Adsorption Immobilization of (*S*)-Norcoclaurine Synthase on Low-Cost Materials Applied to the Stereoselective Pictet–Spengler Reaction

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RESUMO

The enzyme (*S*)-norcoclaurine synthase ((*S*)-NCS) stereoselectively catalyzes the Pictet–Spengler reaction between dopamine and carbonyl compounds, generating pharmacologically relevant tetrahydroisoquinolines (THIQs). However, its low thermal and solvent stability, along with limited reusability, restricts its application. This study evaluated the adsorption immobilization of Δ19*Tf*NCS (a variant of (*S*)-NCS) expressed in *E. coli* onto Kaolin and Celite 545. Kaolin showed higher adsorption capacity (0.224 $mg_{NCS}/mg_{support}$) than Celite 545 (0.008 $mg_{NCS}/mg_{support}$), attributed to its higher specific surface area (11.063 m²/g vs. 0.756 m²/g) and more negative zeta potential (−36.3 mV vs. −15.4 mV). Both supports provided thermal stability and reusability, but only Kaolin enhanced stability against DMSO. Monte Carlo simulations revealed the key role of positively charged residues in the enzyme's unstructured N-terminal region in its strong affinity for Kaolin.

Palavras-chave: (S)-norcoclaurine synthase, Biocatalysis, Enzyme Immobilization, Tetrahydroisoquinolines, Pictet-Spengler Reaction

Introduction

The enzyme (S)-norcoclaurine synthase ((S)-NCS) catalyzes the Pictet–Spengler reaction between dopamine and carbonyl compounds, such as aldehydes and ketones, leading to the formation of tetrahydroisoquinolines, an important class of compounds with a wide range of pharmaceutical applications (1).

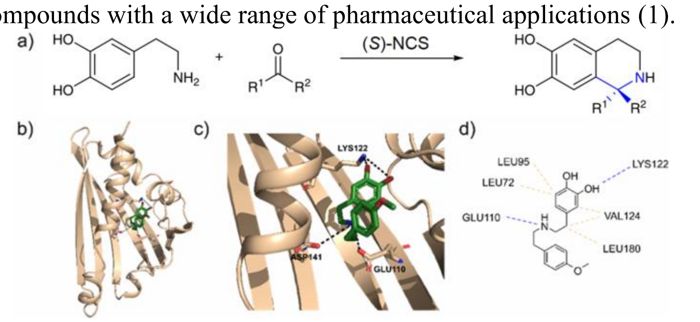

Figure 1. (*S*)-NCS and its catalized reaction scheme. **a)** reaction scheme, **b)** (*S*)-NCS structure (PDB: 5NON), **c)** Catalytic site with transition-state mimetic compound, **d)** Interactions between the catalytic site residues and transition-state mimetic compound.

To enhance the practical use of this biocatalyst, enzyme immobilization was employed as a strategy to improve its stability and reusability (2). The recombinant variant Δ19*Tf*NCS was immobilized by adsorption onto Kaolin and Celite 545. The biocatalysts were evaluated under different temperatures, co-solvent concentrations, and for their reusability.

Experimental

Δ19TfNCS expression and purification.

The gene encoding His₆-tagged Δ19*Tf*NCS was cloned into pET28a and transformed into E. coli BL21(DE3). Protein expression was induced with IPTG (0.5 mM) at OD_{600} = 0.6–0.8 (18 °C, 16 h). Cells were lysed by sonication and centrifuged. Purification was done via Ni^{2+} affinity and size exclusion chromatography.

