

Transaminations with isopropyl amine: Equilibrium displacement with yeast alcohol dehydrogenase coupled to *in situ* cofactor regeneration

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Cloning, Protein Expression and Purification

The mutant gene for *Arthrobacter citreus* ω -Transaminase (TA-S9, Cambrex Karlskoga AB) was inserted in the plasmid pET28a(+) with an N-terminal His₆-tag. The gene was PCR-amplified with the primers:

Forward primer with *NheI* restriction site:

5'-CAT **ATGGCTAGC** GGTTTAACAGTGCAAAAAATAAACTGGGAG-3'

Reverse primer with *HindIII* restriction site:

5'-GCAG **AAGCTT** TTAGGATTGCTGCCATTCTCCACTTTC-3'

After digestion (*NheI*, *HindIII*) and sequence verification the construct was transformed into *E. coli* BL21(DE). Expression was done by mixing a 20 ml over night culture with 180 ml of Luria-Bertoni (LB) medium with 50 mg/L Kanamycin and 0.4 mM IPTG, and incubating for 24 h at 25 °C (150 rpm, baffled flask). The cells were thereafter separated from the medium by centrifugation and resuspended in IMAC binding buffer (20 mM sodium phosphate, 500 mM sodium chloride, pH 7.4), disrupted by addition of BugBuster® 10X (Merck) and applied to a column with Chelating Sepharose FastFlow (GE Healthcare) resin treated with a saturated water solution of Cobalt(II) chloride. After washing with the binding buffer, the His₆-tagged enzyme was eluted with IMAC elution buffer (20 mM sodium phosphate, 500 mM sodium chloride, 500 mM Imidazole, pH 7.4). An excess of cofactor (PLP) was added before desalting on a PD10 column (GE Healthcare). The simple procedure of adding PLP before buffer change ensures that the amount of cofactor is balanced with the enzyme concentration, assuming that a negligible amount of enzyme is in apo form during the desalting. Adding more PLP resulted in lower activity, as presented in the main article. This enzyme variant, denoted S9, is included in the enzyme kit available from Cambrex Karlskoga AB, Sweden.

Enzyme Reactions

The reaction was performed in 50 mM sodium phosphate buffer, pH 7.0, in 1 ml scale. All components were dissolved separately in the buffer and pH corrected. These solutions were then combined to final concentrations of 1.8 mM acetophenone or 20 mM of either 4-methylacetophenone, 4-nitroacetophenone, 2,4-dihydroxyacetophenone (SigmaAldrich), 560 mM isopropylamine (SigmaAldrich), transaminase (1.0 mg/ml, 0.97 U, purified TA-S9 from Cambrex, or 2 mg/ml, 0.90 U ATA-113 from Codexis). Equilibrium displacement was done by adding 600 U/ml yeast alcohol dehydrogenase (SigmaAldrich), 0.2 U/ml formate dehydrogenase (SigmaAldrich), 0.2 mM NADH (SigmaAldrich), 25 mM sodium formate (SigmaAldrich). The unit definition of the yeast alcohol dehydrogenase refers to the activity towards ethanol. For acetone the activity is lower, therefore the apparent excess is a balanced amount. The equilibrium displaced reaction was compared with a non displaced reaction with the same concentrations of amine acceptor and donor, as well as transaminase.

Determination of Kinetic Constants

The reaction rates were measured spectrophotometrically at various substrate concentrations by the consumption of acetophenone at 285 nm. The choice of wavelength was based on the observation that the molar extinction was 1.0 mM⁻¹cm⁻¹ which enables measurement within the acceptable range. All continuous measurements were done on a dual beam spectrophotometer (Cary 300 UV-Vis, Varian Inc.) with appropriate blank correction. The molar absorbance of the formed acetone is negligible.

Determination of Conversion and Enantiomeric Excess

The samples were subjected to high pressure liquid chromatography (HPLC) with a CrownpakCR(+) column (Daicel), after filtration (0.22 μ m) and acidification by perchloric acid. Ultrapure water with addition of perchloric acid to pH 1.6 was used as mobile phase. Retention times: 1-phenylethylamine at flow rate 0.3 ml/min: 41 min (S), 55 min (R); 1-(4-methylphenyl)-ethylamine at flow rate 0.4 ml/min: 45 min (S), 56 min (R); 1-(4-nitrophenyl)ethylamine at flow rate 0.4 ml/min: 44 min (S), 55 min (R); 1-(2,4-dihydroxyphenyl)ethylamine at flow rate 0.3 ml/min: 78 min (S), 89 min (R). All analysed at 254 nm. A standard curve was made for determination of formed product in the cases of 1-phenylethylamine and 1-(4-nitrophenyl)-ethylamine, with 40 μ L injection loop and 1:10 dilution the curve was linear with a coefficient of determination of 0.9997.