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STEREOSPECIFICITY OF HYDROGEN TRANSFER CATALYZED BY D-GALACTOSE DEHYDROGENASE FROM *PSEUDOMONAS SACCHAROPHILA* AND *PSEUDOMONAS FLUORESCENS*

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Summary

D-Galactose dehydrogenase (D-galactose: NAD^+ 1-oxidoreductase EC 1.1.1.48) from *Pseudomonas saccharophila* and from *Pseudomonas fluorescens* is shown to be B-side stereo-specific, using D-[1-^{3}H]galactose as well as [4-^{3}H]NAD^+ as substrates.

Introduction

D-Galactose, L-arabinose and D-glucose 6-phosphate are metabolized in bacteria of the genus *Pseudomonas* by pathways which show distinct similarities, each starting with a pyridine nucleotide-dependent dehydrogenation of the corresponding sugar at carbon-1 [1—4]. In order to test for an evolutionary relationship [5—7] among these sugar dehydrogenases catalyzing the same type of reaction, we have studied the steric specificity of hydrogen transfer. This paper describes the determination of the B-side stereospecificity for the D-galactose dehydrogenases from *Ps. saccharophila* and from *Ps. fluorescens*.

Materials and Methods

[4-^{3}H]NAD^+ (spec. act. 10.8 mCi/mmole) and sodium [^{3}H]borohydride (spec. act. 7.6 Ci/mmole) were obtained from Radiochemical Centre (Amersham, England). D-[1-^{3}H]galactose was prepared by reduction of D-galactono-1,4-lactone with sodium [^{3}H]borohydride [8,9] and purified by paper chromatography. The yield of D-[1-^{3}H]galactose related to the sodium [^{3}H]borohydride used was 43% with a specific activity of approximately 2 Ci/mmole.

D-Galactose dehydrogenase from *Ps. saccharophila* and from *Ps. fluorescens* were isolated and assayed as described [10,11].

Enzymatic conversions

The oxidation of D-[1-^{3}H]galactose was carried out in 1 ml 50 mM triethanolamine · HCl buffer, pH 8.0, containing 50 nmoles labelled D-galactose ($2.2 \cdot 10^8$ dpm) and 1 μmole NAD^+. 50 Units D-galactose dehydrogenase from *Ps. saccharophila* or 100 units of the enzyme from *Ps. fluorescens* were used. When no further increase in absorption at 334 nm was observed, either the D-galactose dehydrogenase was denatured by heating (70°C for 4 min) or the subsequent oxidation of the [4-^{3}H]NADH formed was carried out without preceding denaturation of the enzyme. The reduction of [4-^{3}H]NAD^+ was performed using 2 μmoles D-galactose and 100 nmoles labelled NAD^+ ($2.4 \cdot 10^6$ dpm). All other conditions were as described above.

For oxidation of [4-^{3}H]NADH by way of the alcohol dehydrogenase catalyzed reaction 20 μmoles acetaldehyde and 100 units alcohol dehydrogenase were used. For oxidation by way of the glutamate dehydrogenase-catalyzed reaction, 50 μmoles 2-oxoglutarate, 200 μmoles NH_4Cl and 100 units glutamate dehydrogenase were used. After the reactions stopped, the enzymes were inactivated either by the addition of 0.7 μmoles *p*-chloromercuribenzoate or by heating.

Separation of reaction products

[1-^{3}H]Ethanol was separated by lyophilization of 0.5 ml of the reaction mixture, using small Thunberg tubes. The radioactivity of the lyophilisate and of the residue was determined. [4-^{3}H]NAD^+ was separated by thin layer chromatography on silica gel plates, L-(2-^{3}H)glutamate by paper chromatography on MN filter paper 719 (Macherey and Nagel, Düren, G.F.R.) using in both cases acetone/water (3 : 1, by vol.) as solvent. Radioactivity was detected with a radiochromatogram scanner (Packard, Downers Grove, Illinois, U.S.A.).

