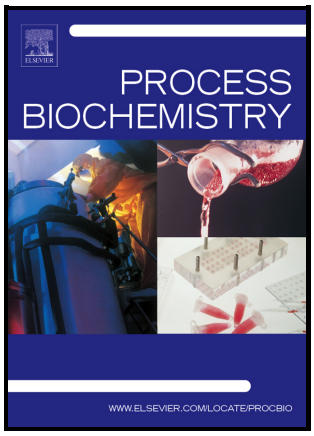

Immobilization of ArRMut11 omega-transaminase for increased operational stability and reusability in the synthesis of 3α-amino-5α-androstan-17β-ol

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Immobilization of ArRMut11 omega-transaminase for increased operational stability and reusability in the synthesis of 3α-amino-5α-androstan-17β-ol

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Abstract

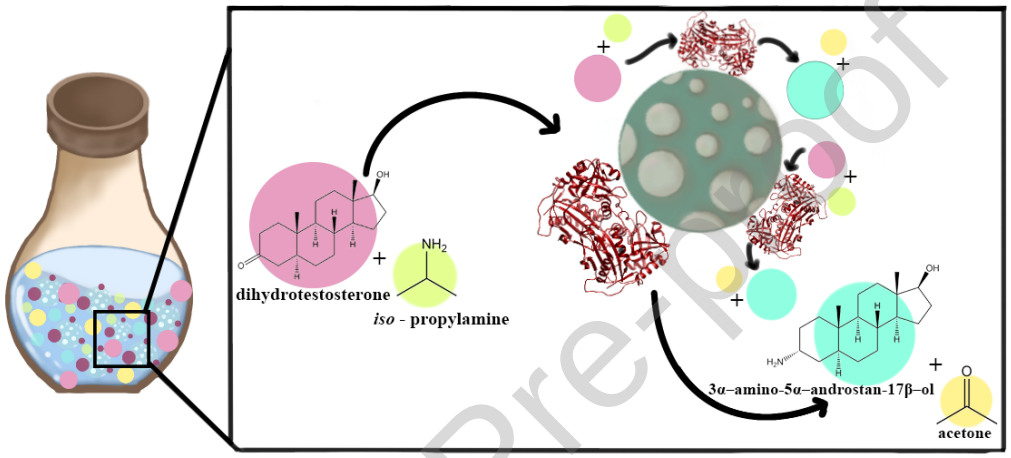
The aim of this research was to improve the operational stability and enable the reusability of ω-transaminase for synthesis of new enantiopure chiral amines of steroids.

Dihydrotestosterone was used to optimize the synthetic procedure of corresponding amino-steroid on a larger scale. The obtained product 3α-amino-5α-androstan-17β-ol was isolated and characterized. The enzyme was immobilized on a methacrylate-based carrier, giving the specific activity of 1.84 U/g of dry polymer. Higher residual activity of the immobilized enzyme in comparison to the soluble form (100% versus 35%) after 24 h incubation in 35% dimethylformamide (DMF) was obtained. The soluble enzyme retained 19% of the initial activity after 2 h incubation in 35% DMF at 70 °C, while the activity of the immobilized enzyme decreased only to 75%. Immobilized retained 85% of initial activity after ten consecutive cycles of 3α-amino-

5α-androstan-17β-ol synthesis. We have tested the specificity of the ArRMut11 variant, further increased its stability by immobilization, and used it in several cycles for the synthesis of 3α-amino-5α-androstan-17β-ol.

We showed that the enzyme previously evolved for higher stability as the immobilized variant showed more increased stability and high reusability that can more effectively be applied for the biosynthesis of amino steroids.

Graphical abstract



Keywords: steroid, macroporous, dihydrotestosterone, biocatalysts, immobilization

Introduction

Productivity, economic efficiency, and technical performance of industrial processes have been improved with the establishment of immobilized enzyme catalysts [1]. Immobilization leads to a biocatalyst with increased operational and long-term stability with a lower likelihood of product contamination [2, 3]. The use of immobilized enzyme also s allows for a reduced number of processing steps, multiple use, reaction control, and the easier development of a multi-enzyme reaction system [1, 4].

