Biochimica et Biophysica Acta, 370 (1974) 354–357

BBA 67380

STEREOCHEMISTRY OF THE L-IDITOL DEHYDROGENASE CATALYZED HYDROGEN TRANSFER TO NAD

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(Received July 29th, 1974)

SUMMARY

The stereochemistry of the hydrogen transfer to NAD catalyzed by L-iditol dehydrogenase from sheep liver (EC 1 1 1 14) was investigated [4-^{3}H]NAD was enzymatically reduced with non-labeled D-sorbitol and the position of the label at C_4 of the isolated [4-^{3}H]NADH was determined The label was found to be exclusively confined to the B-position which suggests that the transferred hydrogen from D-sorbitol must have entered into the A-position of the NADH produced These data imply that L-iditol dehydrogenase from sheep liver is an A-enzyme

INTRODUCTION

NAD-linked dehydrogenases promote direct stereospecific transfer of hydrogen between the substrate and the C_4 position of the nicotinamide ring of the nucleotide [1] Examination of the steric course of reactions catalyzed by a large number of NAD-linked dehydrogenases has revealed that they promote hydrogen transfer specifically to and from either one or the other side of this prochiral position [1] The enzymes fall into two classes, those which catalyze the reversible addition of hydrogen to the same side of the nicotinamide ring as does alcohol dehydrogenase (EC 1 1 1 1), and those which catalyze transfer to the opposite side of the nicotinamide ring such as glutamate dehydrogenase (EC 1 4 1 3) [2]

The alcohol dehydrogenase type has been classified as a transfer to the A-position at C_4 of the nicotinamide ring, this position is identical with pro-*R* [1, 3] In contrast, the glutamate dehydrogenase type transfer to the B-position or pro-*S* [1–3] Since no transfer of hydrogen into or out of water has been observed, the transfer must be a direct one [1–3] In this communication the stereospecificity of the transfer of hydrogen from D-sorbitol to NAD catalyzed by L-iditol dehydrogenase [4] from sheep liver (L-iditol NAD$^+$ 5-oxidoreductase, EC 1 1 1 14) was investigated [4-^{3}H]NAD was enzymatically reduced with non-labeled D-sorbitol and the resultant [4-^{3}H]NADH isolated and the chirality at C_4 of the nicotinamide ring determined

MATERIALS AND METHODS

D-Sorbitol, L-iditol dehydrogenase (EC 1 1 1 14), L-lactate dehydrogenase

(EC 1 1 1 27), L-malate dehydrogenase (EC 1 1 1 37) and L-glutamate dehydrogenase (EC 1 4 1 3) were purchased from Sigma, NAD^+ nucleosidase (EC 3 2 2 5) from Worthington, [4-^{3}H]NAD with a specific radioactivity of 50 Ci/mole from the Radiochemical Center

(1) Isolation of [4-^{3}H]NADH 20 μmoles non-labeled D-sorbitol were incubated at 25 °C with 0 8 μmole [4-^{3}H]NAD with a specific radioactivity of 1 1 10^8 dpm/μmole and 500 μmoles glycine–NaOH buffer (pH 9 5) per ml of reaction mixture The reaction was started with 3 units L-iditol dehydrogenase with a spec act of 3 7 units/mg protein, and after 30 min when 0 25 μmole NADH/ml was formed, the mixture was poured onto a 1 cm × 5 cm DEAE-cellulose anion-exchange column in the bicarbonate form and washed first with 50 ml water (less than 2% of the total radioactivity appears in this fraction), and second with 100 ml 3 5 mM NH_4HCO_3 (this displaces NAD off the column) Finally, the NADH was eluted with 10–15 ml of 0 2 M NH_4HCO_3 [5], and concentrated in vacuo

(2) Analysis of the chirality of the isolated [4-^{3}H]NADH The ^{3}H content of the A-position of 0 4 μmole [4-^{3}H]NADH was transferred to L-malate with 2 1 μmoles oxaloacetate and 5 units of L-malate dehydrogenase, an A-enzyme [6], in 1 ml of 1 M NH_4HCO_3 at pH 7 and 25 °C After the reaction had reached equilibrium, the enzyme was deactivated by heating for 1 min at 90 °C and the incubation mixture divided into two parts in order to separately determine the specific radioactivities of the concomitantly produced L-malate and NAD