Optimization of enzyme immobilization

Kaolin and Celite 545 (10–110 mg) were incubated with (S)-NCS solutions (1.0–7.5 mg·mL⁻¹) for 1 or 3 h in HEPES buffer (pH 7). After centrifugation (11,000 rpm, 4 °C), supernatants were analyzed at 280 nm. Immobilization yield (IY) was calculated (3):

$$\mathrm{IY}(\%) = \frac{C_i - C_s}{C_i} \times 100\% \qquad \text{eq. 1}$$

Immobilized enzyme performance evaluation

Pictet–Spengler reactions with dopamine (0.25 mM) and hexanal (2.5 mM) were used to test activity in 10% DMSO at 37 °C. Reusability was tested for 3 cycles. Reactions were also evaluated at 37, 47, and 57 °C and with 10–20% DMSO.

Quantitative NMR experiments

Samples (600 μL) were prepared with DSS (139 μM) and analyzed via ¹H NMR using noesygppr1d sequence with 20 s delay. Reaction yield was calculated:

$$\mathrm{RY}(\%) = \frac{I4'}{I4' + I4} \times 100\% \qquad \text{eq. 2}$$

Where I_4 and I_4' are integrals of H-4 in dopamine and product, respectively. Dopamine was assumed to be fully converted.

Stereoselectivity assessment

Reactions with dopamine (10 mM), hexanal (20 mM), and (S)-NCS (0.05 mg·mL⁻¹) were analyzed by chiral HPLC using a CHIROBIOTIC T column at 25 °C, 0.350 mL·min⁻¹.

Surface characterization

Samples were degassed (200 °C) and analyzed by N_2 adsorption at 77 K (BET method). Zeta potential was measured at 1 mg·mL⁻¹ in HEPES buffer using a Zetasizer Nano ZS.

Coarse-Grained Monte Carlo Simulation

Kaolin was modeled as a negatively charged cylinder. Enzyme structure was based on PDB 5N8Q with added N-terminal residues. Simulations at constant pH used the Metropolis criterion, with sampling of conformational and protonation states. Electrostatic interactions were included in the energy model (4).

Results and Discussion

The reaction between dopamine and hexanal catalyzed by (S)-NCS, was used to assess the biocatalysts' performance. Reaction yields were determined by ^{1}H NMR, using the integral of dopamine H-4 and product H-4' (Figure 2a).

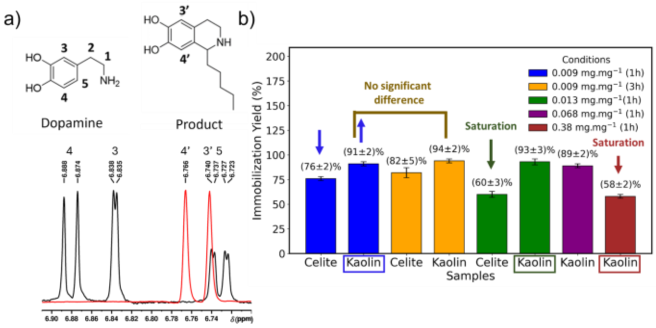

Figure 2. a) ^{1}H NMR spectrum (ppm) zoomed at the aromatic ring region showing dopamine signals (black) and product signals (red). **b)** Immobilization yield of Δ19*Tf*NCS in the Kaolin and Celite 545 at different conditions.

Kaolin showed greater (*S*)-NCS immobilization than Celite 545. The optimized protocol used 130 mg Celite or 10 mg Kaolin, 0.95 mg·mL⁻¹ enzyme, and 1 h incubation. Both supports maintained consistent yields over three reuse cycles, with minor variation after the first reuse.

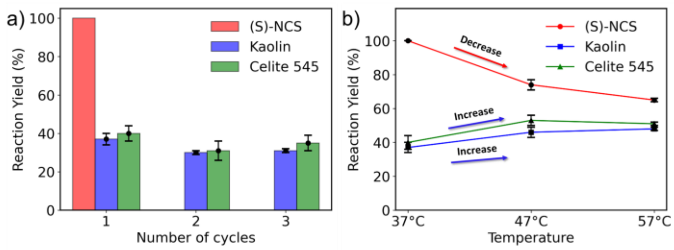

Figure 4. a) Reusability of Kaolin- and Celite 545-immobilized Δ19*Tf*NCS over three consecutive reaction cycles. **b)** Temperature profile.