Transaminases catalyse the transfer of an amino group from the suitable amino donor to the targeted amino acceptor, utilizing the pyridoxal-5'-phosphate (PLP) cofactor, generating the chiral carbon in a stereoselective manner and producing enantiomerically pure products which could be final products or used as building blocks for the synthesis of bioactive compounds [5-7]. Omega-transaminases (ω-transaminases) belong to the class III transaminases that can accept structurally different carbonyl compounds, both aldehydes and ketones [2, 8].

Moreover, they exhibit high enantioselectivity, substrate specificity, and no requirement for cofactor regeneration. Therefore, due to their catalytic properties, ω-transaminases have a great potential in providing a green alternative to metal-catalysed reductive aminations [9, 10].

Over the last years transaminases have been immobilized in their fully or partially purified form, or even as a whole cell catalyst on various carriers such as polymeric resins [11], chitosan [12], macrocellular silica monoliths [13], porous glass metal affinity supports [3], MnO_2 nanorods [14], functionalized cellulose [15], inorganic-based nanoflowers [16] and different sol-gel matrices [17]. One example shows immobilization of *Escherichia coli* (*E. coli)* whole cells expressing ArRMut11 by the sol-gel entrapment method for kinetic resolution of various *rac*-amines [17].

Amino steroids have been used as key mediators in the synthesis of biologically active steroid compounds [18]. Some of those compounds, such as 17β-aryl sulphonamide substituted steroids, exhibit an anticancer activity by acting as a steroid sulfatase inhibitors in breast cancer treatment [19, 20]. The other compounds exert an anticoagulant effect in rodents as has been shown for 17β-aminoestrogens [21]. Some 3-aminosteroids have been proven to display a strong antitrypanosomal activity, which makes them very potent steroid alkaloids for clinical use [22]. Additionally, amino steroids can also serve as precursors for the synthesis of derivatives that have expressed antimalarial activity [23, 24].

The aim of this study was to develop a procedure for the efficient synthesis of new amino steroids using ArRMut11 variant of (R) selective ω-transaminase from *Arthrobacter sp.* [25] by testing the specificity of the ArRMut11 variant for different steroids. An additional aim was to further increase the thermo- and organic-solvent stability of the enzyme that was already previously evolved by 11 rounds of protein engineering for higher stability in organic solvents and possibility for the conversion of bulky substrates, resulting in a new and further immobilized biocatalyst. We also endeavored to optimize synthetic protocol and enable reusability of the enzyme by immobilization onto macroporous methacrylate-based carrier.

In this study, we have chosen methacrylate-based material as the carrier for immobilization of this enzyme since it has been proven to have favorable porous properties, and provides more hydrophilic environment to the enzyme compared to other macroporous polystyrene and other previously published carriers, which is crucial for higher activity and stability in the presence of organic solvents.

Materials and methods

Materials

The *Escherichia coli* BL21 (DE3) pLysS strain was used for protein production. All chemicals used were of analytical grade purity. Solvents for chromatographic purification of 3α-amino-5α-androstan-17β-ol were obtained from commercial suppliers and distilled prior to use. For details, see Supplementary Information Section 1.

Heterologous expression and purification of ω-transaminase

Expression of ArRMut11 ω-transaminase was done in *E. coli* BL21 (DE3) pLysS cells in Luria Bertani medium by using IPTG for induction. Expression was done at 30 °C, after 24-hour harvested cells were disrupted by ultra-sonication (4x30 s) and cell free crude extract was obtained by centrifugation. Bacterial lysate was used either directly, in the form of lyophilized powder, or as purified protein for immobilization.

The protein purification was performed on a Ni-NTA affinity column by using the AKTA purifier system. Elution was done with a linear gradient (from 0 to 100%) of 300 mM imidazole. The purification process was monitored using 12% denaturing polyacrylamide gel. Fractions containing pure enzyme were pooled and dialysed against PBS buffer (pH 7.5). Protein concentration was determined using Bradford assay. For a detailed explanation of the expression and purification process, see Supplementary Information Section 1.

