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Supporting information for article:

The structure of an iron-containing alcohol dehydrogenase from a hyperthermophilic archaeon in two chemical states

Steven B. Larson, Jesse A. Jones and Alexander McPherson

S1. Experimental

Cα atom superpositioning was performed in Coot (Emsley & Cowtan, 2004) using the LSQ algorithm for the three *T. thioreducens* models presented here. Superpositionings of the *T. thioreducens* models with respect to the *T. maritima* structure were also performed in Coot but by the secondary structure matching algorithm to identify corresponding Cα atoms. The structures were then renumbered accordingly and least-squares fitting of Cα atoms for model combinations were calculated in *CNS* (Brünger *et al.*, 1998).

For the five *T. thioreducens* models, the results indicate that the B domains are fairly closely matched with an average rms deviation of 0.34 Å versus 0.78 Å for the A domains (Table 3C). To look at the possible changes in the models when NADP and iron bind in the coenzyme binding pocket in the molecules, the B domains of the various models were superimpose by Cα atom fitting and then the rms deviations and rotation angles between the A domains were calculated using *CNS*.

Table S1 Structural comparisons by Cα atom superposition of various molecular components.

6C75, 6C76, 6C7L and 1O2D are identified in Table 2 and the A or B following refer to chain A or B. Superpositioning calculations are described in Supporting Information.

Part A: RMS deviations (Å) of Cα atoms after Cα atom superposition

	378 Cα atoms					341 Cα atoms	
	6C75-A	6C76-A	6C75-B	6C76-B	6C7L-A	1O2D-A	1O2D-B
	RMS deviations						
6C75-A		0.27	1.02	1.00	1.29	1.68	1.77
6C76-A	0.97		1.09	1.04	1.33	1.69	1.79
6C75-B	5.36	5.47		0.43	0.60	1.76	1.77
6C76-B	5.71	5.88	1.49		0.65	1.79	1.82
6C7L-A	6.85	6.96	2.87	3.62		1.83	1.83
	Maximum deviations						

Part B: RMS deviations (Å) of Cα atoms after Cα atom superposition of ADH dimers

		RMS deviations between dimers			
	6C75	6C76	6C7L	1O2D	aligned aa
6C75		0.42	1.06	2.01	675
6C76	1.59		1.11	1.93	676
6C7L	6.88	7.19		2.08	675
	Max deviations				

Part C: RMS deviations (Å) of C_α atoms after C_α atom superposition of ADH domains A or B

	RMSDs of 182 C_α atoms in Domain A after LSQ C_α fit				
	6C75-A	6C76-A	6C75-B	6C76-B	6C7L-A
6C75-A		0.27	0.90	1.01	1.08
6C76-A	0.20		0.93	1.00	1.08
6C75-B	0.32	0.30		0.41	0.53
6C76-B	0.28	0.26	0.29		0.63
6C7L-A	0.46	0.45	0.40	0.43	
	RMSDs of 196 C_α atoms in Domain B after LSQ C_α fit				

Part D: RMS deviations (Å) of A domains after superposition of B domains

	6C75-A	6C76-A	6C75-B	6C76-B	6C7L-A
6C75-A		0.38	1.96	1.79	2.40
6C76-A			2.06	1.83	2.45
6C75-B				0.77	0.84
6C76-B					1.14

Table S2 Interatomic distances between the residues of FeADH and NADP or ATR.

			Interatomic Distances			
NADP/ATR atom	ADH residue and atom		Monoclinic Chain A	Monoclinic Chain B	Orthorhombic Chain A	Tetragonal Chain A
O7N	S148	OG	3.09	-	2.89	-
N7N	S145	OG	3.69	-	3.43	-
N7N	D96	OD1	3.51	-	2.87	-
N7N	A150	O	3.41	-	3.00	-
O2D	K159	NZ	3.44	-	2.95	-
O3D	K159	NZ	3.43	-	3.05	-
O1N	G92	N	2.87	2.80	2.84	2.78
O1N	T140	OG1	2.55	2.62	2.47	2.66
O2A	S93	N	3.04	3.13	3.02	3.46
O1A	S93	OG	2.68	4.01	2.71	3.74
O2A	S93	OG	3.30	3.27	3.38	2.75
O2X	R38	NH2	3.32	3.42	2.97	2.86
O3X	S35	N	2.83	2.72	2.84	3.33
O3X	S35	OG	2.61	2.53	2.65	2.61
N1A	T181	OG1	2.71	2.88	2.69	2.80
N6A	S139	O	3.39	3.49	3.24	3.54
N6A	L178	O	2.99	2.98	2.71	3.15
N7A	S139	OG	3.15	3.29	2.92	3.28

Table S3 Iron coordination in alcohol dehydrogenases from *T. thioreducens* and *T. maritima*..

