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Supporting Information

Structural Insights into Substrate Specificity and Solvent Tolerance in Alcohol Dehydrogenase ADH-'A' from *Rhodococcus ruber* DSM 44541

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S1. Crystallisation, data collection and refinement

Alcohol dehydrogenase ADH-'A' was purified after over-expression in *E. coli* and disruption using ultrasonication (Branson Digital Sonifier, 102C, cells of 250 mL LB-Amp medium, 50 ml Tris/HCl-buffer, 50 mM, pH 7.5) by heat treatment (65 °C for 25 min), ion exchange chromatography (DEAE, diethylaminoethyl cellulose, Sigma, dm 26 mm, 6 cm height), and hydrophobic interaction chromatography (phenyl-sepharose) using procedures similar as described previously.[1] After concentrating to 10 mg mL^{-1}. NAD^+ was then added to a final concentration of 4 mM. Crystals of ADH-'A' were grown under two different conditions using the vapour batch method and the hanging-drop diffusion method respectively. The crystallisation conditions were: At pH 5.5: 100 mM BisTris pH 5.5, 17% (w/v) PEG MW 10000, 100mM (NH_4)-acetate; at pH 8.5: 100 mM BisTris propane buffer pH 8.5, 20% (w/v) PEG MW 4000, 5 mM $CdCl_2$, 6% (v/v) 2-propanol and 5% (v/v) 1, 4-butanediol. In both cases, crystallisation drops consisted of 1 µL of reservoir solution mixed with 1 µL of enzyme solution. Data were collected on beamline X06DA at the Swiss Light Source (SLS), Villingen, Switzerland on the 15th July 2009 at a wavelength of 1.0815 Å (pH 5.5) and at beamline i03 at the Diamond Synchrotron Source, Oxford, U.K. on the 10th September 2009 at a wavelength of 0.9789 Å (pH 8.5) (manuscript text, Table 1). Each dataset was collected at a temperature of 120K. Data were integrated and scaled using the XDS and HKL2000 suites of programs.[2,3] The pH 5.5 structure was solved by molecular replacement using the higher resolution SLS-dataset. The automatic molecular replacement pipeline BALBES[4] yielded a first model based on known structures of Zn-dependent ADHs (PDB-codes: 2EER, 1JVB, 2H6E, 1RJW and 2HCY), which was completed and refined using Arp/wArp.[5]

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The pH 8.5 structure was solved by isomorphous replacement using the coordinates of the pH 5.5 structure of ADH-'A'. Both structures were further refined using the programs Coot,[6] Refmac[7] and Phenix[8] respectively. After refinement of the protein, NAD^+ and solvent, examination of difference maps revealed clear unmodelled density associated with the catalytic zinc in both structures. In the pH 5.5 structures, the density features were interpreted as originating from a Zn-bound acetate ion and a (4*S*)-2-methyl-2,4-pentanediol molecule. In the pH 8.5 structure, the crystallization additive 1,4-butanediol was modelled and refined into this density. The structures were finally refined to R_{cryst}/R_{free}-values of 0.159/0.206 (pH 5.5) and 0.188/0.258 (pH 8.5) and were validated using PROCHECK.[9] The Ramachandran plot for the pH 5.5 structure had 99.5% of residues in the favoured and allowed regions with 0.5% in the "generously allowed regions" and no outliers. The corresponding plot for ADH-'A' at pH 8.5 with 1,4-butanediol bound in subunits B, C and D featured 94.8% of residues in the favoured region; 4.7% in the allowed region and 0.4% in outlier regions. The coordinates for both structures have been deposited in the Protein Databank under the accession numbers 3JV7 (pH 5.5) and 2XAA (pH 8.5) respectively.

Table S1. Data collection and refinement statistics for ADH-'A' collected on crystals grown at pH 5.5 and 8.5

	ADH-'A' pH 5.5 (3JV7)	ADH-'A' pH 8.5 (2XAA)
Wavelength (Å)	1.0815	0.9789
Resolution (Å)	40.-2.0 (2.17-2.00)	109.5-2.8 (2.87-2.80)
Space Group	$P2_1$	$P2_1$
Unit cell	a=65.82 Å, b=106.1 Å, c=109.1 Å, β=91.3°	a=65.68 Å, b=105.10 Å, c=109.18 Å, β=91.3°
Unique reflections	99493 (21640)	34356 (2238)
No. of water molecules	1184	148
Completeness (%)	98.4 (98.9)	98.7 (87.3)
Multiplicity	3.7 (3.7)	3.6 (3.1)
R_{sym} (%)	0.075 (0.334)	0.10 (0.36)
$<I/\sigma(I)>$	11.9 (3.6)	7.5 (2.0)
R_{cryst}/ R_{free}	0.159/0.206	0.188/0.258
r.m.s.d 1-2 bonds (Å)	0.016	0.014
r.m.s.d 1-3 angles (Å)	1.1	1.6
Average B (Å^2)	30	40
Ligand average B (Å^2)	28 (acetate) 34 (MPD)	42 (butane-1,4-diol)

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S2. Modelling

A molecular model of 2-octanone was docked into the low-pH structure of ADH-'A' using AutoDock 4.0[10] as implemented in YASARA Structure.[11,12] All water molecules and ligands (except the NAD^+ cofactor and the Zn ions) were removed from the structure. The cofactor was modelled as NADH. The charge of the catalytic Zn-ion was set to +2, the ligating sidechain of Cys-28 was treated as deprotonated and negatively charged. The protonation and tautomerisation states of His-residues were chosen according to their hydrogen bonding interactions, while Asp-, Glu-, Arg- and Lys-residues were treated as charged. The model of the substrate was built and optimized using YASARA, and partial atomic charges were calculated using the AM1-BCC protocol.[13] During docking the position, orientation and torsion angles of the ligand were allowed to vary, while the protein was kept rigid. The search was restricted by a 15 $Å^3$ box around the C4 atom of the NADH. Twenty independent simulations were performed employing a genetic algorithm (population size 150, number of generations 20000). The lowest energy structures of each independent run were clustered with an r.m.s-tolerance of 2.0 Å. The docking mode with the overall lowest energy was subjected to an additional molecular mechanics optimization in YASARA.

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Comparative Analysis of Structures of ADH-'A' (3JV7) and Tb-ADH (1YKF) using PISA[14]

	ADH-A (3JV7)	TbADH (1YKF)
Sequence identity	22%	
R.M.S.D. (all Cαs, Å)	2.5 (DALI score of 36.0)	
No. amino acids (in tetramer)	345 (x 4)	352 (x 4)
% surface accessible area non-polar residues (in intact tetramer)*	53%	50%
Surface area contact A-B /C-D dimer interfaces (Å^2)**	1404/1388	1793/1860
No. interfacial H-bonds between A-B or C-D dimers **	15	30
No. interfacial salt-bridges between A-B or C-D dimers **	10	0

Table S2 . * - calculated using the program ccp4mg.[15]; ** calculated using the program PISA.[14]. Owing to the symmetry of the oligomers in 1YKF and ADH-'A', interfaces A-B and C-D are equivalent.

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