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Note

## Enzymatic Oxidation of NADH by Streptomycin

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Streptomycin was established as a novel substrate of yeast alcohol dehydrogenase (EC 1.1.1.1). Kinetic parameters and the primary deuterium isotope effect for the forward enzymatic reaction were determined.

NADH, in the presence of alcohol dehydrogenase (EC 1.1.1.1), can readily reduce an aldehyde function attached to an aliphatic or an aromatic carbon atom in a wide variety of compounds.<sup>1</sup> In addition to the known carbonyl compounds, we have detected a novel carbonyl substrate of the NAD-dependent alcohol dehydrogenase — the antibiotic streptomycin.

Streptomycin has a single free aldehyde function in the streptose moiety of its molecule. This aldehyde function was not reduced non-enzymatically by NADH, in a 50 mM sodium phosphate buffer, pH 7.5 at 24 °C. In the presence of yeast alcohol dehydrogenase (0.1—1 μM; Sigma Chemie GmbH, Taufkirchen, Germany), under identical experimental conditions, NADH (10—200 μM; Sigma) was visibly oxidized by streptomycin (10—100 μM; Sigma). Reaction obeyed the Michaelis kinetics, affording the estimation of kinetic parameters. Figure 1 illustrates the estimation of kinetic parameters for streptomycin, and Table I summarizes the kinetic parameters for the forward reaction, oxidation of NADH by streptomycin and acetaldehyde.

All kinetic parameters in the Table I were estimated spectrophotometrically, from the disappearance of the NADH-band at 340 nm. Due to the limited solubility of streptomycin in neutral buffer solutions ( $\leq 0.1$  M), kinetic parameters for streptomycin were determined under the conditions  $[S] \leq 0.5 K_m$  (Figure 1).

Enzymatic reaction was specific for alcohol dehydrogenase, and did not occur in the presence of lactate, malate, glutamate or glyceraldehyde-3-phosphate dehydrogenases. Streptomycin was a poor substrate of yeast alcohol dehydrogenase. Specific activity of YADH on streptomycin was approx. 300-fold lower compared to acetaldehyde, and the Michaelis constant for streptomycin was approx. 300-fold higher compared to acetaldehyde. The forward reaction on streptomycin afforded a small primary deuterium isotope effect (Table I); this indicated that the direct transfer of hydro-

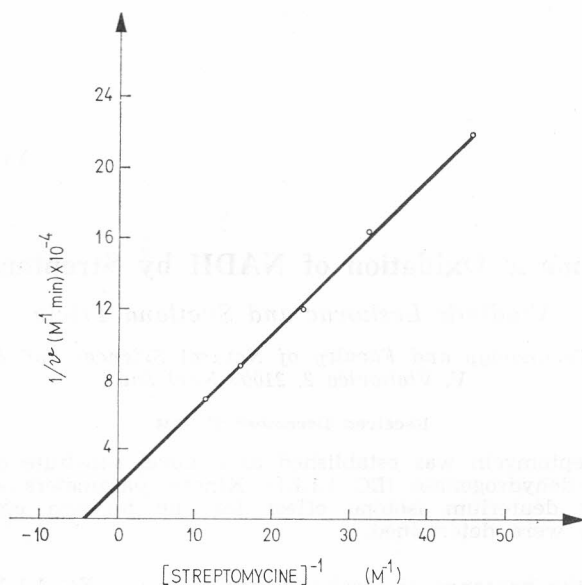


Figure 1. Lineweaver-Burk plot for the oxidation of NADH (150  $\mu$ M) by streptomycin, in the presence of yeast alcohol dehydrogenase (0.5  $\mu$ M in active sites). Experimental details are given in Table I.

TABLE I  
Steady-state Kinetic Parameters for the Forward Reaction<sup>a</sup>

Parameter	Acetaldehyde	Streptomycin
Michaelis constant	0.8 $\pm$ 0.2 mM	0.23 $\pm$ 0.02 M
Specific activity of alcohol dehydrogenase on <sup>b</sup>	1000 U/mg	3 U/mg
Primary deuterium KIE <sup>c</sup>	2.00 $\pm$ 0.20	1.31 $\pm$ 0.10

<sup>a</sup> Measured in a 50 mM sodium phosphate buffer, pH 7.5 at 24 °C. Average values of three experiments are shown.

<sup>b</sup> U (international unit of enzyme activity) was defined as a quantity of YADH capable of transforming one  $\mu$ mole of substrate per minute, under specified conditions.

<sup>c</sup> Primary deuterium kinetic isotope effect (KIE) was calculated from the  $V_{max}$ ; NAD (D/H) was synthesized by the reduction of NAD<sup>+</sup> with dithionite in D<sub>2</sub>O<sup>2</sup>. KIE was determined experimentally and corrected for the isotope purity of reduced coenzyme.<sup>3</sup>

gen nucleus from the C-4 position of nicotinamide moiety of NADH to the substrate was the rate-limiting step in the forward reaction. At higher concentrations of streptomycin (> 10 mM) and NADH (> 1 mM), only 15% of NADH was oxidized; this probably indicates that the equilibrium was shifted towards the oxidized substrate, streptomycin.

The purpose of this communication was to establish streptomycin as a novel substrate of alcohol dehydrogenase. The technical feasibility of this novel reaction for the production of dihydro-streptomycin seems unlikely,

due to the low yield and high costs of NADH. Eventual application of this reaction for the enzymatic determination of streptomycin requires further detailed investigation.

## REFERENCES

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## SAZETAK

*Enzimatska oksidacija NADH streptomicinom*

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Utvrđeno je da je streptomycin novi supstrat alkohol dehidrogenaze kvasca (EC 1.1.1.1). Određeni su kinetički parametri i primarni deuterijski izotopni efekat za ovu enzimsku reakciju.