PLOS ONE

RESEARCH ARTICLE

Gene mining, codon optimization and analysis of binding mechanism of an aldo-keto reductase with high activity, better substrate specificity and excellent solvent tolerance

Wei Jiang*, **Xiaoli Fu, Weiliang Wu**

College of Chemical Engineering, Huaqiao University, Xiamen, China

* wjiang@hqu.edu.cn, tianya416@126.com

Abstract

The biosynthesis of chiral alcohols has important value and high attention. Aldo–keto reductases (AKRs) mediated reduction of prochiral carbonyl compounds is an interesting way of synthesizing single enantiomers of chiral alcohols due to the high enantio-, chemo- and regioselectivity of the enzymes. However, relatively little research has been done on characterization and apply of AKRs to asymmetric synthesis of chiral alcohols. In this study, the AKR from *Candida tropicalis* MYA-3404 (*C. tropicalis* MYA-3404), was mined and characterized. The AKR shown wider optimum temperature and pH. The AKR exhibited varying degrees of catalytic activity for different substrates, suggesting that the AKR can catalyze a variety of substrates. It is worth mentioning that the AKR could catalytic reduction of keto compounds with benzene rings, such as cetophenone and phenoxyacetone. The AKR exhibited activity on *N,N*-dimethyl-3-keto-3-(2-thienyl)-1-propanamine (DKTP), a key intermediate for biosynthesis of the antidepressant drug duloxetine. Besides, the AKR still has high activity whether in a reaction system containing 10%-30% V/V organic solvent. What's more, the AKR showed the strongest stability in six common organic solvents, DMSO, acetonitrile, ethyl acetate, isopropanol, ethanol, and methanol. And, it retains more that 70% enzyme activity after 6 hours, suggesting that the AKR has strong solvent tolerance. Furthermore, the protein sequences of the AKR and its homology were compared, and a 3D model of the AKR docking with coenzyme NADPH were constructed. And the important catalytic and binding sites were identified to explore the binding mechanism of the enzyme and its coenzyme. These properties, predominant organic solvents resistance and extensive substrate spectrum, of the AKR making it has potential applications in the pharmaceutical field.

1 Introduction

The synthesis of chiral alcohols and their derivatives has great significance and great market demand as they are key intermediates in many important drugs, such as the anti-tumor drug

Irutinib, the anti-depression drug Duloxetine, and the lipid-lowering drug Atorvastatin [1–12]. Biocatalysis has shown advantages and concerns due to its high chiral selectivity, stereoscopic and regional selectivity, environmental friendliness, mild conditions and so on. Enzymes, such as alcohol dehydrogenase, ketone reductase, etc, are the key to the synthesis of chiral compounds by biocatalysis [7–15]. Therefore, it is very important to obtain enzymes with good performance, but relatively few of them can be applied to the industrialized production of chiral compounds.

The aldo-keto reductases (AKRs) could catalyze the reduction of ketones or different acids to chiral alcohols [16–18]. The AKRs are one of the three super families that perform oxidoreduction on a variety of natural and synthetic substrates [19]. AKRs mediated stereoselective reduction of prochiral carbonyl compounds is an efficient way of preparing single enantiomers of chiral alcohols due to the high enantio-, chemo- and regioselectivity of the enzymes. But only a few of AKRs have been found, characterized, and applied to biosynthesis of chiral alcohols. A novel AKR, Tm1743, was obtained from *Thermotoga maritimeit*, and it shown broad substrate specificity towards many ketones, keto esters, and aldehydes [20]. The AKR, YOL151W, from *Saccharomyces cerevisiae* was hired to asymmetric synthesis of (*S*)-3-chloro-1-phenyl-1-propanol [21]. Besides, the AKRs from *Penicillium citrinum* IFO4631, *C. albicans*, and *Kluyveromyces lactis* XP1461, was also used for synthesis chiral alcohols [22–24]. Nevertheless, the living example of the synthesis of chiral alcohols by AKRs are few compared to alcohol dehydrogenase and carbonyl reductase, and, it was deserved to be studied [25, 26].

In this study, a aldo-keto reductase AKR was obtained from *C. tropicalis* MYA-3404, its codon was optimized and its performance was analyzed. It was showed that the AKR own high activity, organic solvent tolerance, and broad substrate spectrum, making it a promising catalyst for the biosynthesis of chiral alcohols. In addition, in order to explore the binding mechanism of the AKR with coenzyme, molecular docking was also used.

2 Materials and methods

2.1 Chemicals and strains

All chemicals are analytical or chromatographic grade, and they were purchased from Sinopharm Group or Aladdin (Germany) without further purification. All enzymes were purchased from TaKaRa Co., Ltd. (Dalian, China). The Gel Extraction Kit(200) and Plasmid Mini Kit I (200) were obtained from OMEGA Co.(USA). NADP(H) were obtained from Sigma-Aldrich Co. (Shanghai, China). *Escherichia coli* (*E. coli*) DH5α and *E. coli* BL21 (DE3) was used cloned and heterologous expression, respectively.

2.2 Gene discovery and homologous protein-searching analysis

The keto reductase sequence (GenBank accession number MK503097) was obtained in our laboratory. And it was used as template to search for similar target sequences in *C. tropicalis*. The BLAST tool, https://blast.ncbi.nlm.nih.gov/Blast.cgi, was used in this progress. As HummePankaj Soni and U. C. Banerjee reported that *Candida tropicalis* has the ability to synthesize duloxetine, but the specific protein has not been identified [27]. We hypothesized that keto reductase might play an important role in this, so the scope of retrieval was determined in the *C. tropicalis*.

