

Article

# Efficient Synthesis of Cis-4-Propylcyclohexanol Using a Mutant Alcohol Dehydrogenase Coupled with Glucose Dehydrogenase

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**Abstract:** *cis*-4-Propylcyclohexanol is an important intermediate for synthesizing *trans*-2-(4-propylcyclohexyl)-1,3-propanediol, which is widely used in the manufacture of liquid crystal displays. In this study, *cis*-4-propylcyclohexanol was prepared using a mutant alcohol dehydrogenase from *Lactobacillus kefir* (LK-TADH, A94T/F147L/L199H/A202L) coupled with glucose dehydrogenase. Using the optimal catalytic conditions, 125 g/L (250 g) of 4-propylcyclohexanone was completely transformed after 5 h, and 225.8 g of *cis*-4-propylcyclohexanol (*cis/trans* ratio of 99.5:0.5) was obtained through extraction and rotary evaporation at a yield of 90.32%. This study reports a potential method for the green production of *cis*-4-propylcyclohexanol as the key intermediate of *trans*-2-(4-propylcyclohexyl)-1,3-propanediol at an industrial level.

**Keywords:** alcohol dehydrogenase; liquid crystal display; 4-propylcyclohexanone; *cis*-4-propylcyclohexanol; biotransformation

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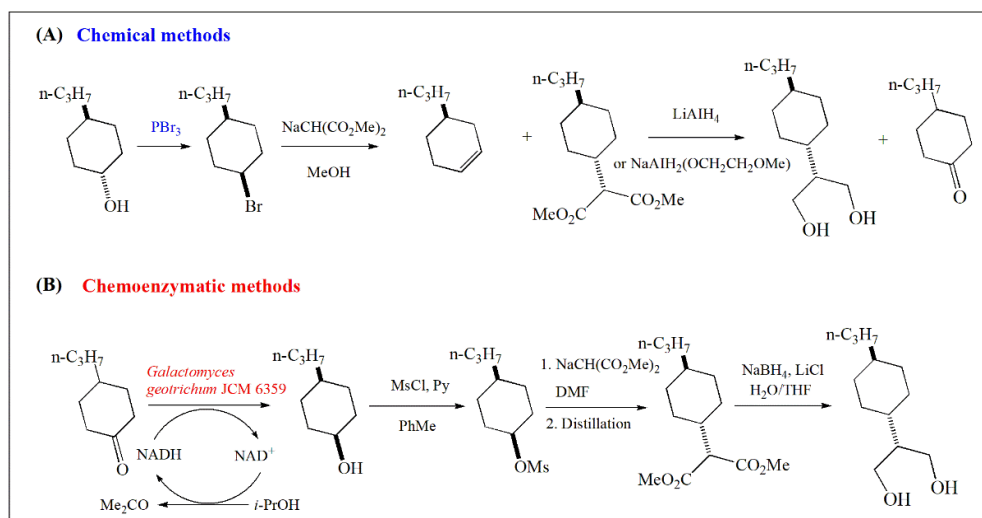
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## 1. Introduction

With an improvement in living standards and the progress of technology, liquid crystal displays (LCDs) have become an indispensable part of our daily life. The wide use of electronic equipment has created a strong demand for LCDs [1–3]. Recent research has shown that *trans*-2-(4-alkylcyclohexyl)-1,3-propanediol exhibits liquid crystalline properties and can be used as an immediate precursor to synthesize *trans*-1,3-dioxane-based liquid crystals [4,5]. *trans*-2-(4-Alkylcyclohexyl)-1,3-propanediol can be chemically synthesized from *trans*-4-alkylcyclohexanol (Figure 1A); however, the strong reducing agent (PBr<sub>3</sub>) and high concentration of the elimination product (4-propylcyclohexene) used during the process hamper the industrial production of *trans*-2-(4-alkylcyclohexyl)-1,3-propanediol [4]. Therefore, there is an urgent requirement to identify safe and reliable methods for the industrial production of *trans*-2-(4-alkylcyclohexyl)-1,3-propanediol.

In previous studies, Ikonaka et al. [4] reported that the synthesis of *trans*-2-(4-propylcyclohexyl)-1,3-propanediol can be chemoenzymatic using *cis*-4-propylcyclohexanol (Figure 1B). They discovered that *Galactomyces geotrichum* JCM 6359 could effectively catalyze the conversion of 4-propylcyclohexanone (69 g/L) to *cis*-4-propylcyclohexanol with a *cis/trans* ratio of 99.5:0.5. However, the amino acid sequence of the enzymes catalyzing the reaction was unknown, and 22% of the substrate was unused after the reaction. Thus, it is difficult to use this strain for the industrial production of *cis*-4-propylcyclohexanol.

