Biocatalytic oxidation of *rac*-borneol to (+)-camphor catalysed by NAD⁺-dependent borneol dehydrogenase

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Introduction

Borneol dehydrogenases offer potential for the kinetic resolution of racemic alcohols.

Plant-based borneol dehydrogenase from *Salvia rosmarinus* (***Sr*BDH1**) catalyses the (+)-camphor production, a valuable ingredient for the pharmaceutical industry.

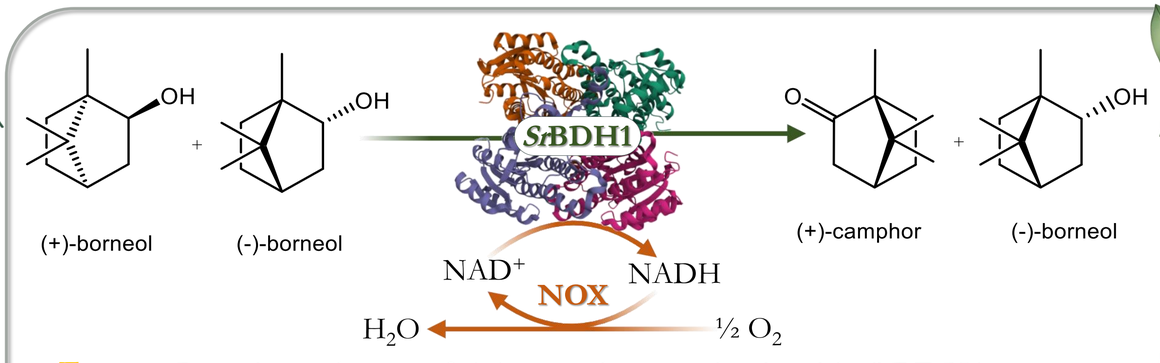

Figure 1. Reaction scheme of (+)-camphor synthesis with *Sr*BDH1 enzyme.

Research goal

Develop an efficient (+)-camphor synthesis route (***Fig 1***) coupled with an NAD⁺ cofactor regeneration system to mitigate the high expense of cofactor addition.

Results

1. KINETIC STUDIES

measured spectrophotometrically

$$V.A. = \frac{\frac{dA}{dt} \cdot V_{reactor}}{\varepsilon_{NADPH} \cdot d \cdot V_{enzyme}} \qquad S.A. = \frac{V.A.}{\gamma_{enzyme}}$$

