

## 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-<sup>3</sup>H]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  $^3\text{H}$  content of the B-position of about  $0.2\ \mu\text{mol}$   $[4\text{-}^3\text{H}]$  NADH was transferred to (S)glutamate with  $0.2\ \mu\text{mol}$  2-oxoglutarate and 3 U of the B-type (S)glutamate dehydrogenase in 1 M  $\text{NH}_4\text{CO}_3$ . 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  $0.5\ \mu\text{mol}$   $[4\text{-}^3\text{H}]$  NAD at  $30^\circ\text{C}$  with  $4.0\ \mu\text{mol}$  nonlabelled substrate,  $100\ \mu\text{mol}$  glycine-NaOH buffer pH 10.0 and  $0.2\ \text{U}$  D-xylulose reductase per ml of reaction medium. After 15 min incubation between  $0.08$  to  $0.13\ \mu\text{mol}$   $[4\text{-}^3\text{H}]$  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-ribitol 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:  $2.1\ \mu\text{mol}$   $[4\text{-}^3\text{H}]$  NAD with a specific radioactivity of  $1.1 \times 10^6$  dpm/ $\mu\text{mol}$ ,  $5.2\ \mu\text{mol}$  ATP,  $10\ \mu\text{mol}$   $\text{MgCl}_2$ ,  $12\ \mu\text{mol}$  nonlabelled xylitol,  $0.1\ \text{U}$  NAD-kinase and  $0.08\ \text{U}$  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,  $0.14\ \mu\text{mol}$   $[4\text{-}^3\text{H}]$  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 <sup>a</sup> (dpm/ $\mu\text{mol}$ )		
	NAD <sup>1</sup>	(S)glutamate <sup>2</sup>	NADH <sup>2</sup>
Ribitol	$1.3 \times 10^7$	$1.1 \times 10^7$	$0.8 \times 10^5$
Xylitol	$1.1 \times 10^7$	$1.2 \times 10^7$	$1.3 \times 10^5$
D-Sorbitol	$1.4 \times 10^7$	$1.6 \times 10^7$	$3.1 \times 10^5$

<sup>a</sup> Specific radioactivities referring to the following sequence of reactions:

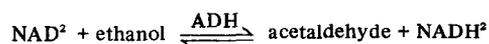
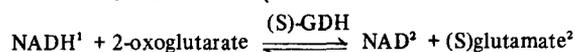
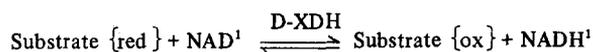


Table 2  
Stereochemistry of the hydrogen transfer from xylitol to NAD  
catalyzed by L-iditol dehydrogenase from sheep liver

Substrate	Specific radioactivity <sup>a</sup> (dpm/μmol)		
	NAD <sup>1</sup>	(S)Glutamate <sup>2</sup>	NADH <sup>2</sup>
Xylitol	1.3 × 10 <sup>7</sup>	2.0 × 10 <sup>7</sup>	5.1 × 10 <sup>5</sup>

<sup>a</sup> 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-<sup>3</sup>H]NADH or [4-<sup>3</sup>H]NADPH showed that the label of [4-<sup>3</sup>H]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-<sup>3</sup>H]NAD must be located at the (4R) position of the produced (4S) [4-<sup>3</sup>H]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-iditol 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.

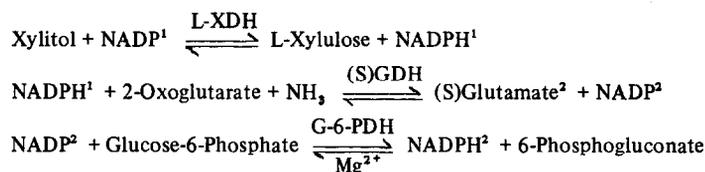
#### 4. Discussion

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

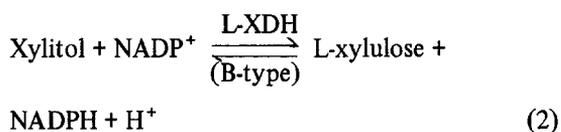
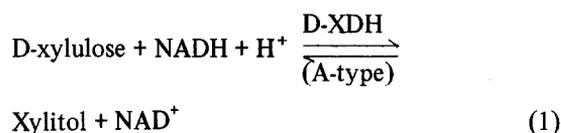
Table 3  
Stereochemistry of the hydrogen transfer from xylitol to NADP catalyzed  
by L-xylulose reductase from guinea pig liver

Substrate	Specific radioactivity (dpm/μmol)		
	NADP <sup>1</sup>	(S)Glutamate <sup>2</sup>	NADPH <sup>2</sup>
Xylitol	2.1 × 10 <sup>7</sup>	8.1 × 10 <sup>5</sup>	2.0 × 10 <sup>7</sup>

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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