2.3 Codon optimization, expression and purification of the AKR

In order to improve the protein expression level, the *akr* gene sequence was optimized according to codon preference of *E. coli*. The Optimized gene sequence was synthesized by Shanghai Sangon Co., Ltd., and the gene was subcloned into the vector of pET-28a, generating a

recombinant expression vector pET-28a-*akr*, then it was transformed into *E. coli* BL21 (DE3) cells for express the recombinant protein. The *E. coli* BL21 (DE3) was cultured in 100 mL LB medium containing 100 µg/mL of ampicillin at 200 rpm, 37˚ C. The IPTG was added with a final concentration of 0.1 mM when an optical density of 600 nm (OD 600) of the culture reached 0.6–0.8, the inducing temperature was 18˚ C. It was cultured for another 12 hours. Then, the culture was then harvested (8000 rpm, 5 minutes), and washed three times by using PBS buffer at 4˚C. The cell pellet was suspended and disrupted by using sonication (JY92-IIN ultrasonic cell crusher, Ningbo Xinzhi Biotechnology Co., LTD). After that, the disrupted cell debris was centrifuged for 30 minutes at 12,000 rpm, 4˚C. Then, the tagged enzyme was puri-fied by an AKTA Prime system equipped with a 10-mL Ni-IDA column (GE Healthcare, USA). Finally, the results and concentration of purification of the AKR were determined by 12% SDS-PAGE and Bradford Protein Assay Kit.

2.4 Characterization of the AKR

2.4.1 Substrate spectrum of AKR.   In order to investigate the substrate specificity of the AKR, the enzyme was selected to catalyzed reduction of ketones, ketoesters and aldehydes under standard conditions. One unit of AKR was defined as the content of AKR that catalyzed the oxidation of 1 µmol NADPH PM (Per Minute). The rates of reactions were assayed at indoor temperature by detecting the change in absorption peak of NADPH at 340 nm ($\varepsilon =$ 6.22 mM^{-1} cm^{-1}). The reaction mixture (220µl) contained 10 µl substrates, 170µl PBS buffer, 10µl NADPH, and 30µl enzyme AKR. The residual maximum activity was defined as 100%. The substrate were 3-methylcyclohexanone, methyl pyruvate, phenoxy ethyl ketone, ethyl levulinate, 4-octanone, acetylacetone, 5-methyl-2-hexanone, methyl isobutyl ketone, acetophe-none, *N*,*N*-dimethyl-3-keto-3-(2-thienyl)-1-propanamine (DKTP).

2.4.2 Effect of pH and temperature.   To detect the pH eect of the AKR, the enzyme activ-ity was measured in 50 mM buffers systems of pH 5.0–6.0 (CH$_3$COOH-CH$_3$COONa), 6.0–8.0 (NaH$_2$PO$_4$-Na$_2$HPO$_4$) and 8.0–9.0 (Tris-HCl buffers). To detect the optimum temperature of the AKR, the reaction system (without NADPH) was preincubated at the respective tempera-ture for 5 minutes, then, the enzyme activity was detected in different temperature, 30–50˚C. The substrate used for enzyme activity determination is the optimal substrate, acetylacetone, for this enzyme.

2.4.3 Organic solvent tolerance of AKR.   Different kinds of organic solvents (10%, 20%, 30%) V/V were introduced into the reaction system for detecting the performance of tolerate organic solvents and the effect of organic solvents on the AKR. Common organic solvents, methanol, isopropanol, ethanol, ethyl acetate, Dimethyl sulfoxideDMSO), and acetonitrile were chosen as the object of study. The residual activity of the enzyme in incubated samples was detected according to standard assay methods. Besides, the performance of tolerate organic sol-vents of AKR was executed by detect the residual activity of AKR in the reaction system with 10% v/v of different kinds of organic solvents for 6 hours. The original AKR's activity was desig-nated as 100% and the optimum substrate, acetylacetone, was selected as the substrate.

2.5 Molecular docking and key site resolution

The BLAST tool (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi) was selected to discover sequences similar to the AKR. Nine other AKRs were obtained from blast results, and in order to analyze the source of the AKR, DNAMAN was used to draw the alignment homologous tree. Homology modeling was built by using SWISS MODEL (http://swissmodel.expasy.org/) according to the sequence information of AKR [28]. In addition, the Docking service for molec-ular docking was employed to explore the binding mechanism of the enzyme and coenzyme.

3 Result and discussion

3.1 Gene discovery and codon optimization

The *C. tropicalis* was determined as the scope of retrieval for it shown the ability to synthesize duloxetine [27]. However, the specific protein has not been identified. And combined with other relevant studies, we hypothesized that AKR might play an important role in this progress. The AKR sequence, (GenBank accession number MK503097) which was obtained in our laboratory, were used as templates to mine the target sequences in *C. tropicalis*. After digging and analysis, the *akr* gene (GenBank accession number NW_003020038.1) from *C. tropicalis MYA-3404* was obtained. The *akr* gene sequence was optimized according to codon preference of *E. coli* to improve the protein expression level. The results of codon optimization are shown in Fig 1. After optimization, the frequency of rare codons was greatly reduced. And it was demonstrated that codon optimization can significantly improve the expression level of recombinant protein.

3.2 Substrate specificity

The substrate specificity of AKR was investigated by detecting the catalytic efficiency of AKR for various keto acids and ketones. It was shown in the Fig 2 that the AKR showed varying degrees of catalytic activity, suggesting that the AKR can catalyze a variety of substrates, but it has a good substrate specificity. The activity of acetylacetone is the highest.

