CHIRALITY OF XYLITOL-OXIDIZING ENZYMES FROM MAMMALIAN LIVER

Miguel A. ALIZADE, Klaus BRENDEL* and Karl GAEDE

Instituto Venezolano de Investigaciones Científicas (IVIC), Apartado 1827, Caracas 101, Venezuela, and
**Department of Pharmacology, College of Medicine, University of Arizona, Tucson, Arizona 85724, USA*

Received 1 June 1976

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 nictonamide 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-iditol 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-iditol 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 disconcord, we decided to elucidate the chirality of the hydrogen transfer from xylitol to NADP catalyzed by L-iditol 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.

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).

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-^{3}H]NAD with a specific radioactivity of 50 Ci/mol from Amersham-Searle Corp., Arlington Heights, Ill.

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-^{3}H] NADH was isolated by chromatography on a DEAE-cellulose column in the bicarbonate form as by Silverstein [13]. The ^{3}H content of the B-position of about 0.2 μmol [4-^{3}H] NADH was transferred to (S)glutamate with 0.2 μmol 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 recristallized from water to constant specific radioactivity. Control experiments with a mixture of [4-^{3}H] NAD and (S)glutamic acid showed that already after one cristallization, 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 μmol [4-^{3}H] NAD at 30°C with 4.0 μmol nonlabelled substrate, 100 μmol 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 μmol [4-^{3}H] NADH per ml medium were isolated.

Determination of the stereochemistry of the hydrogen transfer from xylitol to [4-^{3}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-^{3}H] NADP catalyzed by L-xylulose reductase from guinea pig liver was determined with [4-^{3}H] NADP generated in situ from [4-^{3}H] NAD and ATP in the reaction catlyzed by NAD-kinase. The reaction solution contained per ml medium: 2.1 μmol [4-^{3}H] NAD with a specific radioactivity of 1.1 × 10 dpm/μmol, 5.2 μmol ATP, 10 μmol $MgCl_2$, 12 μmol nonlabelled xylitol, 0.1 U NAD-kinase and 0.08 U L-xylulose reductase. The production of [4-^{3}H] NADPH was optically followed at 340 nm. After an incubation period of 20 min, 0.14 μmol [4-^{3}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

	Specific radioactivity[a] (dpm/μmol)		
Substrate	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:

$$\text{Substrate}\{\text{red}\} + \text{NAD}^1 \xrightleftharpoons{\text{D-XDH}} \text{Substrate}\{\text{ox}\} + \text{NADH}^1$$

$$\text{NADH}^1 + \text{2-oxoglutarate} \xrightleftharpoons{\text{(S)-GDH}} \text{NAD}^2 + \text{(S)glutamate}^2$$

$$\text{NAD}^2 + \text{ethanol} \xrightleftharpoons{\text{ADH}} \text{acetaldehyde} + \text{NADH}^2$$

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

	Specific radioactivity[a] (dpm/μmol)		
Substrate	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-^{3}H]NADH or [4-^{3}H]NADPH showed that the label of [4-^{3}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-^{3}H]NAD must be located at the (4R) position of the produced (4S) [4-^{3}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

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

Specific radioactivies referring to the following sequence of reactions:

$$\text{Xylitol} + \text{NADP}^1 \overset{\text{L-XDH}}{\rightleftharpoons} \text{L-Xylulose} + \text{NADPH}^1$$

$$\text{NADPH}^1 + \text{2-Oxoglutarate} + \text{NH}_3 \overset{\text{(S)GDH}}{\rightleftharpoons} \text{(S)Glutamate}^2 + \text{NADP}^2$$

$$\text{NADP}^2 + \text{Glucose-6-Phosphate} \overset{\text{G-6-PDH}}{\underset{\text{Mg}^{2+}}{\rightleftharpoons}} \text{NADPH}^2 + \text{6-Phosphogluconate}$$

[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-iditol 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)

$$\text{D-xylulose} + \text{NADH} + \text{H}^+ \xrightleftharpoons[\text{(A-type)}]{\text{D-XDH}} \text{Xylitol} + \text{NAD}^+ \quad (1)$$

$$\text{Xylitol} + \text{NADP}^+ \xrightleftharpoons[\text{(B-type)}]{\text{L-XDH}} \text{L-xylulose} + \text{NADPH} + \text{H}^+ \quad (2)$$

proceed with opposite chirality of hydrogen transfer to the coenzyme.

Acknowledgements

Work was supported in part by the Regional Program of Scientific and Technological Development of the Organization of the American States (OAS), Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT), and USPHS Grant NIH AM 14977.

References

[1] Krakow, G., Ludowieg, J., Mether, J. H., Normore, W. M., Tosi, L., Udaka, S. and Vennesland, B. (1963) Biochemistry 2, 1009–1014.
[2] Vennesland, B. (1974) Stereospecificity in Biology, in: Topics in Current Chemistry, Vol. 48, pp. 39–55, Springer Verlag, Berlin, New York.
[3] Bentley, R. (1970) Molecular Asymmetry in Biology Vol. 2, pp. 1–89, Academic Press, New York.
[4] Popjack, G. (1970) Stereospecificity of Enzyme Reaction, in: The Enzymes (P. D. Boyer, ed.) Vol. 2, pp. 115–215, Academic Press, New York.
[5] Alizade, M. A. and Brendel, K. (1975) Die Naturwissenschaften 62, 346–348.
[6] Davies, D. D., Teixeira, A. and Kenworthy, P. (1972) Biochem. J. 127, 335–343.
[7] Do Nascimento, K. H. and Davies, D. D. (1975) Biochem. J. 149, 553–557.
[8] Hickman, J. and Ashwell, G. (1959) J. Biol. Chem. 234, 758–761.
[9] Smith, M. G. (1962) Biochem. J. 83, 135–144.
[10] Alizade, M. A., Gaede, K. and Brendel, K. (1976) in press.
[11] England, S., Kaysen, G. and Avigad, G. (1970) J. Biol. Chem. 245, 1311–1318.
[12] Alizade, M. A., Bressler, R. and Brendel, K. (1974) Biochim. Biophys. Acta 370, 354–357.
[13] Silverstein, E. (1965) Anal. Biochem. 12, 199–212.
[14] Alizade, M. A., Bressler, R. and Brendel, K. (1975) Life Sci. 17, 787–792.