# Chirality of the Hydrogen Transfer to NAD Catalyzed by *myo*-Inositol Dehydrogenase from *Klebsiella pneumoniae*

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The chirality of the hydrogen transfer to NAD catalyzed by *myo*-inositol dehydrogenase (*myo*-inositol: NAD 2-oxydoreductase, EC 1.1.1.18) from *Klebsiella pneumoniae* (formerly classified taxonomically as *Aerobacter aerogenes* or *Klebsiella aerogenes*) was investigated. |4-$^3$H|NAD was enzymatically reduced to |4-$^3$H|NADH with non-labeled *myo*-inositol and *myo*-inositol dehydrogenase. The stereochemistry of the prochiral center at C4 of the NADH produced was determined. It was found that the label was exclusively located at the (4S) position of the produced NADH. Since the hydrogen transferred from non-labeled *myo*-inositol to |4-$^3$H|NAD must have entered the opposite or (R) position, *myo*-inositol dehydrogenase from *K. pneumoniae* should be classified as an (R) or A-type enzyme with respect to the stereochemistry of the hydrogen transfer to NAD.

The absolute streochemistry of direct hydrogen transfer from substrates to the prochiral $C_4$ position of the nicotinamide ring of the coenzyme, and vice versa, catalyzed by pyridine nucleotide-linked dehydrogenases has been conclusively demonstrated [2–5]. Dehydrogenases able to catalyze the hydrogen transfer to the pro(R) or pro(S) position of the $C_4$ prochiral center of the nicotinamide ring of the coenzyme [4] were classified as A or B-type enzymes, respectively [2–5].

A large number of NAD and NADP-linked dehydrogenases have been investigated with regard to their stereochemistry of hydrogen transfer to the coenzyme. Results are summarized in references 6 to 8.

In this communication, the chirality of hydrogen transfer catalyzed by the inducible NAD-linked dehydrogenase *myo*-inositol dehydrogenase (EC 1.1.1.18) from *K. pneumoniae* [1] was investigated. |4-$^3$H|NAD was enzymatically reduced to |4-$^3$H|-NADH with *myo*-inositol and *myo*-inositol dehydrogenase from *K. pneumoniae*. The chiralty at the $C_4$ position of the produced |4-$^3$H|NADH was analyzed by transfer of the hydrogen of the (4S) position to α-ketoglutarate with the B-type (S) glutamate dehydrogenase. From Table I one can ascertain that more than 95% of the label originally present in the (4S)-position of the generated |4-$^3$H|NADH is transferable to α-ketoglutarate by the reaction catalyzed by (S) glutamate dehydrogenase. This outcome was confirmed in another experiment, in which the specific radioactivity of the concomitantly produced NAD was determined. For that purpose the NAD content of another aliquot from the incubation mixture was enzymatically reduced to NADH with nonlabelled (S) lactate and (S) lactate dehydrogenase. As expected only a small fraction (3%) of the original label remains attached to the newly formed NADH (NADH$^c$ from Table I). These results prove that the label of the originally produced |4-$^3$H|NADH is located at the (4S) position of the nicotinamide ring. Hence the hydride transferred from nonlabelled *myo*-inositol to |4-$^3$H|NAD catalyzed by *myo*-inositol dehydrogenase must have entered the (4R)-position of the produced (4S)|4-$^3$H|NADH. This result allows the classification of the investigated *myo*-inositol dehydrogenase from *K. aerogenes* as an (R) or A-type enzyme.

Table I. Stereochemistry of the enzymic hydrogen transfer from *myo*-inositol to |4-$^3$H|NAD catalyzed by *myo*-inositol dehydrogenase from *Klebsiella pneumoniae*.

| Specific radioactivities * [dpm/μmol] | | | |
|---|---|---|---|
| \|4-$^3$H\|NAD$^a$ | \|4-$^3$H\|NADH$^a$ | (S) glutamate$^b$ | \|4-$^3$H\|NADH$^c$ |
| $9.2\times10^6$ | $9.0\times10^6$ | $8.7\times10^6$ | $2.8\times10^5$ |

* The specific radioactivities of Table I refer to the following steps in the following reaction scheme:

|4-$^3$H|NAD$^a$ + *myo*-inositol $\xrightarrow{\text{IDH}}$ 2-keto-*myo*-inositol + |4-$^3$H|NADH$^a$

|4-$^3$H|NADH$^a$ + α-ketoglutarate + $NH_3$ $\xrightarrow{\text{GDH}}$ (S) glutamate$^b$ + |4-$^3$H|NAD$^c$

|4-$^3$H|NAD$^c$ + (S) lactate $\xrightarrow{\text{LDH}}$ pyruvate + |4-$^3$H|NADH$^c$

## *Experimental Section*

*Myo*-Inositol, pyruvic acid, (S) lactic acid, (S)-glutamic acid, (S) lactate dehydrogenase (EC 1.1.1.27) from rabbit muscle, (S) glutamate dehydrogenase (EC 1.4.1.3) from beef heart, and *myo*-inositol dehydrogenase (EC 1.1.1.18) from *Klebsiella pneumoniae* [9, 10] were purchased from Sigma Chemical Co. |4-$^3$H|NAD with a specific radio-

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*Abbreviations used:* IDH, *myo*-inositol dehydrogenase (EC 1.1.1.18); GDH, (S) glutamate dehydrogenase (EC 1.4.1.3); LDH, (S) lactate dehydrogenase (EC 1.1.1.27).

activity of 50 Ci/mol was obtained from The Radiochemical Center, Amersham. *Myo*-inositol was recrystallized three times from water by addition of ethanol. *Myo*-Inositol dehydrogenase was further purified by chromatography at 5 °C on a $1 \times 30$ cm P2-column (Bio-Rad Lab.) previously equilibrated with 0.05 M Tris HCl buffer of pH 8.2 (measured at 25 °C) and obtained by elution with the same buffer.

The enzymatic hydrogen transfer from *myo*-inositol to $|4\text{-}^3H|$NAD was performed incubating 9.0 $\mu$mol of $|4\text{-}^3H|$NAD with a specific radioactivity of $9.2 \times 10^6$ dpm/$\mu$mol with 48 $\mu$mol of non-labelled *myo*-inositol and 0.4 units of *myo*-inositol dehydrogenase (19 U/mg) in a total volume of 3.0 ml of reaction medium containing in addition 600 $\mu$mol of Tri-HCl buffer previously adjusted to pH 8.4 at 25 °C. After a 10 minute incubation, 0.6 $\mu$mol $|4\text{-}^3H|$NADH was isolated [11, 12] and its chirality at $C_4$ determinated as already described [12].

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