Recombinant alcohol dehydrogenase (ADH) has been widely used in the reduction of ketones to obtain related key chiral intermediate alcohols such as chiral delta-lactones, (*R*)-ethyl 3-hydroxy-4,4,4-trifluorobutanoate, and (*R*)-(-)-3-quinuclidinol [6–8]. Therefore, it is necessary to find a suitable recombinant ADH for the catalytic production of *cis*-4-propylcyclohexanol.



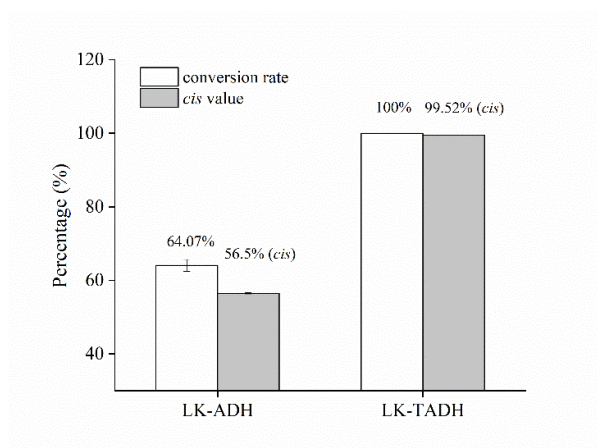
**Figure 1.** Chemical (A) and chemoenzymatic methods (B) in the synthesis of *trans*-2-(4-alkylcyclohexyl)-1,3-propanediol.

The mutant ADH from *Lactobacillus kefir* (LK-TADH, A94T/F147L/L199H/A202L) has been effectively used for the asymmetric reduction of *t*-butyl-6-chloro-3,5-dioxohexanoate [9], methyl-2-benzamidomethyl-3-oxobutyrate [10], and 2-chloro-1-(2,4-dichlorophenyl) ethenone [11]. In this study, recombinant LK-ADH coupled with recombinant glucose dehydrogenase (GDH) was used for the production of *cis*-4-propylcyclohexanol. The catalytic conditions of the enzyme were optimized, and molecular docking was used to illustrate the reason for the increased catalytic ability and the *cis/trans* ratio. Our study findings could likely serve as a suitable reference for the green production of *cis*-4-propylcyclohexanol, especially for the LCD industry.

## 2. Results and Discussion

### 2.1. Using ADH to Produce *Cis*-4-Propylcyclohexanol

*cis*-4-Propylcyclohexanol was biosynthesized by coupling LK-ADH or LK-TADH with GDH. After 6 h of reaction, the conversion rate achieved using recombinant LK-ADH was close to 64.1%, and the *cis/trans* ratio was 56.5:43.5 (Figure 2). However, the conversion rate achieved using recombinant LK-TADH was close to 100%, and the *cis/trans* ratio was 99.5:0.5 after a 6 h reaction. Therefore, LK-TADH was selected for subsequent studies.

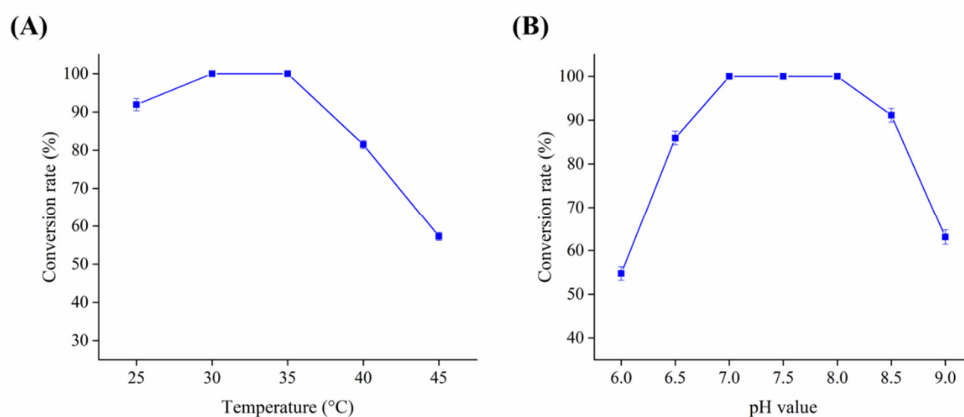


**Figure 2.** Comparison of the conversion rate and *cis/trans* ratio of the product between recombinant LK-ADH and LK-TADH. The reaction system (50 mL) was as follows: 4-propylcyclohexanone (50 g/L), 50 g/L of LK-ADH or LK-TADH (wet cell lysate), 10 g/L GDH (wet cell lysate), 0.1 g/L NAD<sup>+</sup>, glucose: substrate ratio (1.2 mol/mol). The reaction mixture was stirred at 35 °C, and the pH was adjusted to 7.0 using 2 M sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>).