It is worth mentioning that the AKR could catalytic reduction of keto compounds with benzene rings, such as acetophenone and phenoxyacetone. And, the AKR exhibited about 20% relative activity on DKTP, the key intermediate for biosynthesis of the antidepressant drug duloxetine [27, 29]. These results indicate that the AKR could be employed to synthesis of chiral alcohol compounds containing a benzene ring and duloxetine, so the enzyme has a high application potential.
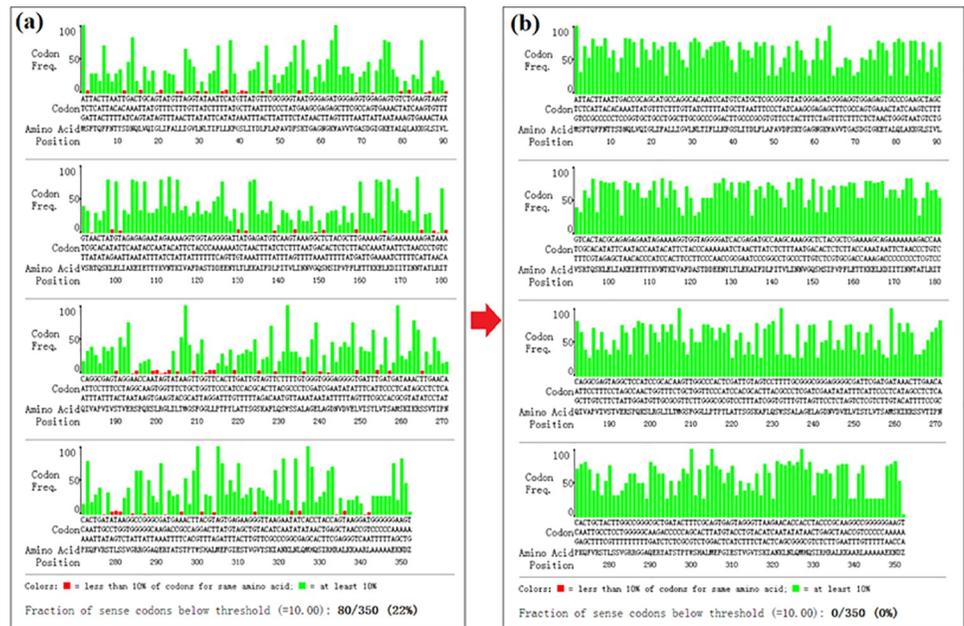

Fig 1. Codon optimization of the *akr* gene. (a) Before optimization; (b) After optimization.
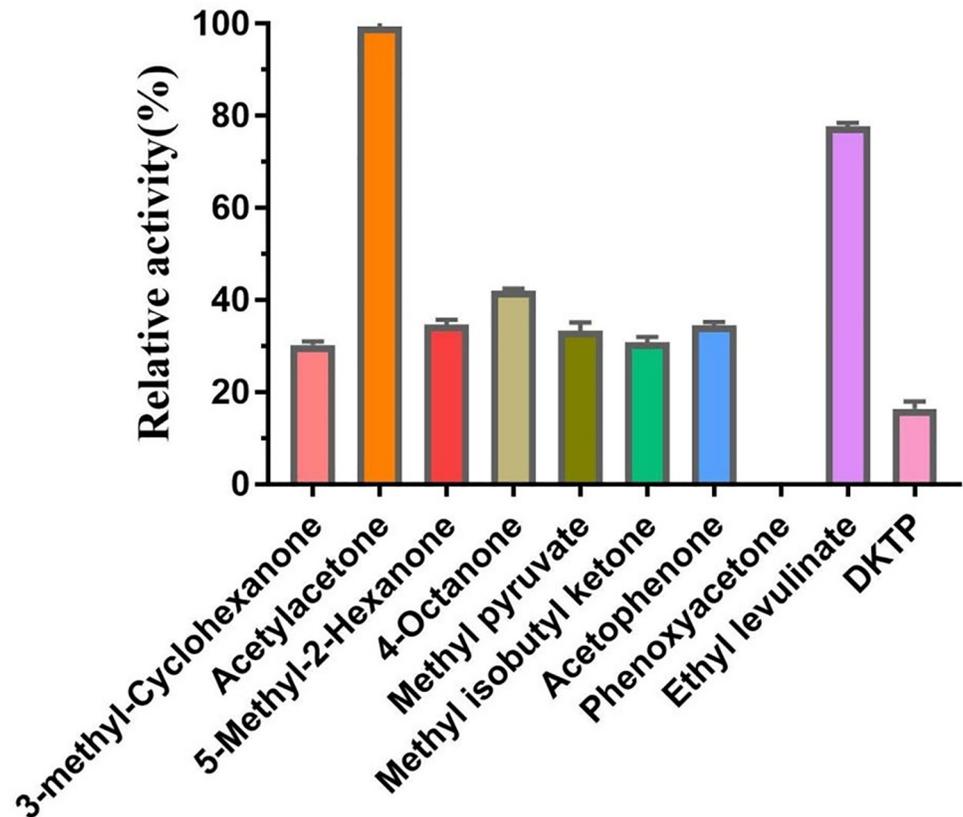
Fig 2. Substrate specificity of the AKR.