The free enzyme and Celite-immobilized reaction yield with the increase of DMSO concentration while kaolin-immobilized enzyme remained stable (Table 1).

Table 1. Reaction yields of biocatalytic Pictet-Spengler reactions performed in HEPES buffer with 10% and 20% (v/v) DMSO concentration.

Sample	Reaction yield at 10% v/v DMSO	Reaction yield at 20% v/v DMSO
(*S*)-NCS	100%	56±4%
Kaolin	37± %	35±3%
Celite 545	40±4%	28±3%

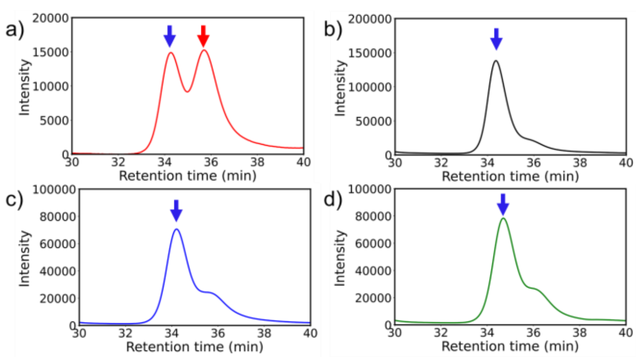

Figure 5. Chiral HPLC chromatograms expanded in the product detection region: a) racemic b) free (S)-NCS, c) Kaolin, d) Celite 545.

Chiral HPLC was used to assess the qualitatively the stereoselectivity of the Pictet–Spengler reaction. The non-enzymatic reaction gave a racemic mixture. The free enzyme showed high stereoselectivity. Immobilized enzymes showed slight loss but retained similar profiles (Figure 5).

Kaolin showed higher surface area (11.063 m²/g) than Celite 545 (0.756 m²/g), supporting its superior immobilization. Its zeta potential (−36.3 mV) was also more negative than Celite's (−15.4 mV).

Table 2. Supports' surface properties

Samples	Surface area B.E.T (m²/g)	Zeta-Potential (mV)
Kaolin	11.063	-36 ± 1
Celite 545	0.756	-15 ± 1

Molecular simulations showed strong adsorption of (*S*)-NCS to Kaolin at pH 7 via electrostatic interactions with the positively charged N-terminal His-tag. Residues His-6, His-7, His-8, His-10, and Arg-17 played key roles, while Lys-46 and His-49 remained distant (Figure 6).

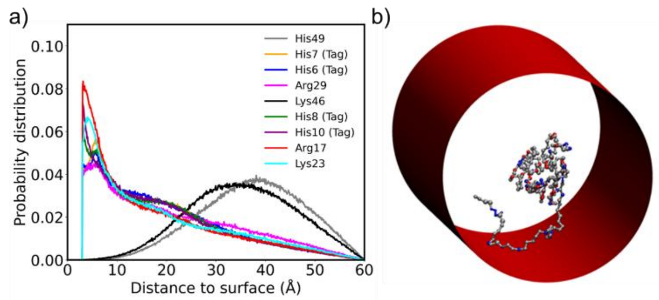

Figure 6. a) Probability distribution of the nine closest titratable residues **b)** Snapshot of (*S*)-NCS adsorbed on the kaolin surface, highlighting positively charged (blue) and negatively charged (red) residues at pH 7.

Conclusions

Immobilization enhanced (*S*)-NCS stability, solvent tolerance, and reusability. Kaolin and Celite 545 were effective low-cost supports, with kaolin showing higher yield and DMSO resistance due to greater surface area. Both preserved stereoselectivity. Simulations revealed key N-terminal histidines aid kaolin binding without blocking the active site.

Aknowlegments

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