Screening of enzyme substrate acceptance

In order to screen the ω-transaminase substrate acceptance conversion of (*R*)-(+)-α,-methylbenzylamine (*(R)*-MBA) to acetophenone was monitored by HPLC detection on reverse phase column NucleosilC18. In the buffered solution of *(R)*-MBA and PLP, ArRMut11 enzyme was added in the form of crude bacterial extract, and the reaction was started by adding an appropriate steroid compound dissolved in dimethyl sulfoxide (DMSO). After 24 hours at 37 °C, the reaction was stopped and samples were analysed to detect the presence of acetophenone. A gradient was run from 30% acetonitrile/70% 0.1% TFA to 95% acetonitrile/5% 0.1% TFA (flow rate 1 mL/min). UV detection was carried out at 245, 254, and 280 nm. Acetophenone eluted at a retention time of 4.6 minutes. For details, see Supplementary Information Section 1.

Biocatalytic synthesis of 3α-amino-5α-androstan-17β-ol

Dihydrotestosterone was dissolved in DMF and added to the previously prepared solution of pyridoxal-5'-phosphate (PLP) and *iso*-propylamine in distilled water. pH of the solution was set at 9.9. Expressed ArRMut11

was added in the form of lyophilized bacterial lysate and the reaction mixture was shaken at 42 °C for four days, with a daily addition of fresh amount of the enzyme (Fig. 1).

After four days the reaction was stopped, and amino analogue of dihydrotestosterone was isolated by extraction. The crude product was obtained as a brownish solid in 58% yield (29 mg). Pure product was obtained after preparative chromatographic separation by dry-flash silica gel (Merck silica gel 60 (0.063–0.200 mm)) chromatography using the two-step elution system. For details, see Supplementary Information Section 1.

Enzymatic assay

For enzyme activity measurement, reaction mixtures were incubated for 2 hours at 37 °C in a thermo-shaker at 900 rpm shaking in a solution consisting of 12.5 mM *(R)*-MBA and 30 mM sodium-pyruvate in 0.1 M HEPES buffer pH 8.0, with the addition of 0.25 mM PLP. Absorbance was measured every 30 minutes (MAPADA UV3100PC Spectrophotometer, Shanghai) at 245 nm by following the conversion of *(R)*-MBA to acetophenone, which can be detected with high sensitivity at this wavelength (ε=12 $mM^{-1}cm^{-1}$) [26]. An enzyme unit is defined as the amount of the enzyme that catalyzes the conversion of 1.0 μmol of (*R*)-MBA to acetophenone per minute.

Immobilization of ω-transaminase on a GMA-EGDMA based carrier

Synthesis of carrier for immobilization

The macroporous carrier for the enzyme immobilization was prepared according to the slightly modified previously published procedure [27]. Continuous phase (125 mL, 1wt% of PVP, Mw=360 000 g/mol, in demineralized water) was placed in a 250 mL reactor equipped with an anchor stirrer and heated to 70 °C. Subsequently, the monomer phase, containing 0.43 g of AIBN, 10.33 g of GMA, 7.68 g of EGDMA, 2.38 g of 1-dodecanol and 21.38 g of cyclohexanol was added to the continuous phase under stirring. Reaction was stopped after 6 hours. The obtained copolymer beads were washed 5 times with ethanol and dried in the oven at 40 °C. The particle size distribution was determined by passing the obtained polymer through a number of sieves of different mesh sizes. A fraction with particle sizes between 150 and 500 μm was further used for enzyme immobilization.

Copolymer was subjected to amination using ethylenediamine as follows: Four grams of copolymer were suspended into 100 mL of demineralized water, 10 g of ethylenediamine were added and the reaction mixture

was stirred for 24 hours at room temperature. Subsequently, the reaction mixture was heated to 80 °C and stirred for another 6 hours. Polymer was filtered, washed with demineralized water, and dried in the oven at 40 °C.

Immobilization of ω-transaminase

The enzyme was immobilized on the GMA-co-EGDMA carrier, previously aminated with ethylenediamine. The copolymer was initially washed with 0.1 M sodium phosphate buffer pH 8.0 and then incubated in 2.5% (v/v) glutaraldehyde in the same buffer for 2 hours at room temperature with periodic mixing. The excess of glutaraldehyde was removed from the polymer suspension with 0.1 M sodium phosphate buffer pH 7.0. An adequate amount of ω-transaminase solution with 0.25 mM PLP (0.64, 1.6, 3.2, 8.0 or 32.0 U) was added to the activated polymer and left at 4 °C for 48 hours with occasional mixing. The polymer was washed with PBS buffer pH 7.5, containing 1 M sodium chloride. Subsequently, free protein binding groups were blocked by incubating the carrier with the immobilized enzyme in the solution of 0.1 M ethanolamine in 0.1 M sodium phosphate buffer pH 7.0, for 2 hours at room temperature. The polymer carrying immobilized enzyme was washed with 0.1 M sodium phosphate buffer pH 7.0 and kept in the same buffer at 4 °C until use. Specific activity of immobilized ω-transaminase was determined by the previously described enzyme assay. The obtained results were fitted into a graph using Graph Pad Prism 6