	Monoclinic ADH	Orthorhombic ADH	1O2D (average)
Axial bonds	(Å)	(Å)	(Å)
Fe···H197-NE2	2.14	2.49	2.30
Fe···H272-NE2	2.54	2.44	2.19
Equatorial bonds/contacts			
Fe···H260-NE2	2.23	2.37	2.14
Fe···D193-OD1	2.38	2.50	2.14
Fe···NADP-HC5N	1.81	1.90	-
Fe···NADP-C5N	2.87	2.93	2.50
Axial angles around Fe	(°)	(°)	(°)
H197-NE2···H272-NE2	165.81	171.81	176.24
H197-NE2···H260-NE2	84.50	95.42	91.19
H197-NE2···D193-OD1	98.52	97.92	89.44
H197-NE2···NADP-HC5N	89.59	75.70	-
H197-NE2···NADP-C5N	94.01	83.63	88.05
H272-NE2···H260-NE2	83.03	86.06	89.64
H272-NE2···D193-OD1	91.38	90.19	87.62
H272-NE2···NADP-HC5N	88.49	97.07	-
H272-NE2···NADP-C5N	84.57	89.28	93.63
Equatorial angles around Fe			
H260-NE2···D193-OD1	105.74	87.81	108.58
H260-NE2···NADP-HC5N	110.62	128.51	-
H260-NE2···NADP-C5N	112.55	130.30	129.88
D193-OD1···NADP-HC5N	143.35	143.25	-
D193-OD1···NADP-C5N	140.63	141.73	121.48

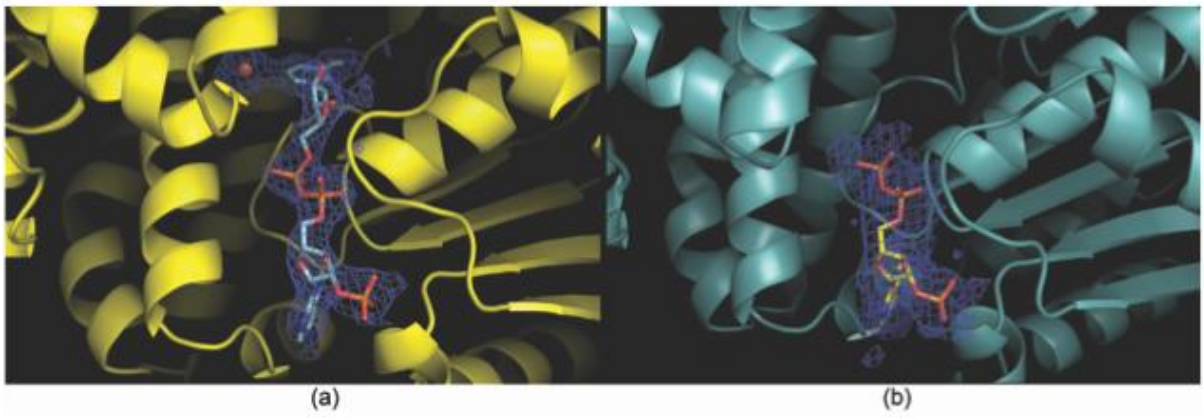

Figure S1 In (*a*) is the NADP and iron bound to chain A of the monoclinic cell of iron-containing alcohol dehydrogenase (FeADH) from *Thermococcus thioreducens* with $2F_o$-F_c electron density contoured at 1.0 σ surperposed. In (*b*) is chain B of the same cell with 2'-monophosphoadenosine-5'-diphosphate (ATR) modeled at 75% occupancy included. Superposed on ATR is the $2F_o$-F_c electron density contoured at 0.5 σ.