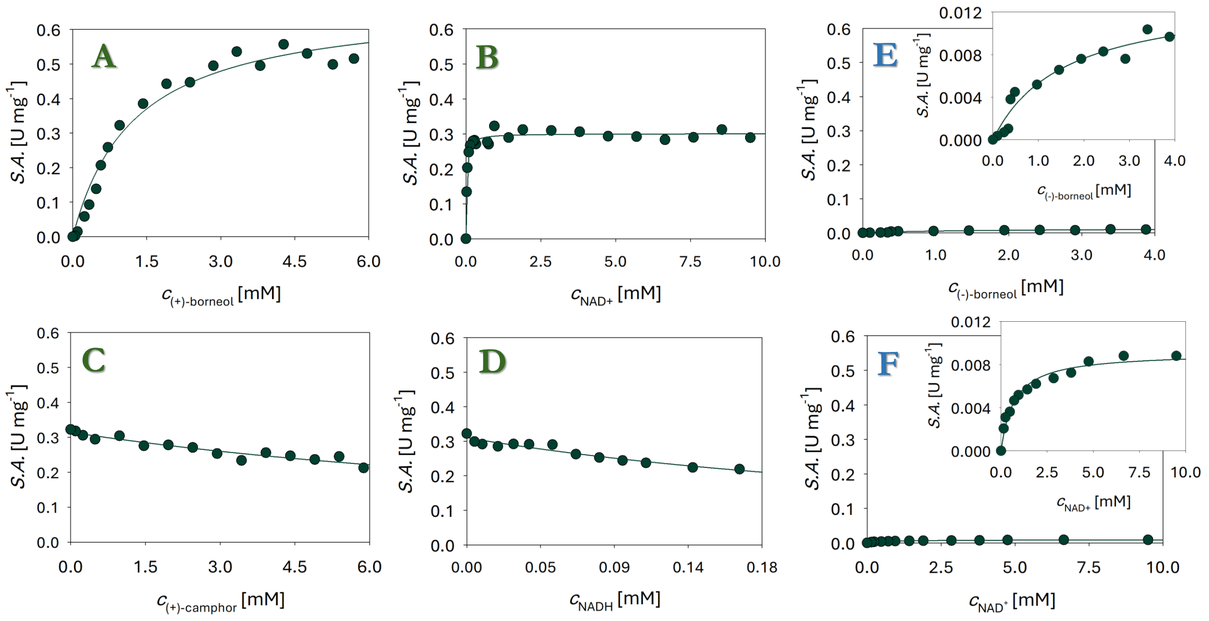

Figure 2. Influence of substrates and products on the specific activity (*S.A.*) of the ***Sr*BDH1** enzyme in the reaction with (+)-borneol *A* (+)-borneol, *B* NAD⁺, *C* (+)-camphor, *D* NADH and comparison with the reaction with (-)-borneol *E* (-)-borneol, *F* NAD⁺

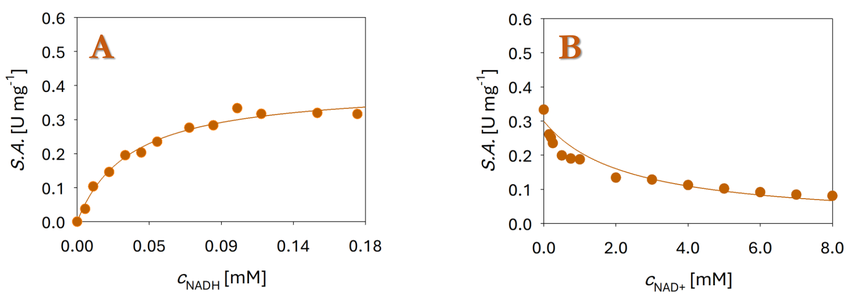

Figure 3. Influence of *A* NADH and *B* NAD⁺ on the specific activity (*S.A.*) of the **NOX** enzyme

V.A.–volume activity, *S.A.*–specific activity

2. PARAMETER ESTIMATION AND KINETIC MODEL SETUP

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$$S.A. \;(c_{substrate}) \rightarrow V_m, K_m \qquad S.A. \;(c_{product}) \rightarrow K_i$$

$$r = \frac{V_m \cdot c_{substrate}}{\left(K_m^{substrate} \cdot \left(1 + \frac{c_{product}}{K_i^{product}}\right) + c_{substrate}\right)}$$

Table 1. Kinetic parameters estimated from data in Figure 2

parameter	*value*	*parameter*	*value*
with (+)-borneol		**with (-)-borneol**	
V_m [U mg⁻¹]	0.696 ± 0.021	V_m [U mg⁻¹]	0.024 ± 0.003
$K_m^{(+)\text{-borneol}}$ [mM]	1.250 ± 0.086	$K_m^{(-)\text{-borneol}}$ [mM]	1.563 ± 0.293
K_m^{NAD+} [mM]	0.024 ± 0.005	K_m^{NAD+} [mM]	0.708 ± 0.125
$K_i^{(+)\text{-camphor}}$ [mM]	8.087 ± 0.814	$K_i^{(-)\text{-camphor}}$ [mM]	not measured
K_i^{NADH} [mM]	0.009 ± 0.001	K_i^{NADH} [mM]	not measured

$$r_1 = \frac{V_m \cdot c_{(+)-borneol} \cdot c_{NAD^+} \cdot \gamma_{SrBDH1}}{\left(K_m^{(+)-borneol} \cdot \left(1 + \frac{c_{(+)-camphor}}{K_i^{(+)-camphor}}\right) + c_{(+)-borneol}\right) \cdot \left(K_m^{NAD^+} \cdot \left(1 + \frac{c_{NADH}}{K_i^{NADH}}\right) + c_{NAD^+}\right)}$$