## 2.2. Optimization of Reaction Temperature and pH

The change in temperature in the catalytic system has a considerable influence on the reaction rate, catalytic capacity, and stability of the enzyme [12–14]. Therefore, to better maintain the catalytic efficiency and stability of the enzyme, it is essential to determine the optimal catalytic temperature of the enzyme. As shown in Figure 3A, the substrate conversion rate was 91.63% at 25 °C and increased to 100% at 30 °C and 35 °C. However, with a further increase in the catalytic temperature, the substrate conversion rate decreased sharply and was found to be 82.5% at 40 °C and 57.4% at 45 °C. This was likely because an increase in the catalytic temperature led to increased diffusion and availability of the substrate around the enzyme molecules, thus increasing contact with the enzyme and improving the catalytic efficiency. However, a further increase in the catalytic temperature or prolonged exposure to high catalytic temperatures leads to denaturation of some or all enzyme molecules, resulting in a sharp decline in the substrate conversion rate [15,16]. After a comprehensive assessment, 35 °C was selected as the optimal catalytic temperature for further studies.

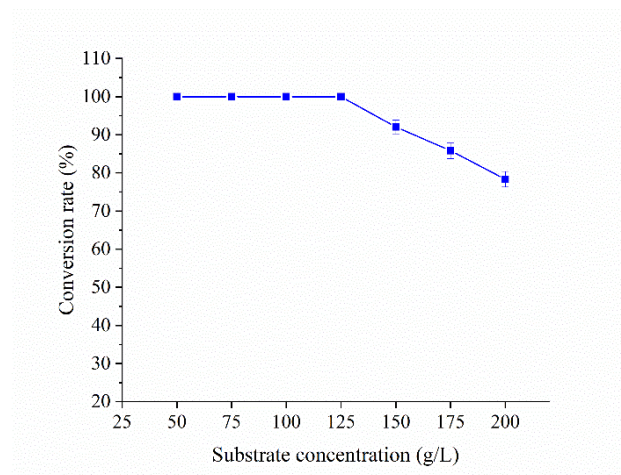
The change in pH of the catalytic system also has a considerable influence on the reaction rate, catalytic capacity, and stability of the enzyme [16,17]; therefore, determination of the optimal catalytic pH of the enzyme is essential. In this study, we performed a coupling reaction between recombinant LK-TADH and GDH to yield *cis*-4-propylcyclohexanol. During the reaction, GDH catalyzed the dehydrogenation of glucose to gluconic acid; thus, the pH of the reaction system showed a continuous decrease. Enzyme catalysis is hampered if the pH is not adjusted on time. As shown in Figure 3B, the substrate conversion rate was 56.3% at pH 6.0, 87.6% at pH 6.5, and 100% at pH 7.0, 7.5, and 8.0. However, the substrate conversion rate decreased with a further increase in pH (92.6% at pH 8.5; 63.1% at pH 9.0). Our results show that the coupling reaction between LK-TADH and GDH could maintain a high substrate conversion rate at neutral and weakly alkaline conditions, whereas, consistent with previous studies, a decrease in the conversion rate was observed when the conditions were too acidic or too alkaline [11,17]. Therefore, we used an automatic flow system to maintain the pH of the reaction system between 7.0 and 8.0 in subsequent studies.



**Figure 3.** Effect of temperature (A) and pH (B) on the production of *cis*-4-propylcyclohexanol. The reaction system (50 mL) was as follows: 100 g/L 4-propylcyclohexanone, 50 g/L LK-TADH (wet cell lysate), 10 g/L GDH (wet cell lysate), 0.1 g/L  $\text{NAD}^+$ , glucose: substrate ratio (1.2 mol/mol). For temperature optimization, the reaction mixture was stirred at 25, 30, 35, 40, and 45 °C, and the pH was adjusted to 7.0 using 2 M  $\text{Na}_2\text{CO}_3$ . For pH value optimization, the reaction mixture was stirred at 35 °C, 0.1 M phosphate-buffered saline was used, and the pH was maintained between 6.0 and 9.0 by adding 1 N HCl or 2 M  $\text{Na}_2\text{CO}_3$ .