3.3 Effect of pH and temperature

pH and temperature play an important role in the influence and regulation of enzyme activity. As shown in Fig 3A, the relative activity of the AKR was more than 80% from 30°C to 50°C. At 42.3°C, the AKR exhibited the highest activity. After the temperature exceeded to 42.3°C, the enzyme activity began to decrease. The optimum temperature for the AKR was higher than that of the aldo-keto reductase, which exhibited the highest activity at 30°C, from *Kluyveromyces lactis* XP1461 [24]. As shown in Fig 3B, the AKR has maximum activity at pH 7.7. The enzyme maintained more than 85% activity at pH 5.3–8.7, demonstrating that it has relatively
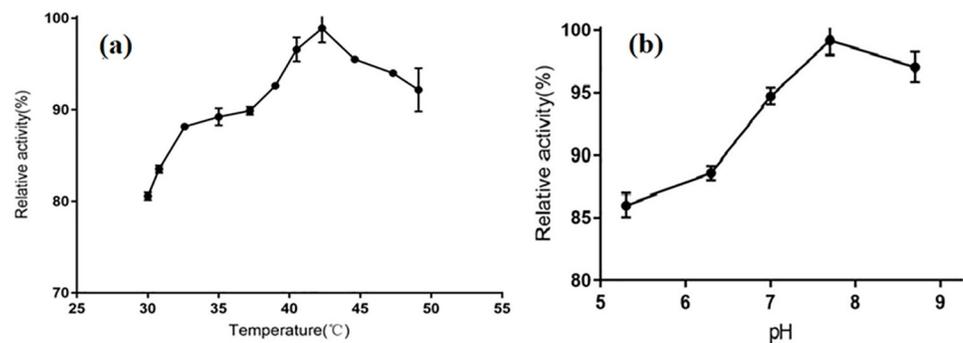

Fig 3. Effect of pH and temperature of the AKR. (A) Effect of temperature of the AKR; (B) Effect of pH of the AKR.

high activity in a broad pH range. When the pH was 7.0–8.7, the activity of AKR was above more than 95%.

3.4 Organic solvent tolerance of AKR

Synthesis of chiral compounds are usually executed in organic solvents, so it is important that the enzyme own high organic solvent tolerance. So, six common organic solvents, DMSO, acetonitrile, ethyl acetate, isopropanol, ethanol, and methanol were selected to detect the organic solvent tolerance of the enzyme. The result was showed in Figs 4 and 5. 10%-30% (V/V) of organic solvent was added in reaction system, and the result of the activity of AKR in 10% to 30% (V/V) in the six organic solvents was showed in the Fig 4. The enzyme exhibited high activity and it all retained more than 60% activity except in the 20%-30% (V/V) of DMSO (Fig 4). The organic solvent tolerance of the enzyme was very superior compared to the aldo-keto reductase, which can only withstand 5% V/V organic solvent, from *Kluyveromyces lactis* [24]. Interestingly, the activity of the AKR increased from 10% to 30% (V/V) isopropanol, ethanol or methanol. So, it was speculated that an appropriate amount of isopropanol, ethanol or methanol can alter the spatial structure of the AKR, thereby improving enzyme activity. However, the specific reaction mechanism was not clear in this progress. So, it will be detected in the future work.

Besides, the stability of the enzyme in the organic solvents was investigated to examine the effective reaction time of the enzyme. The AKR exhibited the strongest stability in six common organic solvents, DMSO, acetonitrile, ethyl acetate, isopropanol, ethanol, and methanol (Fig 5). And, it retains more that 70% enzyme activity after 6 hours. These results indicated that the AKR own a strong organic solvent tolerance in common organic solvents, suggesting that the enzyme has high potential applications in the high value-added chemicals synthesis and biopharmaceutical fields [30–35].
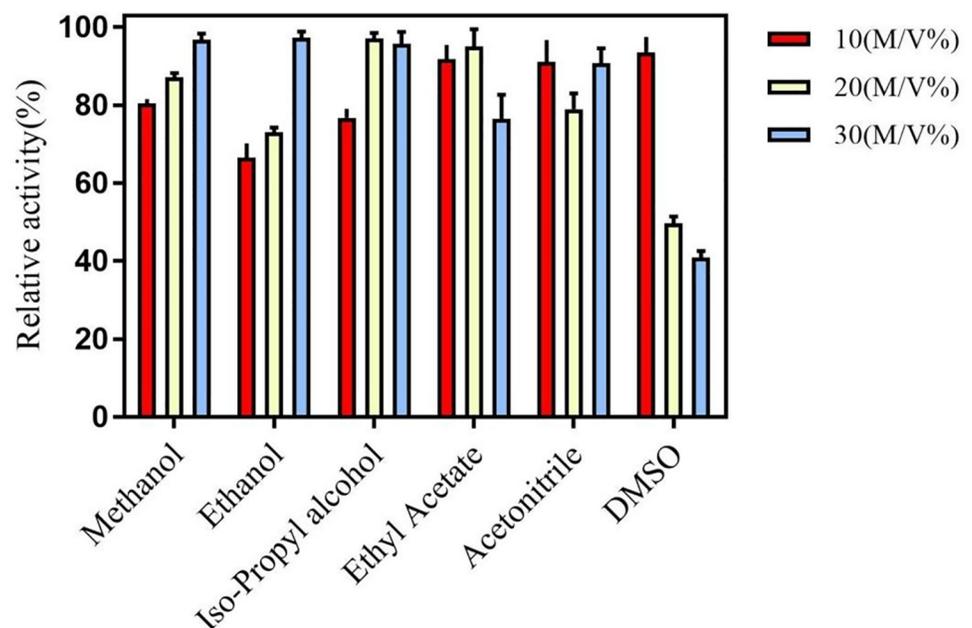