Table 2. Kinetic parameters estimated from data in Figure 3

parameter	*value*
V_m [U mg⁻¹]	0.399 ± 0.016
K_m^{NADH} [mM]	0.033 ± 0.004
K_i^{NAD+} [mM]	0.574 ± 0.095

$$r_2 = \frac{V_m \cdot c_{NADH} \cdot \gamma_{NOX}}{\left(K_m^{NADH} \cdot \left(1 + \frac{c_{NAD^+}}{K_i^{NAD^+}}\right) + c_{NADH}\right)}$$

V_m–maximal reaction rate, K_m–Michaelis constant, K_i–inhibition constant

r_1–reaction velocity for main reaction of (+)-borneol oxidation, r_2–reaction velocity of NAD⁺ regeneration

3. MODEL SIMULATIONS

modelling in SCIENTIST

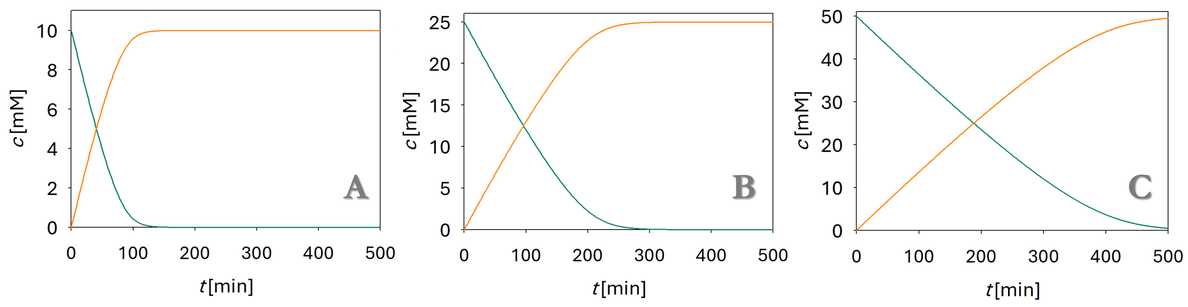

Figure 4. Simulations for (+)-borneol oxidation to (+)-camphor,
A 10 mM (+)-borneol, 0.1 mM NAD⁺, 0.4 mg/ml *Sr*BDH1, 1 mg/ml NOX,
B 25 mM (+)-borneol, 0.1 mM NAD⁺, 0.4 mg/ml *Sr*BDH1, 1 mg/ml NOX,
C 50 mM (+)-borneol, 0.1 mM NAD⁺, 0.4 mg/ml *Sr*BDH1, 1 mg/ml NOX,
– simulation for (+)-borneol, – simulation for (+)-camphor

Highlights and conclusion

The kinetics of the main reaction was investigated (***Fig. 2 A-D***). Besides (+)-borneol, the enzyme activity was tested with (-)-borneol (***Fig. 2 E-F***). From estimated kinetic parameters (***Table 1***) it can be seen that the activity of the enzyme towards (-)-borneol is nearly 30 fold lower, showing ***Sr*BDH1** preference towards (+)-borneol. The reaction's kinetics can be described by double substrate Michaelis-Menten kinetics with included product inhibitions as shown in equation r_1.

For NAD⁺ cofactor regeneration **NOX** enzyme was used. The kinetics of the regeneration reaction can be described by Michaelis-Menten kinetics with product inhibition (***Fig 3***, ***Table 2***, r_2).

According to simulations for batch reactions with NAD⁺ cofactor regeneration (***Fig. 4 A-C***), higher initial (+)-borneol concentration yields more (+)-camphor, which increasingly inhibits enzyme activity and prolongs full conversion time.

Next steps

4. EXPERIMENTS FOR MODEL VALIDATION

5. PROCESS OPTIMISATION AND MODEL APPLICATION

REFERENCES

[1] N. Milčić., M. Česnik, M. Sudar, Z. Findrik Blažević, *Kem. Ind.* 68 (2019) 427–436.

[2] A.M. Chánique, N. Dimos, I. Drienovská, E.Calderini, M.P. Pantín, C.O.P. Helmer, V. Sieber, L.P. Parra, B. Loll, R. Kourist, *ChemCatChem* 13 (2021) 2262-2277.

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