pH optimum

In order to determine the pH optimum of the soluble and immobilized enzyme, the activity was assayed by the protocol described for measuring enzyme activity at various pH values in the range from 5 to 12 (pH = 5-6, citrate-phosphate buffer; pH = 7-9, HEPES buffer; pH = 10-11, carbonate buffer; pH = 12 phosphate buffer). The obtained results were fitted into a graph using Graph Pad Prism 6.

Thermal stability

Thermal stability of both the soluble and immobilized enzyme was determined by incubating of the corresponding enzyme form in 0.1 M HEPES buffer (pH 8.0) at 50, 60 and 70 °C for 30 minutes. After incubation the samples were cooled in the ice for 10 minutes and residual activities of the enzymes were assayed by the protocol previously described. The obtained results were fitted into a graph using Graph Pad Prism 6.

Organic solvent stability

Stability in organic solvent was determined by incubating both the soluble and immobilized enzyme for 24 hours in a solution of 35% DMF in 0.1 M HEPES buffer pH 8.0 at 37 °C, and stirring the mixtures at 180 rpm. The enzyme activity was assayed after 24 hours by the procedure described for measuring enzyme activity. The obtained results were fitted into a graph using Graph Pad Prism 6.

Thermal stability in the organic solvent

Thermal stability in the organic solvent was determined by incubating the corresponding enzyme form in a solution of 35% DMF in 0.1 M HEPES buffer (pH 8.0) at 50, 60 and 70 °C for 2 h. Aliquots were taken at different time points and the samples were cooled in ice for 10 minutes. Residual activities of the enzymes were assayed by the protocol described for measuring enzyme activity. The obtained results were fitted into a graph using Graph Pad Prism 6.

Determination of degree of reusability of immobilized ArRMut11for synthesis of 3α-amino-5α-androstan-17β-ol

Reusability of immobilized ArRMut11 was evaluated by following the conversion of dihydrotestosterone to 3α-amino-5α-androstan-17β-ol in ten consecutive cycles. This was put into a solution of PLP (0.425 mg, 0.017 mmol) and *iso*-propylamine (146 µL,17 mmol) in distilled water, and the pH of the solution was adjusted to 9.9. Subsequently, 595 µL of a solution of dihydrotestosterone (5 mg) dissolved in DMF was added (10 mM, 35% vol, respectively). The reaction was initiated by the addition of immobilized enzyme (0.47 U) and the mixture was shaken at 42 °C and 180 rpm for four days during the first cycle of conversion. In the first cycle, reaction with bacterial lysate containing overexpressed ArRMut11 was set for comparation of the conversion process, under the same conditions as with the immobilized enzyme. Reaction mixture aliquots were analysed every 24 hours by the previously described TLC procedure. The immobilized enzyme was removed from the reaction mixture by centrifugation, then washed twice with the solution of 35% DMF in water, and subsequently washed twice with demineralized water. Then, a new cycle of synthesis was initiated with the recycled enzyme under the same conditions as the first one. After the first cycle, the reaction time was shortened to two days. Residual activity of the immobilized enzyme was determined after each cycle using the procedure described for measuring enzyme activity. The obtained results were fitted into a graph using Graph Pad Prism 6.

Results and discussion

Heterologous expression and purification of ω-transaminase

ArRMut11 was expressed in *E. coli* BL21 (DE3) pLysS cells and purified to homogeneity by the Ni-NTA affinity chromatography. The process of purification was analysed by SDS electrophoresis on a 12% gel and purified ArRMut11 showed only one band in polyacrylamide gel electrophoresis (Fig. S1). The estimated molecular mass of the purified protein is approximately 40 kDa, which is consistent with the data obtained by MALDI-TOF analysis for the weight of one ArRMut11 subunit (36.72 kDa) [25].