Fig 4. Organic solvent tolerance of the AKR. AKR is sensitive to six different organic solvents (methanol,Ethanol, 2-propanol, ethyl acetate, acetonitrile, and DMSO) with a gradient of 10%(v/v) to 30%(v/v) for each organic solvent. Three parallels were set for each experiment, and the error bar indicated the standard error of the mean value.
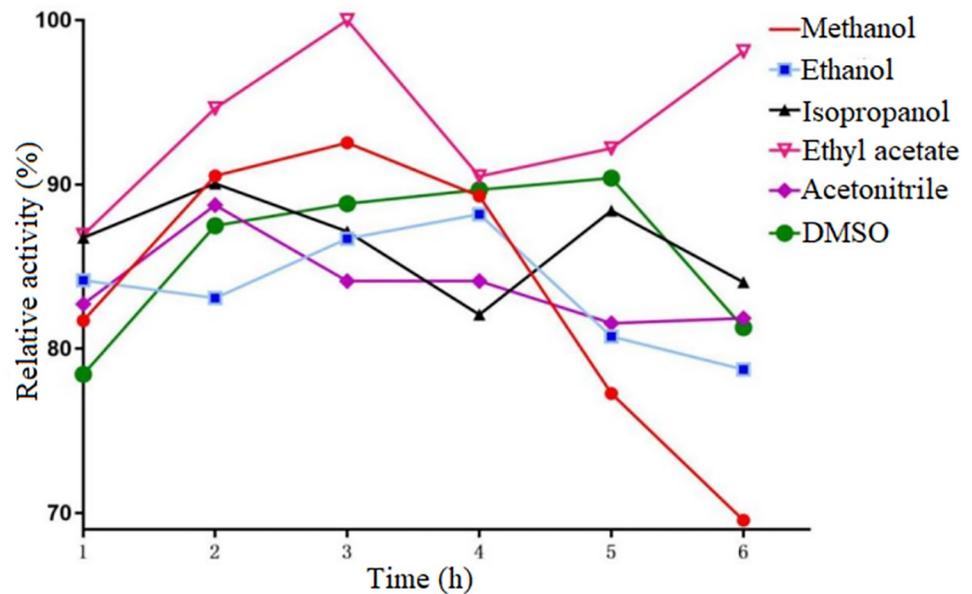
Fig 5. Effects of incubation time on the tolerance of AKR to organic solvents. Time 1h-6h to 6 h, six organic solvents (methanol,Ethanol, 2-propanol, ethyl acetate, acetonitrile, and DMSO), 10%(v/v) organic solvent.

3.5 Molecular docking and key site resolution

The source of the AKR was analyzed and DNAMAN was used to draw the alignment homologous tree, as shown in Fig 6. The results showed that the homology between the AKR and the same kind was low. Homology modeling of the AKR was built by using SWISS-MODEL, which showed the unique three-dimensional structure (Fig 7A). The symmetric 4-subunit structure suggested that the AKR is relatively conservative. Besides, the structure of the AKR was also evaluated (Fig 7B). Key indicators of model evaluation are shown in the red dot in the Fig 7B, and it can be seen from the Fig 7B that the key evaluation factors are concentrated within a limited range. The results indicated that the three-dimensional structure model of the AKR is reliable.

Since the AKR relies on the coenzyme NADPH to provide [H] during the reduction reaction with the substrate, so its conformation will undergo corresponding changes when the AKR combines with the coenzyme to form a holoenzyme. Through molecular docking, the amino acid residues that the AKR interacted with the coenzyme NADPH were predicted, and the molecular docking results were shown in Fig 8. As can be seen from the figure, the coenzyme binds to the tail end of the outer barrel structure of the AKR, and the amino acid residues in contact with it are Lys272, Pro271, and Asn270. Lys272 provides electrostatic stability and helps stabilize the structure of the enzyme coenzyme complex, while Pro271 and Asn270 provide REDOX reaction conditions and transfer protons [36, 37].

Unlike the common AKR 3 subunit structure, the AKR has a 4-subunit structure where NADPH binds to one of the subunits and is exposed outside the protein molecule rather than contained inside the molecule (Fig 8A). The local forces of molecular docking are shown in Fig 8B. Lys272, Pro271 and Asn270 interact with NADPH by hydrogen bonds, while Arg276 and Gln273 stabilize the enzyme coenzyme complex by van der Waals forces. Thus, the AKR may have more than one single active site.
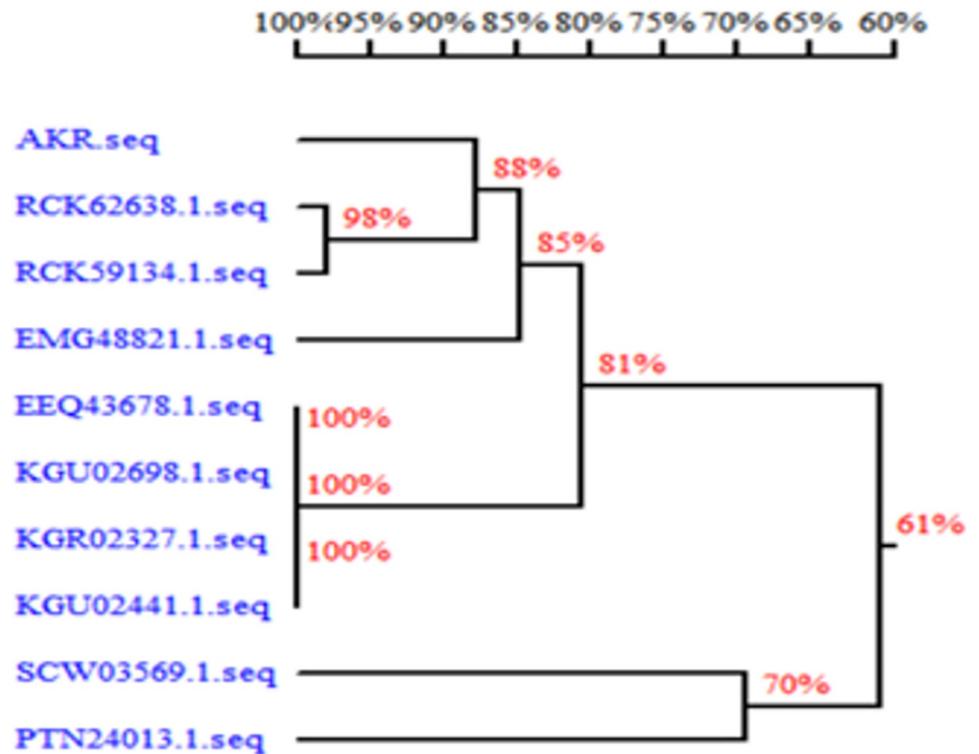
Fig 6. AKR sequence alignment homologous tree.