### 2.3. Optimization of Substrate Concentration

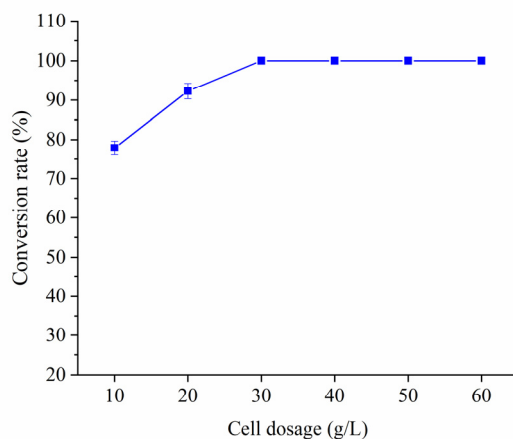
The optimal catalyst concentration was determined at a temperature of 35 °C and pH between 7.0 and 8.0. 4-Propylcyclohexanone concentrations ranging from 50 g/L to 200 g/L were used to determine the optimal concentration for the catalytic production of *cis*-4-propylcyclohexanol. As shown in Figure 4, the conversion rate was 100% when the 4-propylcyclohexanone concentration was 50–125 g/L. However, when a concentration of 150–200 g/L was used, a decline in the conversion rate was observed. The conversion rate was 92.03%, 85.8%, and 78.33% when the 4-propylcyclohexanone concentration was 150 g/L, 175 g/L, and 200 g/L, respectively. This finding was likely because high concentrations of the substrate or product inhibited the catalytic activity of the enzyme, resulting in incomplete substrate transformation. Therefore, 125 g/L of 4-propylcyclohexanone was selected as the optimal concentration for the biosynthesis of *cis*-4-propylcyclohexanol.



**Figure 4.** Effect of substrate concentration on the production of *cis*-4-propylcyclohexanol. The reaction system (50 mL) was as follows: 50–200 g/L 4-propylcyclohexanone, 50 g/L LK-TADH (wet cell lysate), 10 g/L GDH (wet cell lysate), 0.1 g/L  $\text{NAD}^+$ , glucose: substrate ratio (1.2 mol/mol). The reaction mixture was stirred at 35 °C, and the pH was maintained between 7.0 and 8.0 by 2 M  $\text{Na}_2\text{CO}_3$ .

#### 2.4. Optimization of Cell Dosage

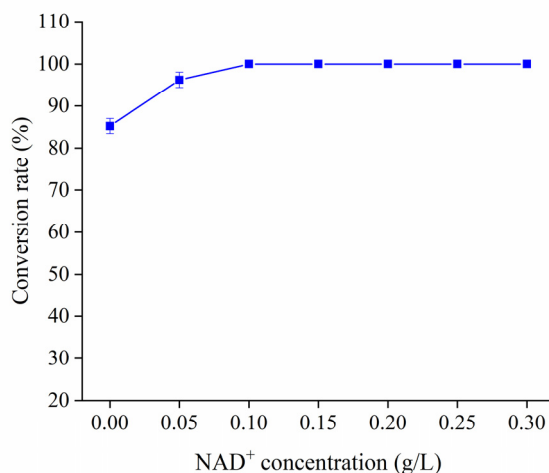
The optimal density of recombinant LK-TADH cells was determined at a catalytic temperature of 35 °C, pH between 7.0 and 8.0, and 4-propylcyclohexanone concentration of 125 g/L. As shown in Figure 5, the substrate conversion rate was 100% when a dosage of 30–60 g/L cells was used for 6 h. The greater the cell density, the higher the reaction rate. However, high cell concentrations affect mass transfer, increase industrial production costs, and pose difficulties during separation and extraction [18]. The lower cell dosage could not completely catalyze the production of *cis*-4-propylcyclohexanol when the substrate concentration exceeded 125 g/L. Therefore, a dosage of 30 g/L of wet cells was chosen for subsequent studies.



**Figure 5.** Effect of cell dosage on the production of *cis*-4-propylcyclohexanol. The reaction system (50 mL) was as follows: 125 g/L 4-propylcyclohexanone, 10–60 g/L LK-TADH (wet cell lysate), 10 g/L GDH (wet cell lysate), 0.1 g/L NAD<sup>+</sup>, glucose: substrate ratio (1.2 mol/mol). The reaction mixture was stirred at 35 °C, and the pH was maintained between 7.0 and 8.0 by 2 M Na<sub>2</sub>CO<sub>3</sub>.

