Supplement

Crystallographic characterisation of the (R)-selective amine transaminase from Aspergillus fumigatus

Maren Thomsen‡, Lilly Skalden‡, Gottfried J. Palm, Matthias Höhne, Uwe T. Bornscheuer and Winfried Hinrichs

‡ These authors contributed equally to this work.

Institute of Biochemistry, University of Greifswald, Felix-Hausdorff-Str. 4, 17489 Greifswald, Germany

Correspondence e-mail: winfried.hinrichs@uni-greifswald.de

Cloning of the amine transaminase mutant of Neosartorya fischeri

To delete 22 amino-acid residues at the N-terminus an additional NdeI restriction site was inserted into the sequence of the amine transaminase of Neosartorya fischeri after the second amino acid alanine. The insertion was made by a QuikChange PCR with the Quik Lightning Multi Enzyme kit (Agilent Technologies). The QuikChange was done as described in the manual with the forward primer CTGCTGGAACGTAGCCATATGGCGTTCTCTAAAGGTATTG. The following PCR program was used: Hold 95°C for 2 min before 30 cycles with 95°C for 20 sec, 55°C for 30 sec and 65°C for 5 min started. Finally 65°C was hold additional for 5 min. After the QuikChange PCR a DpnI digestion from the same kit followed. 1 μl DpnI was mixed with 25 μl PCR reagent and incubated for 5 min at 37°C before the enzyme was inactivated by 80°C for 20 min. The resulting plasmid was verified through sequencing.

The final deletion of the 22 amino acids was done through a restriction digestion with NdeI enzyme (New England Biolabs). 20μl plasmid was mixed with 7 μl NEB-buffer 4, 3 μl NdeI enzyme and 40 μl dist. water. The digestion was made at 37°C for 2h before the enzyme was inactivated for 10 min at 80°C. A PCR purification with the High-Pure PCR Cleanup Micro kit (Roche) followed. The purification was done as described in the manual. Finally the ligation of the plasmid was carried out with the T4-ligase (Thermo Scientific). 5.8 μl purified PCR product was mixed with 0.7 μl T4-ligase buffer, 0.5 μl T4-ligase and 1 μl dist. water. The following program was used: Hold 20°C for 2h, hold 16°C for 4h, hold 14°C for 3h, hold 12°C for 3h, hold 10°C for 2h and hold 72°C for 10 min. The resulting plasmid was verified by sequencing.

Expression of the wild type and the mutant of Neosartorya fischeri

The expression of the amine transaminase mutant and wild type from Neosartorya fischeri was carried out like the expression of the amine transaminase from Aspergillus fumigatus as described (Höhne et al., 2010). To optimize the expression of the mutant, the same expression protocol was used with exception of the following varied parameter. The expressions at different temperatures were additionally carried out at 15°C, 25°C and 30°C. The expression at different induction times were also tested with an induction OD600 of 1.5 and 5. Also different inducer concentrations of 0.1 mM and 0.5 mM IPTG were investigated. The expression optimization was carried out with chaperones present in the Takara chaperone kit (Takara Bio INC.). The plasmids carrying the chaperones were co-transformed with the amine transaminase gene carrying plasmid. During all cultivations and expressions 7/OD600 samples were taken.
**Transformation and co-transformation of chaperones**

Competent *E. coli* BI21 cells were incubated for 30 min on ice with 1 μl of the plasmid. Afterwards a heat shock at 42°C for 45 sec followed. After 5 min incubation on ice, 250 μl LB-Soc was added and incubated at 37°C for 1h. Finally, 150 μl culture was plated on agar-plates with specific antibiotic resistance (ampicillin for wild type and mutant of the amine transaminase of *Neosartorya fischeri*). The co-transformation was done with in each case 1 μl of the plasmid carrying the chaperones and the amine transaminase encoding genes. The resulting culture was plated on agar-plates with ampicillin (100 μg/ml) and chloramphenicol (50 μg/ml).

**SDS-PAGE**

The 7/OD<sub>600</sub> samples were disrupted two times by Fast Prep with 4 ms<sup>−1</sup> for 20 sec. After centrifugation at 13000 × g the supernatant was separated and the insoluble fraction was washed with sodium phosphate buffer (50 mM, pH 7.5, adjusted with KOH). Finally, the insoluble fraction was re-suspended in sodium phosphate buffer (50 mM, pH 7.5, adjusted with KOH).

SDS-PAGEs with a 10% separation gel and 4% collecting gel was used. 15 μl normalized cultivation sample was mixed with 15 μl sample buffer before a denaturation at 95°C for 10 min happened. 20μl prepared sample was loaded to the SDS-PAGE. As protein marker the commercial available protein marker from Carl Roth (200 kDa to 14 kDa) was used. With 200V and 50 mM the SDS-PAGE run for 45 min before it was stained with coomassie blue. After de-staining the gels, the protein bands were visible.

![SDS-PAGE](image)

*Figure S1:* SDS-PAGE of the amine transaminases mutant from *Neosartorya fischeri*. Lanes are labelled according to the induction time t (hour). a) soluble fraction b) insoluble fraction.
**Figure S2:** $2F_{\text{obs}} - F_{\text{calc}}$ map of a representative region of the enzyme centred at Phe 270 calculated at 1.27 Å resolution contoured at 1σ level. The figure was prepared with *PyMOL* (Delano, 2002).

**Figure S3:** Anomalous map contoured at 3σ. Sulphur atoms of Met149, Met271, Cys272, Met279, and Met308 are identified. The figure was prepared with *PyMOL* (Delano, 2002).
Figure S4: Stereo view of additional positive difference electron density ($F_{\text{obs}} - F_{\text{calc}}$ map, 3σ level) in the active site next to the PLP. The figure was prepared with PyMOL (Delano, 2002).

Figure S5: Anomalous map contoured at 3σ around Ile78 and Cys82, ions are shown as spheres (potassium: violet; chloride: green). The figure was prepared with PyMOL (Delano, 2002).

References