Screening of enzyme substrate acceptance

Although ArRMut11 was previously tested for amination of few steroid compounds [18], in this article we wanted to further test its amination performance for other 12-steroid compounds. Therefore, reactivity and selectivity of the enzyme was tested for 12-steroid compounds that significantly differ on position and reactivity of keto-group, as well as the configuration of A and B rings. The reaction yield was expressed as mol% of conversion of *(R)*-MBA to acetophenone (Table S1). The retention times for (*R*)-MBA and acetophenone under applied conditions were 1.96 min and 4.6 min, respectively. From the results given in Table S1, among all tested compounds, the best yield of conversion was observed with dihydrotestosterone (Table S1, compound 1), androstenedione (Table S1, compound 6), 3-oxocholic acid (Table S1, compound 9), and 3,7,12-trioxoholic acid (Table S1, compound 12) (Fig. 2).

Compounds **9** and **12** are well accepted by the enzyme and the conversion rate is high, where yield of transformations were 17% and 35%, respectively. The highest yield of transformation was detected with compound **12**. However, this derivative has three keto groups, and according to results of other derivatives of cholic acid, complex mixture of products could be expected, resulting in their isolation and separation being impeded. Similarly, isolation of product obtained from derivative **9** was highly difficult due to its good solubility in water. With compound **6**, 11% of conversion was observed. However, this derivative possesses two keto groups, which differs not only by their positions, but by their reactivity also. Due to higher reactivity of 17-keto group, it could be expected that the main product will be a corresponding 17-amino derivative [28]. At the same time, the study could not completely exclude the possibility of product that could be observed from the 3-keto group, which means that mixture of products should be expected [18]. Taking into account the above presented results, we decided to use compound **1** for further experimental work.

Biocatalytic production and characterization of 3α-amino-5α-androstan-17β-ol

The obtained product was separated by extraction from the other components of the reaction mixture and subsequently purified by dry-flash silica gel chromatography. Amino product was obtained as a colourless powder (25 mg, 50% yield).

The structure of 3α-amino-5α-androstan-17β-ol was confirmed by ^{1}H and ^{13}C-NMR spectroscopy in perdeuterated methanol (Fig. S2 and Fig. S3). The position and width of signal H-C (3) in 1H NMR spectra (δH = 3.14 ppm) suggested on equatorial (β) orientation of H-C (3) due to the absence of diaxially coupling and the anisotropy effect of cyclohexyl ring. Confirmation was obtained from the 1D NOESY experiment (Fig. S4). The obtained spectrum shows that H-C (3) exhibits NOE interaction with protons of angular methyl group (H-C (19), δH = 0.84 ppm), which means that both the angular methyl group C (19) and H-C (3) are on the same β-side of the steroidal skeleton. This in turn suggests that NH_2-group has α orientation, *i.e.*, that C (3) has R-stereochemistry. In addition, corresponding NOE interactions with H-2β and H-4β protons are much stronger than those with H-2α and H-4α. This result of highly stereospecific forming of amine with *R*-configuration is entirely consistent with the report for small cyclic ketones and *R*-specific transamination with ATA-117, a homolog of an enzyme from *Arthrobacter sp.* [25]. In this report, we showed that ArRMut11 is capable of accepting larger substrates, while simultaneously retaining *R*-stereo induction during transamination reaction.

The observed ion type detected by the mass spectroscopy was $[M-H]^{+}$ ion with the observed mass of 292.2626 g/mol (Fig. S5).

Immobilization of ω-transaminase

ArRMut11 to the best of our knowledge was not previously extensively tested in immobilized form in published literature in order to further increase its stability under operating conditions using our hydrophilic macroporous copolymer. Therefore, it was immobilized on GMA-co-EGDMA carrier to additionally increase its operational stability and to enable its reusability in the biocatalytic processes. In order to determine the optimal enzyme quantity for immobilization, the effect of the amount of enzyme, added per weight of dry carrier, on specific activity and immobilization yield, was observed (Fig. 3).

Fig. 2 shows that the optimal ratio between the specific activity and the immobilization yield was achieved when 8.0 U of the enzyme was added. The enzyme, immobilized under these conditions, has the specific activity of 1.84 U/g of dry polymer and a immobilization yield of 23.4%. Therefore, this amount of the enzyme was chosen for further experiments. Low specific activity of the immobilized enzyme was probably caused by

diffusion limitations, produced when immobilized enzyme act on a substrate or interference of the immobilization procedure with the catalytic site of the enzyme [27].

pH optimum

Both enzyme forms exhibited the pH optimum at 8.0. However, the immobilized enzyme showed a wider pH optimum range from pH 7.0 to pH 9.0 (Fig. S6).