https://doi.org/10.1371/journal.pone.0260787.g006

4 Conclusions

A keto reductase with excellent properties was mined and characterized. The properties of the AKR were exploited and it shown wider optimum temperature and pH. Besides, the AKR has a better substrate specificity and excellent solvent tolerance. More importantly, the AKR showed activity on DKTP, an important intermediate for biosynthesis of the antidepressant duloxetine. To explore the binding mechanism of the enzyme and its coenzyme, a 3D model of the AKR docking with NADPH were constructed. Then, the important catalytic and binding sites were identified. These properties of the AKR demonstrated that it has potential applications in the field of chiral drug synthesis.
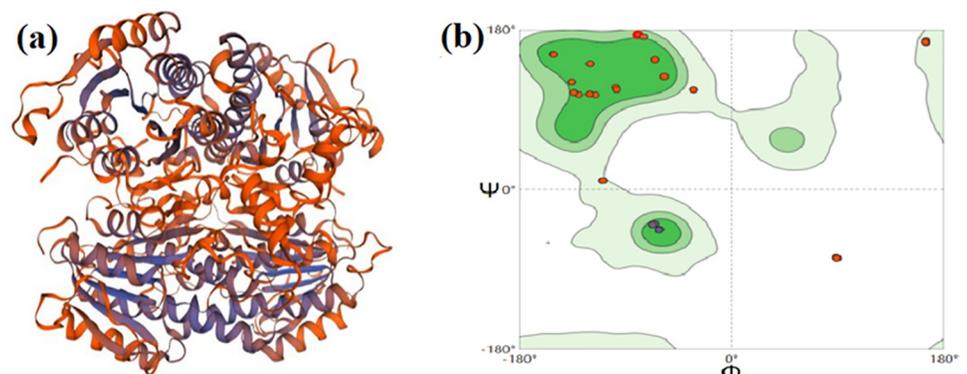

Fig 7. (a) The 3D model of the AKR, (b) Raman diagram for the AKR model evaluation.

https://doi.org/10.1371/journal.pone.0260787.g007
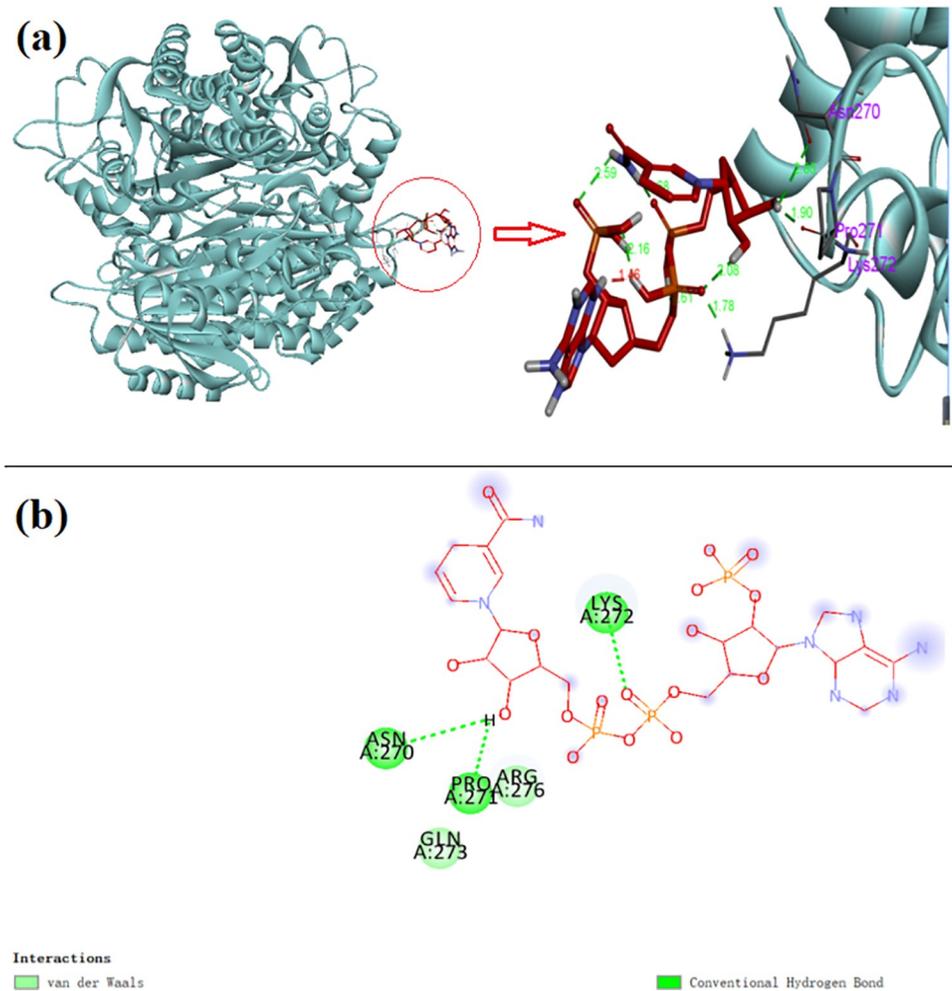
Fig 8. Molecular docking of the AKR with NADPH. (a) The AKR molecular docking model; (b) The AKR molecular docking local forces.

https://doi.org/10.1371/journal.pone.0260787.g008

Supporting information

S1 Data.
(XLSX)

Author Contributions

Conceptualization: Xiaoli Fu, Weiliang Wu.

Data curation: Xiaoli Fu, Weiliang Wu.

