

Chirality of the Hydrogen Transfer to the Coenzyme Catalyzed by Ribitol Dehydrogenase from *Klebsiella Pneumoniae* and D-Mannitol 1-Phosphate Dehydrogenase from *Escherichia coli*

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Summary: The stereochemistry of the hydrogen transfer to NAD catalyzed by ribitol dehydrogenase (ribitol:NAD⁺ 2-oxidoreductase, EC 1.1.1.56) from *Klebsiella pneumoniae* and D-mannitol-1-phosphate dehydrogenase (D-mannitol-1-phosphate:NAD⁺ 2-oxidoreductase, EC 1.1.1.17) from *Escherichia coli* was investigated. [4-³H]NAD was enzymatically reduced with nonlabelled ribitol in the presence of ribitol dehydrogenase and with nonlabelled D-mannitol-1-phosphate and D-mannitol 1-phosphate dehydrogenase, respectively. In both cases the [4-³H]-NADH produced was isolated and the chirality at the C-4 position determined. It was found that after the transfer of hydride, the label was in both reactions exclusively confined to the (4*R*) position of the newly formed [4-³H]NADH. In order to explain these results, the hydrogen transferred from the nonlabelled substrates to [4-³H]NAD must have entered the (4*S*) position of the nicotinamide ring. These data indicate for both

investigated inducible dehydrogenases a classification as B or (*S*) type enzymes.

Ribitol also can be dehydrogenated by the constitutive A-type L-idoitol dehydrogenase (L-idoitol:NAD⁺ 5-oxidoreductase, EC 1.1.1.14) from sheep liver. When L-idoitol dehydrogenase utilizes ribitol as hydrogen donor, the same A-type classification for this oxidoreductase, as expected, holds true. For the first time, opposite chirality of hydrogen transfer to NAD⁺ in one organic reaction – ribitol + NAD⁺ = D-ribulose + NADH + H⁺ – is observed when two different dehydrogenases, the inducible ribitol dehydrogenase from *K. pneumoniae* and the constitutive L-idoitol dehydrogenase from sheep liver, are used as enzymes. This result contradicts the previous generalization that the chirality of hydrogen transfer to the coenzyme for the same reaction is independent of the source of the catalyzing enzyme.

Key words: Chirality, hydrogen transfer, NAD-linked dehydrogenases, ribitol dehydrogenase, D-mannitol-1-phosphate dehydrogenase, L-idoitol dehydrogenase.

Enzymes:

Glutamate dehydrogenase, (NAD(P)⁺), L-glutamate:NAD(P)⁺ oxidoreductase (deaminating) (EC 1.4.1.3);
L-idoitol dehydrogenase, L-idoitol:NAD⁺ 5-oxidoreductase (EC 1.1.1.14);
Lactate dehydrogenase, L-lactate:NAD⁺ oxidoreductase (EC 1.1.1.27);
Mannitol-1-phosphate dehydrogenase, D-mannitol-1-phosphate:NAD⁺ 2-oxidoreductase (EC 1.1.1.17);
Ribitol dehydrogenase, ribitol:NAD⁺ 2-oxidoreductase (EC 1.1.1.56).