Thermal stability

Fig. S7 shows that the immobilized enzyme retained 30.0% of its initial activity even after 30 min of incubation at 70 °C. Unlike to the immobilized enzyme, the soluble form of ArRMut11 retained only 13.0% of its initial activity after incubation at 70 °C. The residual activity of the soluble enzyme decreased to 49.6% of its initial activity after 30 minutes of incubation at 50 °C, whereas the immobilized form retained 66.2% of the initial activity.

Immobilization by covalent binding contributed to the increased thermal stability of the enzyme since the enzyme is fixed to the carrier by the formation of multiple covalent bonds, resulting in reduced of both thermal vibrations and conformational freedom [29]. The thermal stabilization of covalently immobilized enzymes has already been reported for lipase from *Candida rugosa* [30], alcohol dehydrogenase [31] , laccase [32]. and many others.

Organic solvent stability

The presence of an organic solvent during the biocatalytic conversion of bulky substrates such as steroids is essential because of its low substrate solubility in aqueous mixtures [18].

Unlike the soluble enzyme—whose residual activity after only 24 hours incubation in the organic solvent decreased to approximately 35.0% of the initial activity—the immobilized enzyme retained 100.0% of its initial activity (Fig. 4). Immobilization contributed to the stabilization of enzyme structure, thus enabling application in industrial processes demanding high concentrations of a co-solvent.

The immobilized enzyme has more rigid conformation, which prevents denaturation in the presence of organic solvent as has already been reported for α-chymotrypsin, alcohol dehydrogenase, lipase, and others [33-36].

Thermal stability in the organic solvent

The use of enzymes in organic synthesis often requires both the presence of solvents and elevated temperatures that can affect enzyme stability and activity. Therefore, the effect of the organic solvent and increased temperature on soluble and immobilized ArRMut11 was investigated.

The residual activity of soluble and immobilized ArRMut11 at different temperatures within 2 hours of incubation 35.0% DMF is presented in Fig. 5. The residual activity of the soluble enzyme (Fig. 5a) substantially decreased at all temperatures within first 30 minutes and subsequently remained unchanged. With increasing incubation temperature, the residual activity of the soluble enzyme considerably decreased. The most distinct loss of the enzyme activity was observed at 70 °C, where the soluble enzyme lost almost 80.0% of its initial activity. The covalent immobilization provided a more stable enzyme as shown at Fig. 5b. At 50 °C and 60 °C, the immobilized enzyme retained its initial activity for 2 hours, whereas the immobilized enzyme lost only 20% of its initial activity at 70 °C. These results show that the immobilized enzyme has improved properties, which should provide superior reusability in organic synthesis.

Determination of degree of reusability of immobilized ArRMut11

Immobilized enzyme was applied in ten consecutive cycles of dihydrotestosterone conversion retaining 85.0% of initial activity (Fig. 6). Based on the TLC analysis during the first cycle of conversion, we noticed that after the second day the rate of dihydrotestosterone conversion to its amino derivative had not changed significantly, so we shortened the reaction time to 2 days in the next cycles (Fig. S8). TLC analysis showed that during the first 6 cycles the reaction was almost quantitative. However, the conversion rate between the 7^{th} and 10^{th} cycle decreased, probably as a consequence of declined enzyme activity and a slight loss of the polymer between cycles (Fig. S9).

Conclusions

We tested specificity of the ω-transaminase ArRMut11 mutant originating from *Arthrobacter sp.* towards amination of different steroid substrates and found that the highest rate of conversion was observed with derivatives having a C (3) keto moiety (dihydrotestosterone, androstenedione, 3-oxocholic acid, and 3,7,12-trioxocholic acid).

ArRMut11 ω-transaminase was purified after recombinant expression and successfully further thermostabilized in organic solvent such as 35% DMF by immobilization on macroporous methacrylate-based

carrier. For example, after 2 hours of incubation at 70°C ArRMut11, soluble mutant previously stabilized by protein engineering had residual activity of 19%, while the immobilized one had residual activity of 75%.

