

CHIRALITY OF XYLITOL-OXIDIZING ENZYMES FROM MAMMALIAN LIVER

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1. Introduction

The transfer of hydrogen from the reduced substrate to the pro(4R) or pro(4S) position of the nicotinamide ring of the nucleotide catalyzed by NAD(P)-linked dehydrogenases has been studied for a large number of oxidoreductases [1–4]. Tentatively rules correlating chirality with the structure of the substrate, the inducible or constitutive nature and coenzyme specificity of the dehydrogenase have been formulated [1–6]. In addition, Bentley [3,4] proposed rules which state that the chirality of hydrogen transfer to the coenzyme of a particular reaction is independent of the source of the enzyme and “when an enzyme reacts with a range of substrates, the stereospecificity of the hydrogen transfer is the same with each substrate”. Further, in this context Davies et al. [6] submitted the generalization that “when a metabolic sequence involves consecutive nicotinamide adenine dinucleotide dependent reactions, the dehydrogenases have the same stereospecificity”. Recently, Do Nascimento and Davies reported the B-type chirality of D-xylulose reductase (EC 1.1.1.9) and L-xylulose reductase (EC 1.1.1.10), both isolated from guinea pig liver [7]. These results would be in accordance with the last of the cited rules.

Whereas the NADP-linked L-xylulose reductase is quite specific for xylitol, the NAD-specific D-xylulose reductase can utilize, in addition to xylitol, a relative

large number of pentitols and hexitols, such as ribitol and D-sorbitol [8] which are also substrates for L-idoitol dehydrogenase (EC 1.1.1.14) from sheep liver [9]. The chirality of the hydrogen transfer from ribitol [10] and D-sorbitol [11,12] to NAD catalyzed by L-idoitol dehydrogenase has been reported to be of the A-type. This latter outcome would contradict Bentley's generalization in the sense that oxidoreductases from different sources catalyzing the same enzyme reaction should have the same chirality of hydrogen transfer [3,4]. Indeed, constitutive dehydrogenases from different sources acting on an organic substrate, up to date have been found to catalyze hydrogen transfer from and to the coenzyme invariably with the same chirality [2–4]. In order to clarify these points of apparent discord, we decided to elucidate the chirality of the hydrogen transfer from xylitol to NADP catalyzed by L-idoitol dehydrogenase and from ribitol and D-sorbitol to NAD catalyzed by D-xylulose reductase. In this opportunity the chirality of the L-xylitol reductase catalyzed reaction has been reinvestigated.

2. Materials and methods

Xylitol, ribitol, D-sorbitol, glucose-6-phosphate, DEAE-cellulose, alcohol dehydrogenase (EC 1.1.1.1), (S)glutamate dehydrogenase (EC 1.4.1.2) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) from yeast were purchased from the Sigma Chemical Co., St. Louis, Miss.; NAD-kinase (EC 2.2.1.2.3) from pigeon liver was obtained from Boehringer Mannheim Corp., [4-³H]NAD with a specific radioactivity of 50 Ci/mol from Amersham-Searle Corp., Arlington Heights, Ill.

Abbreviations: D-XDH = D-xylulose reductase (EC 1.1.1.9); L-XDH = L-xylulose reductase (EC 1.1.1.10); (S)-GDH = (S)glutamate dehydrogenase (EC 1.4.1.2); ADH = alcohol dehydrogenase (EC 1.1.1.1); G-6-PDH = D-glucose -6-phosphate dehydrogenase (EC 1.1.1.49).

The D- and L-xylulose reductases (EC 1.1.1.9 and EC 1.1.1.10) were isolated from guinea pig liver as described by Hickman and Ashwell [8]. $[4\text{-}^3\text{H}]$ NADH was isolated by chromatography on a DEAE-cellulose column in the bicarbonate form as by Silverstein [13]. The ^3H content of the B-position of about $0.2\text{ }\mu\text{mol}$ $[4\text{-}^3\text{H}]$ NADH was transferred to (S)glutamate with $0.2\text{ }\mu\text{mol}$ 2-oxoglutarate and 3 U of the B-type (S)glutamate dehydrogenase in 1 M NH_4CO_3 . The (S)glutamate formed was diluted with 3.0 mmol of nonlabelled (S)glutamic acid and recrystallized from water to constant specific radioactivity. Control experiments with a mixture of $[4\text{-}^3\text{H}]$ NAD and (S)glutamic acid showed that already after one crystallization, less than 1% of the original counts of NAD are found in (S)glutamic acid.

