. However, the oxidised form of the coenzyme: NAD$^+$ and 3-acetyl PyAD$^+$ contains little radioactivity. This shows that the pro-*R* hydrogen removed by the yeast alcohol dehydrogenase [3] is the one introduced by the lactate dehydrogenase in NAD$^+$ and 3-acetyl PyAD$^+$. Therefore the lactate dehydrogenase from dogfish belongs to the A class of dehydrogenase.

The fact that all the α-hydroxy acid dehydrogenase: glyoxylate dehydrogenase (NAD$^+$) (EC 1.1.1.26) from spinach leaves and (NADP$^+$) from peas [4], L- and D-lactate dehydrogenases (EC 1.1.1.27 and 1.1.1.28) from various sources such as rabbit skeletal muscle [1a] beef heart muscle [1b], dogfish muscle, *Lactobacillus plantarum* and *L. arabinosus* [1c], *Escherichia coli* [1d] and potato tubers [1a], glycerate dehydrogenase (EC 1.1.1.29) from parsley leaf [1a], malate dehydrogenase (EC 1.1.1.37 to 1.1.1.40) from pig heart muscle [4], pigeon liver [4a], wheat germ [6], potatoes [7], maize leaves [7] and cauliflower [7] and isocitrate dehydrogenase (EC 1.1.1.41 and 1.1.1.42) from bovine heart [8] and pig heart muscle [9], belong to the A class of dehydrogenases, must have an evolutionary significance. All these enzymes may have very similar binding sites for the coenzyme. This similarity for the dogfish lactate dehydrogenase and the cytoplasmic malate dehydrogenase from pig heart is apparent from their X-ray diffraction pattern [10]. The fact also that active hybrids may be found from subunits of dogfish and beef heart lactate dehydrogenases, is also revealing [11]. All these α-hydroxy acid dehydrogenases may have evolved from the same precursor: changes in certain amino acids are responsible for the substrate variation and the stereochemistry of the hydrogen transfer has been fixed very early and remained constant throughout the evolution.

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