Using the immobilized enzyme, dihydrotestosterone was transaminated to 3α-amino-5α-androstan-17β-ol in ten consecutive cycles, during which the immobilized enzyme retained 85% of its initial activity.

Results from this study confirmed that ω-transaminase ArRMut11 mutant originating from *Arthrobacter sp.* immobilized onto macroporourous methacrylate-based polymer can be used for efficient amination of steroid substrates having C (3) keto moiety and synthesis of 3α-amino-5α-androstan-17β-ol. Results also showed that even where the enzyme was already previously stabilized by protein engineering for operating under higher temperatures in organic solvents, this stabilization can be further increased by optimizing immobilization on macroporous methacrylate-based polymer. Therefore, we proved that already highly engineered enzymes with respect to the stability could be further stabilized by immobilization on macroporous carrier that we have developed and used it in ten consecutive cycles of 3α-amino-5α-androstan-17β-ol synthesys. Further studies are in progress for obtaining larger quantities of product and testing them for physiological activity.

Declarations:

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Conflict of interest: The authors declare that they have no competing interests.

Availability of data and material: All data and material are available from the corresponding author.

Code availability: Not applicable.

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Figure captions:

Fig. 1 The reaction scheme of dihydrotestosterone conversion catalyzed by transaminase ArRMut11 using *iso*-propylamine as an amine donor

Fig. 2 Structures of steroids accepted by the enzyme

Fig. 3 Effect of the added amount of transaminase activity on the immobilization yield and specific activity of the immobilized enzyme; each data point represents the average value of three independent measurements +/- standard deviation

Fig. 4 Influence of the organic solvent (35.0% dimethylformamide (DMF)) on the stability of the soluble and immobilized enzyme, measured during 24 hours incubation period; each data point represents the average value of three independent measurements +/- standard deviation

Fig. 5 Thermal stability of ArRMut11 incubated in 35% dimethylformamide (DMF) solution; (a) soluble enzyme (b) immobilized enzyme; each data point represents the average value of three independent measurements +/- standard deviation

Fig. 6 Residual activity of immobilized ArRMut11 measured during consecutive cycle synthesis of 3α-amino-5α-androstan-17β-ol; each data point represents the average value of three independent measurements +/- standard deviation