(3) Specific radioactivity of L-malate 0 2 μmole L-malate was incubated with 0 3 unit diaphorase-free NAD^+ nucleosidase for 30 min at 25 °C, diluted with 2 5 μmoles non-labeled L-malate, and the reaction mixture placed onto a 1 cm × 20 cm Dowex-1 X 8, 200–400 mesh, anion exchanger in the formate form The column was washed with 100 ml water and the L-malate eluted with a formic acid gradient according to the procedure of Bush et al [7], was concentrated in vacuo and the specific radioactivity measured [8]

(4) Specific radioactivity of NAD The second part of the above sample containing 0 20 μmole of L-malate originally produced was diluted with 20 4 μmoles of non-labeled L-malate (which corresponds to a 102-fold dilution) Then the NAD present was reduced back to NADH, using 500 μmoles glycine–hydrazine buffer of pH 9 5 [8] and 5 units L-malate dehydrogenase/ml of reaction mixture The NADH thus generated was isolated as described above and its specific radioactivity measured In a second experiment another aliquot of the above solution (see *Analysis of the chirality of the isolated [4-^{3}H]NADH*) containing 0 20 μmole NAD was added to 25 μmoles L-lactate and the NAD reduced to NADH with 6 units L-lactate dehydrogenase in 500 μmoles/ml glycine–hydrazine buffer of pH 9 5 and isolated as described above [5] No significant difference was found in the specific radioactivity of the isolated NADH, using L-malate dehydrogenase or L-lactate dehydrogenase, in the experiments described above

(5) ^{3}H content of the B-position of [4-^{3}H]NADH The ^{3}H content of the B-position of 0 20 μmole of [4-^{3}H]NADH was transferred to L-glutamate with 1 8 μmole oxaloacetate and 3 units L-glutamate dehydrogenase, a B-enzyme [2], in 1 ml of 1 M NH_4HCO_3 at a pH of 7 and 25 °C The L-glutamate formed was then diluted with 2 8 mmoles of non-labeled L-glutamate and recrystallized three times from water to constant specific radioactivity

RESULTS AND DISCUSSION

Table I summarizes the experimental results It can be shown that the transfer of hydrogen from D-sorbitol to NAD catalyzed by L-iditol dehydrogenase in analogy to other NAD-linked dehydrogenases studied is a direct transfer, not involving the hydrogen atoms of the surrounding medium [1] ^3H in NADH produced by oxidation

TABLE I

^3H TRANSFER FROM D-SORBITOL TO [4-^3H]NAD CATALYZED BY L-IDITOL DEHYDROGENASE FROM SHEEP LIVER

The specific radioactivities in this table refer to the following steps of the overall reaction scheme

$$[4\text{-}^3H]NAD^1 + \text{D-sorbitol} \xrightleftharpoons[\text{dehydrogenase}]{\text{L-iditol}} [4\text{-}^3H]NADH^2 + \text{D-fructose}$$

$$[4\text{-}^3H]NADH^2 + \text{oxalacetate} \xrightleftharpoons[\text{dehydrogenase}]{\text{L-malate}} [4\text{-}^3H]NAD^3 + \text{L-malate}^4$$

$$[4\text{-}^3H]NADH^2 + \alpha\text{-ketoglutarate} + NH_3 \xrightleftharpoons[\text{dehydrogenase}]{\text{L-glutamate}} NAD + [2\text{-}^3H]\text{-L-glutamate}^5$$

Specific radioactivity (dpm/μmole)

[4-^3H]NAD^1	NADH^2	NAD^3	L-malate^4	L-glutamate^5
$1\ 10\ 10^8$	$1\ 08\ 10^8$	$9\ 91\ 10^7$	$1\ 5\ 10^6$	$1\ 12\ 10^8$

of D-sorbitol with [4-^3H]NAD can be quantitatively transferred to L-glutamate with L-glutamate dehydrogenase, a B-enzyme [2] In addition, it was shown that when the A hydrogen atom was transferred to L-malate with L-malate dehydrogenase known to be an A-enzyme [5], less than 2% of the original specific radioactivity was transferred to L-malate and 98% was retained in NAD which is concomitantly produced

These experiments conclusively prove that the NADH produced in the L-iditol dehydrogenase reaction has its ^3H content localized in the B-position, whereas, the newly acquired hydrogen atom from non-labeled D-sorbitol must have entered in the opposite or A-position The results allow the enzyme L-iditol dehydrogenase, also known as D-sorbitol dehydrogenase (EC 1 1 1 14), from sheep liver to be classified, in regard to the stereospecificity of the hydrogen transfer to NAD, as an A-enzyme

ACKNOWLEDGEMENTS

This work was supported by U S P H S Grants N I H AM14977 and N I H HL13636

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