#### 2.5. Optimization of NAD<sup>+</sup> Levels

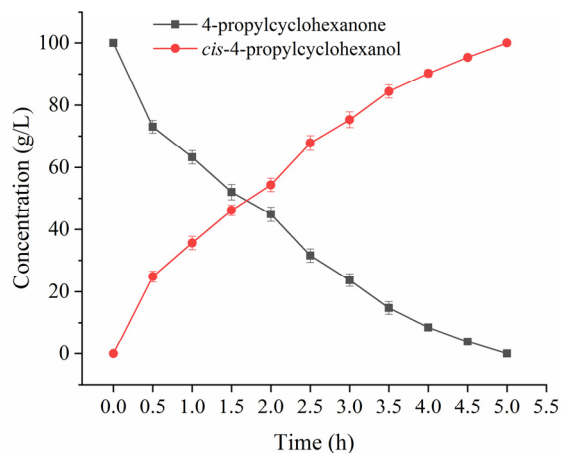
NADH and NADPH are necessary components in preparing target chiral alcohols by the asymmetric reduction of ketone substrates by ADH [19–21]. However, the high cost of NAD and NADPH is one of the bottlenecks during biocatalytic synthesis that hinder the industrial production of target products [22–24]. Therefore, we combined recombinant LK-TADH and GDH and constructed a dual-enzyme coupling system to achieve the cyclic regeneration of NAD<sup>+</sup>. Optimum NAD<sup>+</sup> levels were determined at the following conditions: temperature of 35 °C, pH between 7.0 and 8.0, 4-propylcyclohexanone concentration of 125 g/L, and LK-TADH cell dosage of 30 g/L. As seen in Figure 6, the substrate conversion rate in the catalytic system without NAD<sup>+</sup> was 85.23%. The rate increased to 96.2% after adding 0.05 g/L NAD<sup>+</sup>, and to 100% when the NAD<sup>+</sup> concentration was 0.1–0.3 g/L. It is likely that some amount of NAD<sup>+</sup> or NADP<sup>+</sup> accumulates in recombinant cells; thus, a certain amount of substrate can be transformed into the target product without the exogenous addition of NAD<sup>+</sup> or NADP<sup>+</sup> [18]. However, the NAD<sup>+</sup> content in recombinant cells was not sufficient to catalyze the reaction and yield high concentrations of *cis*-4-propylcyclohexanol. Therefore, 0.1 g/L NAD<sup>+</sup> was selected for subsequent experiments.



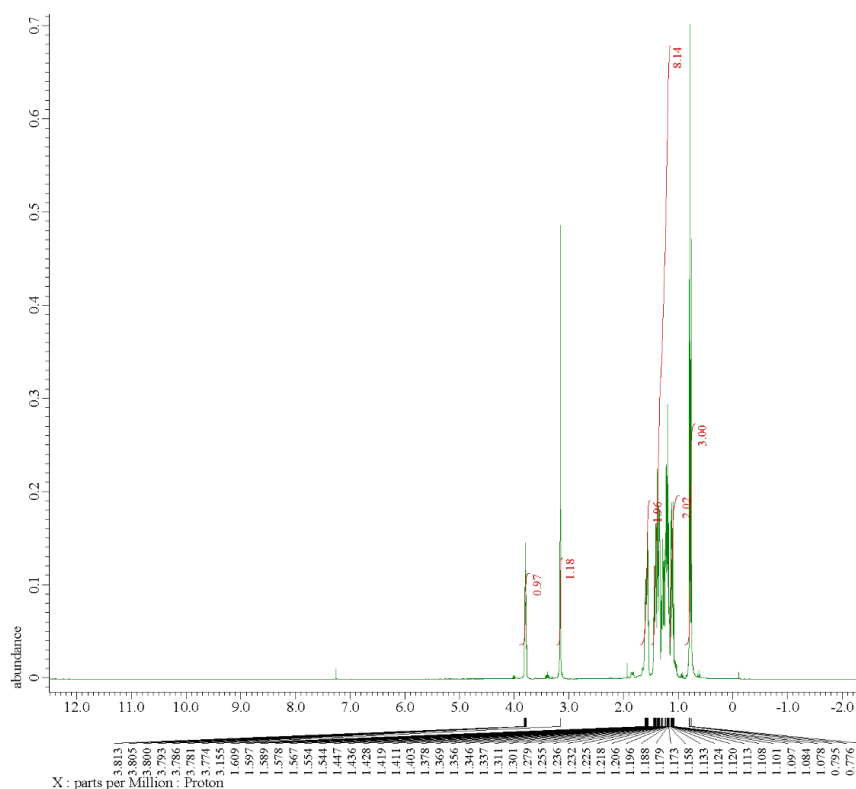
**Figure 6.** Effect of NAD<sup>+</sup> concentration on the production of *cis*-4-propylcyclohexanol. The reaction system (50 mL) was as follows: 125 g/L 4-propylcyclohexanone, 30 g/L LK-TADH (wet cell lysate), 10 g/L GDH (wet cell lysate), 0–0.3 g/L NAD<sup>+</sup>, glucose: substrate ratio (1.2 mol/mol). The reaction mixture was stirred at 35 °C, and the pH was maintained between 7.0 and 8.0 by 2 M Na<sub>2</sub>CO<sub>3</sub>.