Fig. 1

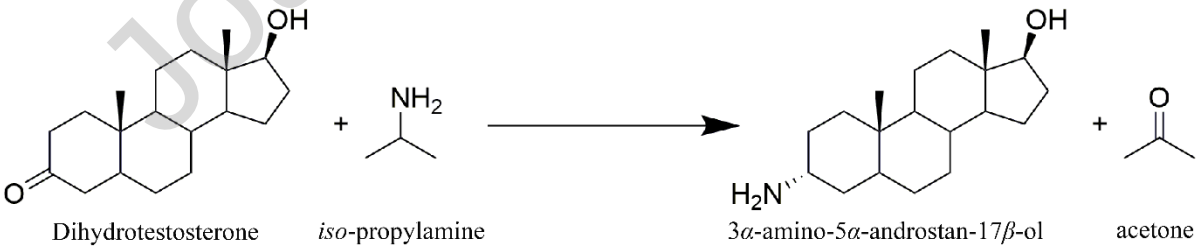


Fig. 2

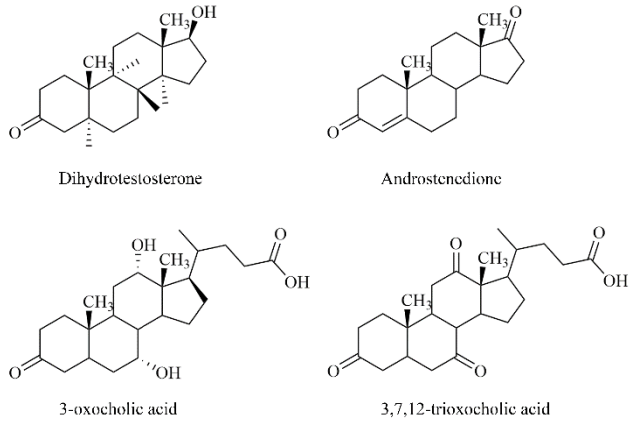


Fig. 3

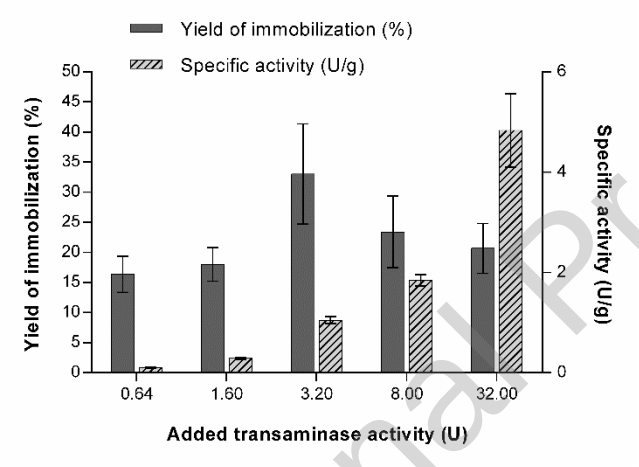


Fig. 4

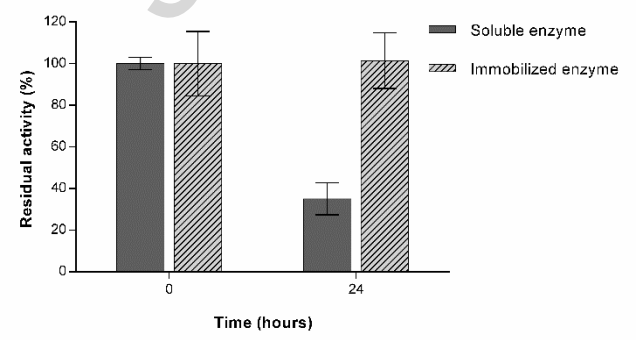


Fig. 5

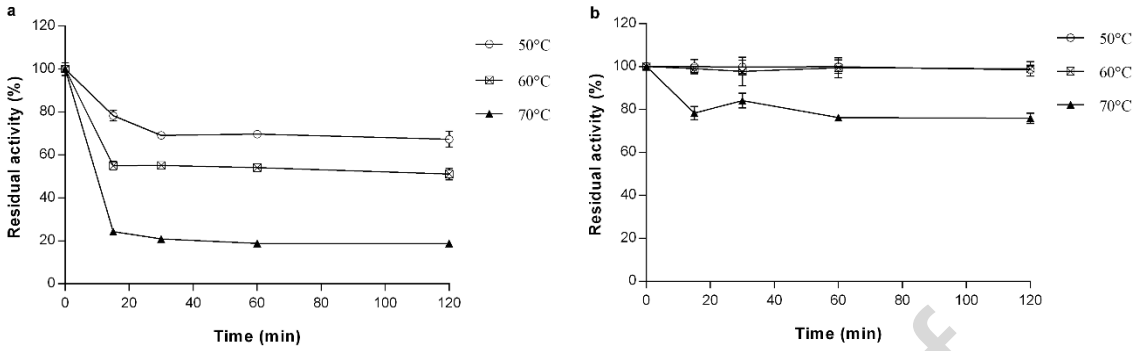
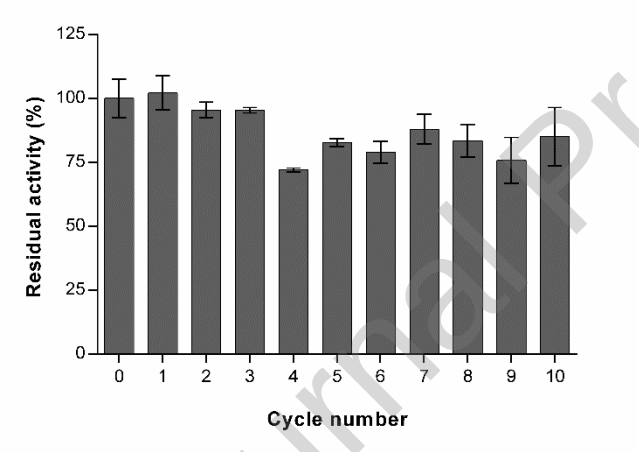


Fig. 6



HIGHLIGHTS

- ArRMut11 omega-transaminase was tested for amination of steroid compounds
- 3α-amino-5α-androstan-17β-ol was synthesized and characterized
- The enzyme was further stabilized by immobilization onto macroporous copolymer
- Synthetic process was performed in ten consecutive cycles with immobilized enzyme