# ON THE INCORPORATION OF TRITIUM IN YEAST ALCOHOLDEHYDROGENASE FROM ETHANOL-T OR NADH-T

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It was found recently, that during the dehydrogenation of ethanol-1-T through yeast alcoholdehydrogenase (YADH) under alkaline conditions, tritium is transfered to the enzyme protein; after hydrolysis of the protein, T is found in the tryptophan residue (Schellenberg 1965). It was postulated, that tryptophan participates as an intermediate in the hydrogen transfer of YADH. In this paper we can show, that incorporation of T into the enzyme takes place both with A-NADH-T (under conditions which do not lead to enzymatic equilibrium) and with B-NADH-T. By studying the time dependence of T incorporation from ethanol-1-T into products it is further shown, that enzyme labeling follows the NADH formation. These findings are not consistent with the hypothesis that tryptophan is an intermediate in enzymatic hydrogen transfer.

## Methods

YADH was purchased from Boehringer. Ethanol-1,1-T was prepared from acetaldehyde and $LiBH_4$-T. A-NADH-T was prepared enzymatically from ethanol-1,1-T. NAD-T was prepared by enzymatic oxidation of A-NADH-T by glutamic dehydrogenase (Krakow et al. 1963) and purified by ion-exchange chromatography.

Isolation of labeled protein and NADH: After denaturation of YADH by $HClO_4$ (final conc. 2 m) the protein was centrifuged and resuspended 3 times in $H_2O$. It was separated from low molecular weight impurities by chromatography on Sephadex G-25 (40 x 1 cm) in 0.2 m NaOH as solvent. Reproducible specific radioactivities were obtained.- NADH-T was isolated by chromatography on DEAE-carbonat (Silverstein and Boyer 1964)

Aqueous samples containing the T-labeled compounds were assayed in the appropiate scintillator solution with a Packard Liquid Scintillation counter.

## Results and discussion

Under conditions which establish a rapid formation of the equilibrium between YADH + ethanol + $NAD^+ \rightleftharpoons$ YADH + acetaldehyde + NADH + $H^+$, Schellenberg (1965) found the maximal incorporation of 2.2 T per mol of enzyme from ethanol-1,1-T. The repetition of this experiment under similar conditions in our laboratory (YADH $4.5 \cdot 10^{-5}$m, NAD $3.6 \cdot 10^{-2}$m, ethanol $2.6 \cdot 10^{-2}$m, 0 °C, pH 11.0) and isolation of the protein as described above, yielded 3.75 T per mol of enzyme ( calculated for the specific radioactivity of the transferable H of ethanol) ("experiment 1").

Consecutively we examined the increase of the specific radioactivity of NADH and of the protein under 10 fold lower concentrations of the reactants, to see, if possible, a time dependence of T incorporation (Table 1). The increase of specific radioactivity of the protein takes place more slowly than the increase of NADH concentration (by optical test) and the increase of the specific radioactivity of NADH-T. The incorporation of T into the enzyme is therefore not due to an enzyme

Table 1. Incorporation of T in YADH from ethanol-1,1-T

| time (min.) | NADH (%*) | NADH cpm/μmol ($\times 10^{-4}$) | protein cpm/μmol ($\times 10^{-4}$) | T per mol of enzyme |
|---|---|---|---|---|
| 0.25 | 50 | | | |
| 0.5 | 80 | | 236 | 1.1 |
| 1.0 | 87 | 184 | | |
| 2.5 | | | 252 | 1.2 |
| 3.0 | 90 | 196 | | |
| 8.0 | 96 | 187 | 339 | 1.6 |
| 30 | 100 | 197 | 360 | 1.7 |

concentrations of the test system: YADH $2 \cdot 10^{-6}$m, NAD $2 \cdot 10^{-3}$m, ethanol-1,1-T $2.4 \cdot 10^{-3}$m, pH 10.7, ice bath. *100% at 30 min.

bound intermediate in the H transfer from ethanol to NAD.

The foregoing experiment indicates, that T labeling of the enzyme is brought about after the formation of NADH-T. This explains the results of Schellenberg (1965) that ethanol-1-T, which is labeled in the nontransferring position, gives no incorporation of T into the enzyme, because no labeled NADH is formed. Therefore we examined the possibility of T labeling of the enzyme under conditions which do not yield an enzymatic equilibrium as in experiment 1 and 2, that is by ommitting acetaldehyde (Table 2). Lyophilized YADH and A-NADH-T were dissolved in buffer. We found the incorporation of 0.5 T per mol of enzyme. The smaller incorporation of T as compared to experiment 1 is nonetheless comparable to the small incorporation from ethanol-T found by Schellenberg, if small concentrations of NAD (or NADH) are present. A similar effect is seen if the

Table 2. Incorporation of T in YADH from A-NADH-T

| time (min.) | NADH Concentration | NADH cpm/μmol ($\times 10^{-4}$) | protein cpm/μmol ($\times 10^{-4}$) | T per mol of enzyme |
|---|---|---|---|---|
| 0 | $2.07 \cdot 10^{-3}$m | 270 | | |
| 2 | $2.07 \cdot 10^{-3}$m | 280 | 153 | 0.55 |
| 10 | $2.07 \cdot 10^{-3}$m | 275 | 145 | 0.53 |

concentrations of the test system: YADH $2.8 \cdot 10^{-5}$m, NADH $2.08 \cdot 10^{-3}$m, pH 10.7, ice bath.

concentration of NADH is further lowered by addition of acetaldehyde ($1 \cdot 10^{-2}$m) under conditions comparable to those in table 2. Incorporation of 0.03 T per mol of enzyme is found.

Finally we examined the incorporation of T into the enzyme from NAD-T, which, in the YADH reaction, forms B-NADH-T. The experimental conditions were those of experiment 1, but NAD was replaced by NAD-T and ethanol-1,1-T by ethanol. Due to the stereospecifity of the enzyme no transfer of T can take place during the dehydrogenation reaction, but B-NADH-T is formed. Incorporation of 1.9 T per mol of enzyme was found.

Therefore, the T labeling of YADH by labeled substrates is mediated by either A- or B-NADH-T in a reaction mechanism which is not an intermediate step in the H transfer from substrate to NAD and which is not characterized by the stereospecifity of YADH. Schellenberg (1965) found a marked pH dependance on T incorporation. This can be explained by its influence on the NADH concentration, but there may be also an influence on the equilibrium $H_{NADH}/H_{protein}$.

Other information about the participation of a tryptophan residue in a dehydrogenase reaction was found for glutamic dehydrogenase, where it is involved in substrate binding (Fisher and Gross 1965).

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## References

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