#### 2.6. Production of *Cis*-4-Propylcyclohexanol under Optimized Conditions

*cis*-4-Propylcyclohexanol was prepared in a 2 L reaction system under the following optimized conditions: temperature of 35 °C, pH maintained between 7.0 and 8.0, 4-propylcyclohexanone concentration of 125 g/L, LK-TADH cell dosage of 30 g/L, NAD<sup>+</sup> concentration of 0.1 g/L. The GDH cell dosage was 10 g/L, and the glucose: substrate ratio was 1.2 mol/mol. Then, 2 M Na<sub>2</sub>CO<sub>3</sub> was added automatically to maintain the pH between 7.0 and 8.0. The reaction solution was extracted three times using a certain volume of ethyl acetate after the reaction was complete. The organic phase was collected after centrifugation, washed with an equal volume of saturated sodium chloride solution, and dried overnight with anhydrous sodium sulfate. After extraction and filtration, the ethyl acetate phase was collected, and the final product was obtained after rotary evaporation at 60 °C. As shown in Figure 7, the substrate (125 g/L; 250 g) was completely transformed after 5 h in the optimized catalytic system, and 225.8 g (yield: 90.32%) of *cis*-4-propylcyclohexanol was obtained after extraction and rotary evaporation. As shown in Figure 8, the NMR result of the final product was as follows: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.90 (1H, ddd, *J* = 8.0, 5.0, 3.0 Hz), 1.73–1.64 (2H, m), 1.56–1.38 (5H, m), 1.37–1.23 (5H, m), 1.25–1.18 (2H, m), 0.88 (3H, t, *J* = 7.2 Hz), which is consistent with a previous study [4]. Compared with that used in previous studies, the substrate concentration used in our study was higher (125 g/L vs. 69 g/L), and there was no residual substrate when the reaction was complete [4]. Moreover, to the best of our knowledge, this is the first study reporting the use of recombinant ADH to produce *cis*-4-propylcyclohexanol.



**Figure 7.** Time course of the biotransformation process of *cis*-4-propylcyclohexanol by recombinant LK-TADH and GDH. The reaction system (2 L) was as follows: 125 g/L 4-propylcyclohexanone, 30 g/L LK-TADH (wet cell lysate), 10 g/L GDH (wet cell lysate), 0.1 g/L NAD<sup>+</sup>, glucose: substrate ratio (1.2 mol/mol). The reaction mixture was stirred at 35 °C, and the pH was maintained between 7.0 and 8.0 by 2 M Na<sub>2</sub>CO<sub>3</sub>.



**Figure 8.** The <sup>1</sup>H NMR spectrum of *cis*-4-propylcyclohexanol.

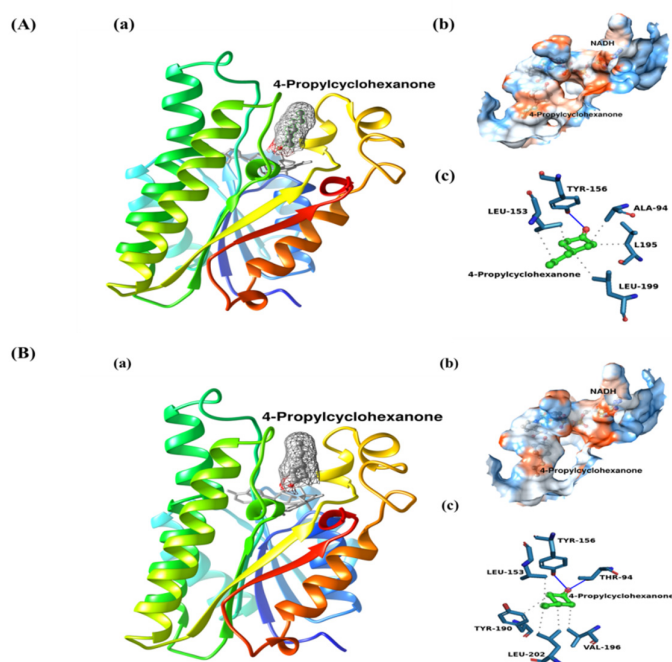
### 2.7. Mechanism of Enhanced Catalytic Activity

Molecular docking simulations are often performed to elucidate the underlying mechanisms of enhanced mutant enzyme activity [25–27]. The interaction model of LK-ADH and 4-propylcyclohexanone was analyzed using molecular docking simulation to determine the driving force behind the binding (Figure 9A). As shown in Figure 9, 4-

propylcyclohexanone could bind near NADH with a binding energy of  $-3.83$  kcal/mol. The substrate molecules were mainly bound to the hydrophobic pockets composed of Ala-94, Leu-153, Tyr156, Leu-195, and Leu-199 and had a good catalytic conformation with NADH, with a catalytic atomic distance of  $4.3$  Å.