The stereochemistry of the hydrogen transfer from xylitol, ribitol, and D-sorbitol to NAD catalyzed by D-xylulose reductase from guinea pig liver was determined by incubation of $0.5\text{ }\mu\text{mol}$ $[4\text{-}^3\text{H}]$ NAD at 30°C with $4.0\text{ }\mu\text{mol}$ nonlabelled substrate, $100\text{ }\mu\text{mol}$ glycine-NaOH buffer pH 10.0 and 0.2 U D-xylulose reductase per ml of reaction medium. After 15 min incubation between 0.08 to $0.13\text{ }\mu\text{mol}$ $[4\text{-}^3\text{H}]$ NADH per ml medium were isolated.

Determination of the stereochemistry of the hydrogen transfer from xylitol to $[4\text{-}^3\text{H}]$ NAD catalyzed by L-iditol dehydrogenase from sheep liver was performed in the same way as already described for the investigation of the chirality of the

hydrogen transfer from D-sorbitol to NAD catalyzed by this oxidoreductase [12]. The general reaction scheme is shown in table 1.

Stereochemistry of the hydrogen transfer from xylitol to $[4\text{-}^3\text{H}]$ NADP catalyzed by L-xylulose reductase from guinea pig liver was determined with $[4\text{-}^3\text{H}]$ NADP generated in situ from $[4\text{-}^3\text{H}]$ NAD and ATP in the reaction catalyzed by NAD-kinase. The reaction solution contained per ml medium: $2.1\text{ }\mu\text{mol}$ $[4\text{-}^3\text{H}]$ NAD with a specific radioactivity of 1.1×10^6 dpm/ μmol , $5.2\text{ }\mu\text{mol}$ ATP, $10\text{ }\mu\text{mol}$ MgCl_2 , $12\text{ }\mu\text{mol}$ nonlabelled xylitol, 0.1 U NAD-kinase and 0.08 U L-xylulose reductase. The production of $[4\text{-}^3\text{H}]$ NADPH was optically followed at 340 nm. After an incubation period of 20 min, $0.14\text{ }\mu\text{mol}$ $[4\text{-}^3\text{H}]$ NADPH per ml reaction medium were isolated [13] and its chirality analyzed as already described [14]. Reaction sequences applied are presented in table 3.

The chirality of the produced reduced nucleotides was analyzed transferring the hydrogen located at the (4S) position to 2-oxoglutarate in the reaction catalyzed by the B-type (S)glutamate dehydrogenase. The specific radioactivities of the produced (S)glutamate and NAD or NADP, were determined [12,14].

3. Results

Data on the stereochemistry of the hydrogen transfer from xylitol, D-sorbitol and ribitol to NAD

Table 1
Stereochemistry of the hydrogen transfer from ribitol, xylitol and D-sorbitol to NAD catalyzed by D-xylulose reductase from guinea pig liver

Substrate	Specific radioactivity ^a (dpm/ μmol)		
	NAD ¹	(S)glutamate ²	NADH ²
Ribitol	1.3×10^7	1.1×10^7	0.8×10^5
Xylitol	1.1×10^7	1.2×10^7	1.3×10^5
D-Sorbitol	1.4×10^7	1.6×10^7	3.1×10^5

^a Specific radioactivities referring to the following sequence of reactions:

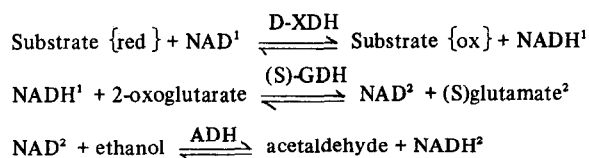


Table 2
Stereochemistry of the hydrogen transfer from xylitol to NAD
catalyzed by L-idoitol dehydrogenase from sheep liver

Substrate	Specific radioactivity ^a (dpm/ μ mol)		
	NAD ¹	(S)Glutamate ²	NADH ²
Xylitol	1.3×10^7	2.0×10^7	5.1×10^5

^a Specific radioactivities referring to the sequence of reactions outlined in table 1.

catalyzed by D-xylulose reductase are shown in table 1. Similarly the stereochemistry of the hydrogen transfer from xylitol to NADP catalyzed by L-xylulose reductase is outlined in table 2.