Formal analysis: Xiaoli Fu, Weiliang Wu.

Funding acquisition: Wei Jiang.

Investigation: Wei Jiang, Weiliang Wu.

Methodology: Weiliang Wu.

Writing – original draft: Wei Jiang.

Writing – review & editing: Wei Jiang.

References

1. Wang J-W, Li Y, Nie W, Chang Z, Yu Z-A, et al. (2021) Catalytic asymmetric reductive hydroalkylation of enamides and enecarbamates to chiral aliphatic amines. Nature communications 12: 1–10. https://doi.org/10.1038/s41467-020-20314-w PMID: 33397941

2. Zhou M, Gridneva T, Zhang Z, He E, Liu Y, et al. (2021) Chiral Bicyclic Imidazole-Catalyzed Acylative Dynamic Kinetic Resolution for the Synthesis of Chiral Phthalidyl Esters. Angewandte Chemie 133: 1665–1669. https://doi.org/10.1002/anie.202012445 PMID: 33009704

3. Wojaczyńska Eb, Wojaczyński J (2020) Modern stereoselective synthesis of chiral sulfinyl compounds. Chemical Reviews 120: 4578–4611. https://doi.org/10.1021/acs.chemrev.0c00002 PMID: 32347719

4. Liao K, Gong Y, Zhu RY, Wang C, Zhou F, et al. (2021) Highly enantioselective CuAAc of functional tertiary alcohols featuring an ethynyl group and their kinetic resolution. Angewandte Chemie 133: 8569–8574. https://doi.org/10.1002/anie.202016286 PMID: 33369828

5. Çolak NS, Kalay E, Şahin E (2021) Asymmetric reduction of prochiral aromatic and hetero aromatic ketones using whole-cell of Lactobacillus senmaizukei biocatalyst. Synthetic Communications: 1–11.

6. Xiang M, Ghosh A, Krische MJ (2021) Diastereo-and Enantioselective Ruthenium-Catalyzed CC Coupling of 1-Arylpropynes and Alcohols: Alkynes as Chiral Allylmetal Precursors in Carbonyl anti-(α-Aryl) allylation. Journal of the American Chemical Society 143: 2838–2845. https://doi.org/10.1021/jacs.0c12242 PMID: 33555867

7. Marcyk PT, Jefferies LR, AbuSalim DI, Pink M, Baik MH, et al. (2019) Stereoinversion of unactivated alcohols by tethered sulfonamides. Angewandte Chemie International Edition 58: 1727–1731. https://doi.org/10.1002/anie.201812894 PMID: 30536739

8. Maji R, Ugale H, Wheeler SE (2019) Understanding the Reactivity and Selectivity of Fluxional Chiral DMAP-Catalyzed Kinetic Resolutions of Axially Chiral Biaryls. Chemistry–A European Journal 25: 4452–4459. https://doi.org/10.1002/chem.201806068 PMID: 30657217

9. Zheng G-W, Liu Y-Y, Chen Q, Huang L, Yu H-L, et al. (2017) Preparation of structurally diverse chiral alcohols by engineering ketoreductase Cg KR1. ACS Catalysis 7: 7174–7181.

10. Luo X, Wang Y-J, Shen W, Zheng Y-G (2016) Activity improvement of a *Kluyveromyces lactis* aldo-keto reductase KlAKR via rational design. J Biotechnol 224: 20–26. https://doi.org/10.1016/j.jbiotec.2016.03.008 PMID: 26959479

11. Wang C-F, Russell G, Wang S-Y, Strichartz GR, Wang GK (2016) R-duloxetine and N-methyl duloxetine as novel analgesics against experimental postincisional pain. Anesthesia & Analgesia 122: 719–729. https://doi.org/10.1213/ANE.0000000000001086 PMID: 26646348

12. Cai S, Shao N, Chen Y, Li A, Pan J, et al. (2019) Enantioselective reduction of α, β-unsaturated ketones and aryl ketones by perakine reductase. Organic letters 21: 4411–4414. https://doi.org/10.1021/acs.orglett.9b00950 PMID: 31045374

13. McFadden CF, Cremer PS, Gellman AJ (1996) Adsorption of chiral alcohols on "chiral" metal surfaces. Langmuir 12: 2483–2487.

14. Wang K, Zhang L, Tang W, Sun H, Xue D, et al. (2020) Asymmetric Guerbet Reaction to Access Chiral Alcohols. Angewandte Chemie 132: 11505–11512. https://doi.org/10.1002/anie.202003104 PMID: 32291869

15. Abdur-Rashid K, Faatz M, Lough AJ, Morris RH (2001) Catalytic Cycle for the asymmetric hydrogenation of prochiral ketones to chiral alcohols: Direct hydride and proton transfer from chiral catalysts trans-Ru (H) 2 (diphosphine)(diamine) to ketones and direct Addition of Dihydrogen to the Resulting Hydridoamido Complexes. Journal of the American Chemical Society 123: 7473–7474. https://doi.org/10.1021/ja015902u PMID: 11472199

16. Drury JE, Hyndman D, Jin Y, Penning T, Weiner H, et al. (2007) The aldo-keto reductase superfamily homepage: 2006 update. Enzymology and Molecular Biology of Carbonyl Metabolism: 184–197.