The binding mode of mutant LK-TADH and 4-propylcyclohexanone is shown in Figure 9B. The substrate molecules were mainly bound in the hydrophobic pocket composed of Thr-94, Leu-153, Tyr-156, Tyr-190, Val-196, and Leu-202, and the binding energy was  $-4.18$  kcal/mol. From the perspective of energy, the affinity of the protein to 4-propylcyclohexanone increased after mutation, indicating that the mutant site was conducive to improving the catalytic efficiency of the enzyme to a certain extent. Moreover, 4-propylcyclohexanone exhibited good catalytic conformation with NADH, with a catalytic atomic distance of  $3.8$  Å.

The results from molecular docking indicate that the volume of the three amino acid side chains increased after mutation, especially the mutation of Leu-199 to His-199 and Ala-202 to Leu-202. Larger side chains can occupy the active cavity of the protein. Thus, the possibility of a conformational reversal in the active site of 4-propylcyclohexanone was reduced, enhancing the efficiency of LK-TADH in catalyzing 4-propylcyclohexanone into its *cis* derivative. In addition, the distance between the NADH-C4 atom and the carbonyl carbon is a key factor that determines the catalytic activity of ADH for ketones [28,29]. In our study, the distance between the carbonyl carbon of 4-propylcyclohexanone and the active hydrogen atom on NADH of LK-ADH and mutants was  $4.3$  and  $3.8$  Å, respectively. The smaller catalytic distance between 4-propylcyclohexanone and NADH in the mutant LK-TADH combined with the L199H and A202L mutations reduced the active pocket volume and restricted the activity of 4-propylcyclohexanone. Thus, the binding between 4-propylcyclohexanone and LK-TADH was tight, which may explain the higher catalytic efficiency of this mutant.



**Figure 9.** Binding pattern of 4-propylcyclohexanone to LK-ADH (A) and LK-TADH (B). (a) The binding position of the substrate in LK-ADH or LK-TADH is shown as a ball and stick model, and the molecular surface is shown as a mesh. (b) The substrate was closely bound to the active cavity of LK-ADH or LK-TADH. The protein cavity is shown as a hydrophobic surface, where blue and orange represent hydrophilic and hydrophobic regions, respectively. The substrate molecule is shown as a ball and stick model. (c) Interaction analysis between the substrate and key residues in



the active cavity of LK-ADH or LK-TADH, where the gray dashed line represents hydrophobic interactions, and the blue solid line represents hydrogen bonding.

### 3. Materials and Methods

#### 3.1. Materials

The BL21 (DE3) strain was used for expression, and pET26b was used as the expression plasmid. 4-Propylcyclohexanone, *cis*-4-propylcyclohexanol, *trans*-4-propylcyclohexanol, isopropyl- $\beta$ -D-thiogalactoside (IPTG), and nicotinamide adenine dinucleotide (NAD<sup>+</sup>) were provided by Zhejiang Zhengshuo Biological Co., Ltd. (Huzhou, China). All other reagents were of analytical grade.

#### 3.2. Cloning and Expression

The gene sequences of LK-ADH (WP\_054768785.1), LK-TADH (A94T/F147L/L199H/A202L), and GDH (WP\_119899028.1) were synthesized and sub-cloned into pET26b by Sangon Biotech (Shanghai, China) and named pET-LK-ADH, pET-LK-TADH, and pET-GDH, respectively. These three plasmids were transformed into BL21 (DE3)-competent cells to obtain the target strains of BL21-LK-ADH, BL21-LK-TADH, and BL21-GDH, respectively. Cells were then cultured in LB medium (50  $\mu$ g/mL kanamycin) at 37 °C, and 0.1 mM IPTG was added when the OD<sub>600</sub> was 0.6. Cells were induced overnight at 25 °C, collected using centrifugation (5000 g, 4 °C, 10 min), and stored at -20 °C until further studies. The activity of ADH was measured with 4-propylcyclohexanone as a substrate as follows: 50 g/L ADH (wet cell lysate), 50 g/L 4-propylcyclohexanone, 0.1 g/L NADH, 10 g/L GDH (wet cell lysate), glucose: substrate ratio (1.2 mol/mol). The reaction was performed at 35 °C for 0.5 h. One unit of the enzyme was defined as the amount of enzyme synthesizing 1  $\mu$ g of *cis*-4-propylcyclohexanol per minute under standard assay conditions.