Analysis of the produced $[4\text{-}^3\text{H}]\text{NADH}$ or $[4\text{-}^3\text{H}]\text{NADPH}$ showed that the label of $[4\text{-}^3\text{H}]\text{NADH}$ generated in the reaction catalyzed by D-xylulose reductase with xylitol, D-sorbitol or ribitol as substrates (table 1) must be located in all cases exclusively at the (4S) position of the nicotinamide ring since it can be transferred quantitatively to 2-oxo-glutarate in the (S)glutamate dehydrogenase reaction. This result was confirmed determining the specific radioactivity of the concomitantly produced NAD. As expected, less than 3% of the original label was found in the accompanying NAD. Hence, the transferred hydride from unlabelled xylitol, D-sorbitol or ribitol to $[4\text{-}^3\text{H}]\text{NAD}$ must be located at the (4R) position of the produced (4S) $[4\text{-}^3\text{H}]\text{NADH}$. This outcome allows the (R) or A-type classification regarding the stereochemistry of the hydrogen transfer from xylitol, D-sorbitol or ribitol to the

coenzyme catalyzed by D-xylulose reductase from guinea pig liver, contradicting the previous report of Do Nascimento and Davies [7].

Table 2 shows similar results obtained, as expected, for the chirality of the hydrogen transfer from xylitol to NAD catalyzed by L-idoitol dehydrogenase which has been classified before as an A-type oxidoreductase with either D-sorbitol [11,12] or ribitol [10] as substrates.

Results summarized in table 3 confirm the observation of Do Nascimento and Davies [7] on the B-type chirality of the hydrogen transfer from xylitol to NADP catalyzed by L-xylulose reductase from guinea pig liver.

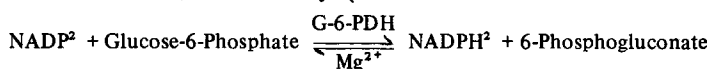
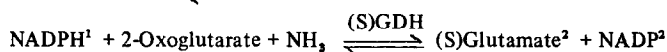
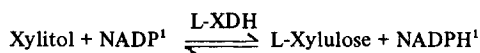
4. Discussion

The data presented in table 1 and 2 harmonizes with Bentley's prediction that different oxidoreductases catalyzing the same enzyme reaction, should have the same chirality of hydrogen transfer to the coenzyme

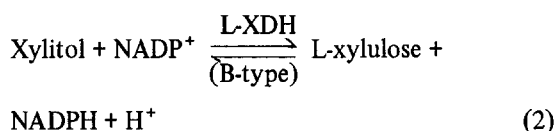
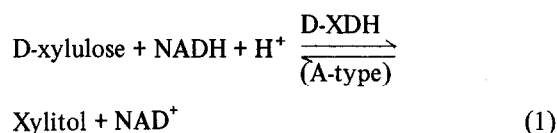
Table 3
Stereochemistry of the hydrogen transfer from xylitol to NADP catalyzed
by L-xylulose reductase from guinea pig liver

Substrate	Specific radioactivity (dpm/ μ mol)		
	NADP ¹	(S)Glutamate ²	NADPH ²
Xylitol	2.1×10^7	8.1×10^5	2.0×10^7

Specific radioactivities referring to the following sequence of reactions:



[3]. The NAD-linked oxidation of D-sorbitol to D-fructose, of ribitol to D-ribulose, or xylitol to D-xylulose catalyzed by either D-xylulose reductase from guinea pig liver or L-itol dehydrogenase from sheep liver [10–12], proceeds with the same A-type chirality. However, this outcome does not agree with prior observations of Do Nascimento and Davies [7] on the B-type chirality of D-xylulose reductase and are not in concord with the generalization proposed by Davies et al. [6] who postulated that dehydrogenases involved in consecutive NAD(P) linked reactions should have the same chirality of hydrogen transfer. Results of the present study (tables 1 and 3) show clearly that the L- and D-xylulose reductase catalyzing the consecutive sequence of reactions shown in eqs. (1) and (2)



proceed with opposite chirality of hydrogen transfer to the coenzyme.

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