Results and Discussion

D-Galactose dehydrogenases catalyze the dehydrogenation of D-galactose at carbon-1 with the favourable apparent equilibrium constant at pH 8.0 of $K_{eq} = 6.2 \cdot 10^3$, because the primarily formed D-galactono-1,5-lactone is rearranged to the corresponding 1,4-lactone [12]. The stereospecificity of hydrogen transfer was investigated using D-(1-^{3}H)galactose as well as [4-^{3}H]NAD^+. The [4-^{3}H]NADH formed either by dehydrogenation of D-[1-^{3}H]galactose or by reduction of [4-^{3}H]NAD^+ was oxidized by the alcohol dehydrogenase-catalyzed reaction. Alcohol dehydrogenase from yeast is known to transfer the hydrogen from the A-side of the dihydronicotinamide ring to the substrate [13,14]. The equilibrium of the reaction strongly favours the oxidized coenzyme [15]. After separation of the reaction products the radioactivities determined indicate clearly, as documented in Table I that both D-galactose dehydrogenases belong to the class of B-side stereospecific enzymes.

The slightly better yields in the experiments with D-galactose dehydrogenase from *Ps. fluorescens* compared with those where the enzyme from *Ps. saccharophila* was used may be due to the omission of heat denaturation in the first case. Heat treatment may account for a loss of β-NADH by reoxidation and/or by racemisation [16,17].

TABLE I

SIDE STEREOSPECIFICITY OF D-GALACTOSE DEHYDROGENASES FROM *Ps. SACCHAROPHILA* AND FROM *Ps. FLUORESCENS*, DETERMINED BY WAY OF ALCOHOL DEHYDROGENASE CATALYZED REACTION

The radioactivity was measured in an aliquot after oxidation of (4-^{3}H)NADH by the alcohol dehydrogenase reaction and calculated for the complete reaction mixture.

D-galactose dehydrogenase, source	labelled substrate	Radioactivity (cpm)			Side stereospecificity
		Before lyophilisation	In lyophilisate	In residue	
Ps. saccharophila	D-[1-^{3}H]-galactose	$5.99 \cdot 10^7$ (100%)	$0.419 \cdot 10^7$ (7%)	$5.39 \cdot 10^7$ (90%)	B
Ps. fluorescens	D-[1-^{3}H]-galactose	$5.99 \cdot 10^7$ (100%)	$0.090 \cdot 10^7$ (1.5%)	$5.69 \cdot 10^7$ (95%)	B
Ps. saccharophila	[4-^{3}H]NAD^+	$6.48 \cdot 10^5$ (100%)	$5.64 \cdot 10^5$ (87%)	$0.71 \cdot 10^5$ (11%)	B
Ps. fluorescens	[4-^{3}H]NAD^+	$6.48 \cdot 10^5$ (100%)	$5.73 \cdot 10^5$ (88.4%)	$0.51 \cdot 10^5$ (7.9%)	B

In contrast to alcohol dehydrogenase which is A-specific glutamate dehydrogenase belongs to the class of B-side stereospecific enzymes [14]. If the [4-^{3}H]NADH formed by dehydrogenation of [1-^{3}H]galactose with unlabelled NAD^+ is oxidized in the glutamate dehydrogenase-catalyzed reaction all radioactivity must be found in the L-glutamate. The products of this reaction were separated by paper chromatography and examined for radioactivity. As expected the only radioactivity indicated by a strip scanner corresponded to L-glutamate. No radioactivity could be found at the position of NAD^+, thus again confirming the B-side stereospecificity of the two D-galactose dehydrogenases.

The enzyme from *Ps. saccharophila* as well as the enzyme from *Ps. fluorescens* transfers the hydrogen from carbon-1 of β-D-galactopyranose [12] to the si face of carbon-4 of the nicotinamide ring and in the reverse reaction the pro-S hydrogen atom from carbon-4 of the dihydronicotinamide to the re face of carbon-1 of D-galactono-1,5-lactone. This same stereospecificity has also been shown for D-glucose dehydrogenase from liver [18] and for D-glucose 6-phosphate dehydrogenase from yeast [19], pointing to an evolutionary relationship of all enzymes which dehydrogenate pyranoses at carbon-1.

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