#### 3.3. Using Recombinant ADH to Produce *Cis*-4-Propylcyclohexanol

4-Propylcyclohexanone (50 g/L) was added into the following reaction system (50 mL): 50 g/L of LK-ADH or LK-TADH (wet cell lysate), 10 g/L GDH (wet cell lysate), 0.1 g/L NAD<sup>+</sup>, glucose: substrate ratio (1.2 mol/mol). The reaction mixture was stirred at 35 °C, and the pH was adjusted to 7.0 using 2 M sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>). The reaction was monitored using thin-layer chromatography, and 10% phosphomolybdic acid was used for color development. The final product was extracted using ethyl acetate and analyzed using gas chromatography. The substrate conversion rate (C%) and *cis* selectivity (%) were calculated as follows:

$$C\% = (C_1 - C_2)/C_1 \times 100\% \quad (1)$$

where C<sub>1</sub>: initial concentration of 4-propylcyclohexanone; C<sub>2</sub>: final concentration of 4-propylcyclohexanone.

$$Cis\ (\%) = (C_{cis} - C_{trans})/(C_{cis} + C_{trans}) \times 100\% \quad (2)$$

where C<sub>cis</sub>: concentration of *cis*-4-propylcyclohexanol; C<sub>trans</sub>: concentration of *trans*-4-propylcyclohexanol.

#### 3.4. Optimization of Catalytic Parameters

The initial reaction system (50 mL) was set up as follows: 100 g/L 4-propylcyclohexanone, 50 g/L LK-TADH (wet cell lysate), 10 g/L GDH (wet cell lysate), 0.1 g/L NAD<sup>+</sup>, glucose: substrate ratio (1.2 mol/mol). The reaction mixture was stirred at 35 °C, and the pH was adjusted using 2M Na<sub>2</sub>CO<sub>3</sub>. To optimize catalytic parameters, the reaction temperature (25–45 °C), pH (6.0–9.0), 4-propylcyclohexanone concentration (50–200 g/L), cell dosage (10–100 g/L wet cell lysate), and NAD<sup>+</sup> addition (0–0.5 g/L) were investigated.

### 3.5. Scale-Up of *Cis*-4-Propylcyclohexanol

4-Propylcyclohexanone was added to the reaction system (2 L) under the optimal catalytic conditions, and the pH was adjusted using 2 M Na<sub>2</sub>CO<sub>3</sub>. After 4-propylcyclohexanone was completely transformed, the reaction solution was extracted three times using ethyl acetate, dried using anhydrous sodium sulfate, and evaporated to obtain the final product.

### 3.6. Homology Modeling and Molecular Docking

The crystal structure of LK-ADH was downloaded from the Protein Data Bank (PDB code: 4RF5) [30], and the point mutation was performed using the rotamers tool of UCSF Chimera 1.15 [31]; the 2000 steps steepest descent algorithm and 2000 steps conjugate gradient algorithm were used to minimize the energy of the structure. Ligand structures were downloaded from the PubChem database. Next, hydrogen atoms and Gasteiger charges of the ligands and receptors were added using AutoDockTools [32]. Molecular docking was performed using Autodock 4.2.6, and the coordinates of the center point of the grid box were defined as 20.64, −s27.36, and 26.58 with dimensions of 40 × 40 × 40 Å in X, Y, and Z, respectively, and a spacing center of 0.375 Å. The results of 100 docking replications were cluster analyzed to obtain the conformation of the ligand–receptor complex with the lowest binding energy, which was then visualized using UCSF Chimera 1.15 and LigPlot + v.2.2.4.

### 3.7. Product Determination

The concentrations of 4-propylcyclohexanone and *cis*-4-propylcyclohexanol were measured using a gas chromatograph (Agilent 7820) equipped with an HP-5 capillary column (30 m × 0.25 mm × 0.25 μm) and a flame ionization detector. The gas phase conditions were as follows: 80 °C for 2 min, then ramped up at 10 °C/min to 250 °C for 30 min. The retention times of 4-propylcyclohexanone, *cis*-4-propylcyclohexanol, and *trans*-4-propylcyclohexanol were 9.93 min, 9.68 min, and 9.88 min, respectively. All tests were conducted in triplicate.

## 4. Conclusions

We found that LK-TADH (A94T/F147L/L199H/A202L) coupled with GDH could efficiently produce *cis*-4-propylcyclohexanol. The optimal catalytic conditions were as follows: temperature of 35 °C, pH between 7.0 and 8.0, 4-propylcyclohexanone concentration of 125 g/L, LK-TADH cell dosage of 30 g/L, NAD<sup>+</sup> concentration of 0.1 g/L, GDH cell dosage of 10 g/L, and glucose: substrate ratio of 1.2 mol/mol. Under these optimized conditions, 125 g/L of 4-propylcyclohexanone was completely transformed into *cis*-4-propylcyclohexanol in 5 h with a yield of 90.32% (the *cis/trans* ratio was 99.5:0.5). Our findings suggest the potential of LK-TADH in the large-scale production of *cis*-4-propylcyclohexanol in an industrial setting.

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