17. Ni Y, Li C-X, Ma H-M, Zhang J, Xu J-H (2011) Biocatalytic properties of a recombinant aldo-keto reductase with broad substrate spectrum and excellent stereoselectivity. Applied microbiology and biotechnology 89: 1111–1118. https://doi.org/10.1007/s00253-010-2941-4 PMID: 20981419

18. Agrawal C, Sen S, Yadav S, Rai S, Rai LC (2015) A novel aldo-keto reductase (AKR17A1) of *Anabaena* sp. PCC 7120 degrades the rice field herbicide butachlor and confers tolerance to abiotic stresses in E. coli. Plos One 10: e0137744. https://doi.org/10.1371/journal.pone.0137744 PMID: 26372161

19. Penning TM (2015) The aldo-keto reductases (AKRs): Overview. Chemico-biological interactions 234: 236–246. https://doi.org/10.1016/j.cbi.2014.09.024 PMID: 25304492

20. Ma Y-H, Lv D-Q, Zhou S, Lai D-Y, Chen Z-M (2013) Characterization of an aldo–keto reductase from *Thermotoga maritima* with high thermostability and a broad substrate spectrum. Biotechnology letters 35: 757–762. https://doi.org/10.1007/s10529-013-1141-6 PMID: 23338701

21. Choi YH, Choi HJ, Kim D, Uhm K-N, Kim H-K (2010) Asymmetric synthesis of (S)-3-chloro-1-phenyl-1-propanol using Saccharomyces cerevisiae reductase with high enantioselectivity. Applied microbiology and biotechnology 87: 185–193. https://doi.org/10.1007/s00253-010-2442-5 PMID: 20111861

22. Asako H, Wakita R, Matsumura K, Shimizu M, Sakai J, et al. (2005) Purification and cDNA cloning of NADPH-dependent aldoketoreductase, involved in asymmetric reduction of methyl 4-bromo-3-oxobuty-rate, from *Penicillium citrinum* IFO4631. Applied and environmental microbiology 71: 1101. https://doi.org/10.1128/AEM.71.2.1101-1104.2005 PMID: 15691974

23. Liang C, Nie Y, Mu X, Xu Y (2018) Gene mining-based identification of aldo–keto reductases for highly stereoselective reduction of bulky ketones. Bioresources and bioprocessing 5: 1–8.

24. Luo X, Wang Y-J, Zheng Y-G (2015) Cloning and characterization of a NADH-dependent aldo-keto reductase from a newly isolated *Kluyveromyces lactis* XP1461. Enzyme Microb Technol 77: 68–77. https://doi.org/10.1016/j.enzmictec.2015.06.004 PMID: 26138402

25. Woodley JM (2008) New opportunities for biocatalysis: making pharmaceutical processes greener. Trends Biotechnol 26: 321–327. https://doi.org/10.1016/j.tibtech.2008.03.004 PMID: 18436317

26. Cao C, Fukae T, Yamamoto T, Kanamaru S, Matsuda T (2013) Purification and characterization of fluorinated ketone reductase from *Geotrichum candidum* NBRC 5767. Biochemical engineering journal 76: 13–16.

27. Soni P, Banerjee UC (2005) Biotransformations for the production of the chiral drug (S)-Duloxetine catalyzed by a novel isolate of *Candida tropicalis*. Applied Microbiology & Biotechnology 67: 771–777. https://doi.org/10.1007/s00253-004-1870-5 PMID: 15660213

28. Biasini M, Bienert S, Waterhouse A, Arnold K, Studer G, et al. (2014) SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. Nucleic acids research 42: W252–W258. https://doi.org/10.1093/nar/gku340 PMID: 24782522

29. Knadler MP, Lobo E, Chappell J, Bergstrom R (2011) Duloxetine. Clinical pharmacokinetics 50: 281–294. https://doi.org/10.2165/11539240-000000000-00000 PMID: 21366359

30. Asako H, Nakajima H, Kobayashi K, Kobayashi M, Aono R (1997) Organic solvent tolerance and antibiotic resistance increased by overexpression of marA in *Escherichia coli*. Applied and Environmental Microbiology 63: 1428. https://doi.org/10.1128/aem.63.4.1428-1433.1997 PMID: 9097440

31. Lee MS, Raig RM, Gupta MK, Lux MW (2020) Lyophilized cell-free systems display tolerance to organic solvent exposure. ACS Synthetic Biology 9: 1951–1957. https://doi.org/10.1021/acssynbio.0c00267 PMID: 32646213

32. Jiang W, Fang B-S (2020) Coenzyme binding site analysis of an isopropanol dehydrogenase with wide substrate spectrum and excellent organic solvent tolerance. Applied Biochemistry and Biotechnology 190: 18–29. https://doi.org/10.1007/s12010-019-03091-1 PMID: 31301008

33. Zhu F, He B, Gu F, Deng H, Chen C, et al. (2020) Improvement in organic solvent resistance and activity of metalloprotease by directed evolution. Journal of Biotechnology 309: 68–74. https://doi.org/10.1016/j.jbiotec.2019.12.014 PMID: 31870685

34. Bollinger A, Molitor R, Thies S, Koch R, Coscolín C, et al. (2020) Organic-solvent-tolerant carboxylic ester hydrolases for organic synthesis. Applied and Environmental Microbiology 86. https://doi.org/10.1128/AEM.00106-20 PMID: 32111588

35. Zhu T, Li R, Sun J, Cui Y, Wu B (2020) Characterization and efficient production of a thermostable, halo-stable and organic solvent-stable cellulase from an oil reservoir. International journal of biological macromolecules 159: 622–629. https://doi.org/10.1016/j.ijbiomac.2020.05.021 PMID: 32387598

36. Shi Lizhen, Ying Bingbing, Wang Y (2017) Structure, function and biocatalytic applications of microbial aldo-keto reductases. Bulletin of Fermentation Science and Technology 046: 198–204.

37. Kim T, Flick R, Brunzelle J, Singer A, Evdokimova E, et al. (2017) Novel aldo-keto reductases for the biocatalytic conversion of 3-hydroxybutanal to 1, 3-butanediol: structural and biochemical studies. Applied and environmental microbiology 83. https://doi.org/10.1128/AEM.03172-16 PMID: 28130301