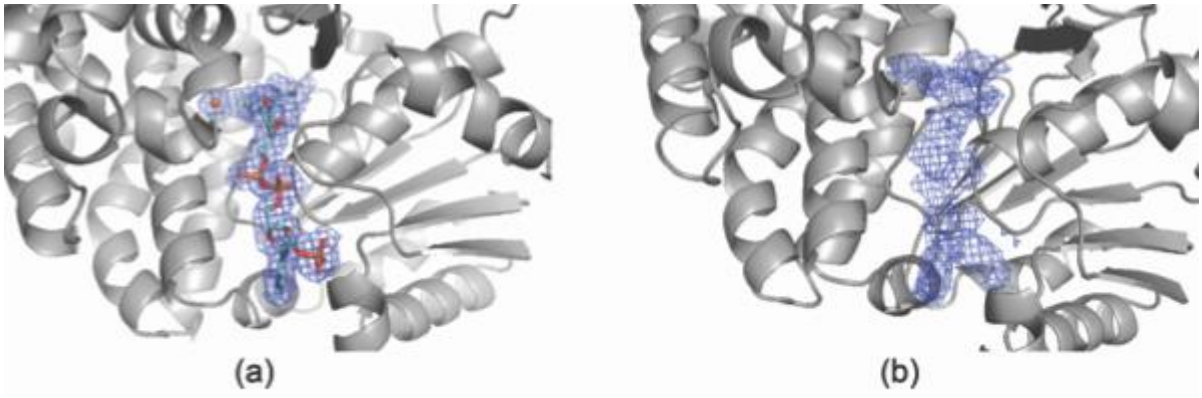

Figure S2 In (*a*) is the NADP and iron bound to chain A of the orthorhombic cell of FeADH from *Thermococcus thioreducens* with $2F_o$-F_c electron density contoured at 1.0 σ surperposed. In (*b*) is chain B of the same cell with $2F_o$-F_c electron density contoured at 0.15 σ. It is apparent that chain B also contains either ATR or NADP in the binding pocket but at a much-reduced occupancy for which cause no coenzyme was modeled.

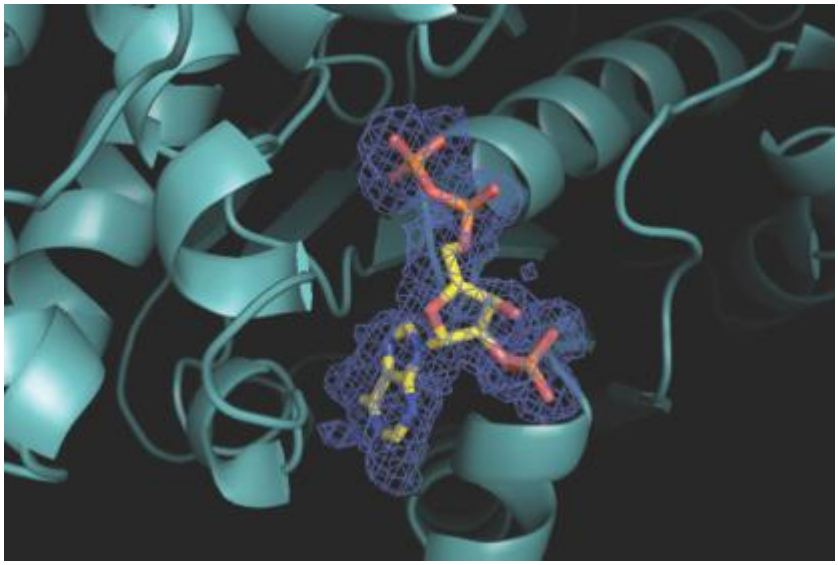

Figure S3 FeADH from the tetragonal cell is shown with ATR modeled at 60% occupancy included. Superposed on ATR is the $2F_o$-F_c electron density contoured at 0.5 σ.

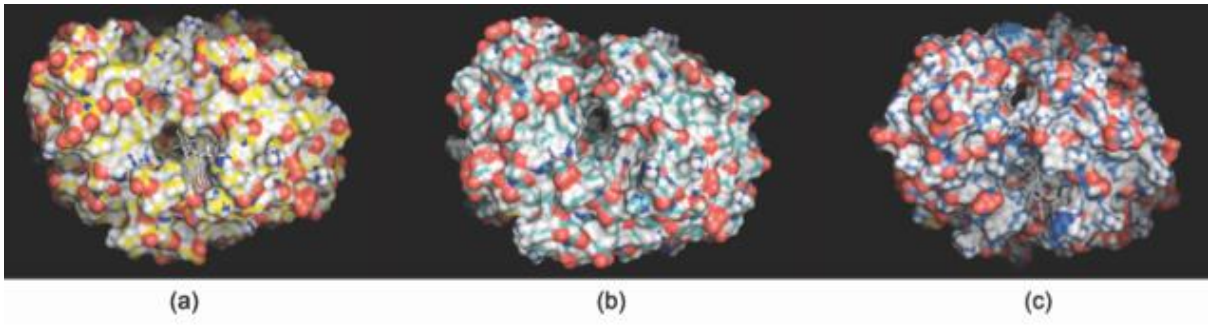

Figure S4 The B domains of the B chains of the (*a*) monoclinic and (*b*) orthorhombic structures and the (*c*) tetragonal structure were superposed on the B domain of chain A of the orthorhombic structure (in dark blue lines). The B domains, on the left side, superpose well with rms deviations of 0.30, 0.26 and 0.43 Å, respectively. The rms deviations in the A domains, as displayed here, are 2.06, 1.83, and 2.45 Å (Table 3D) and the difference in A domain positions represent rotations of 5.7°, 5.4°, and 8.1°, respectively. It is reported that in liver alcohol dehydrogenase, a rotation of 10° occurs when the apoenzyme binds NAD^+ (Hammes-Schiffer and Benkovic, 2006).

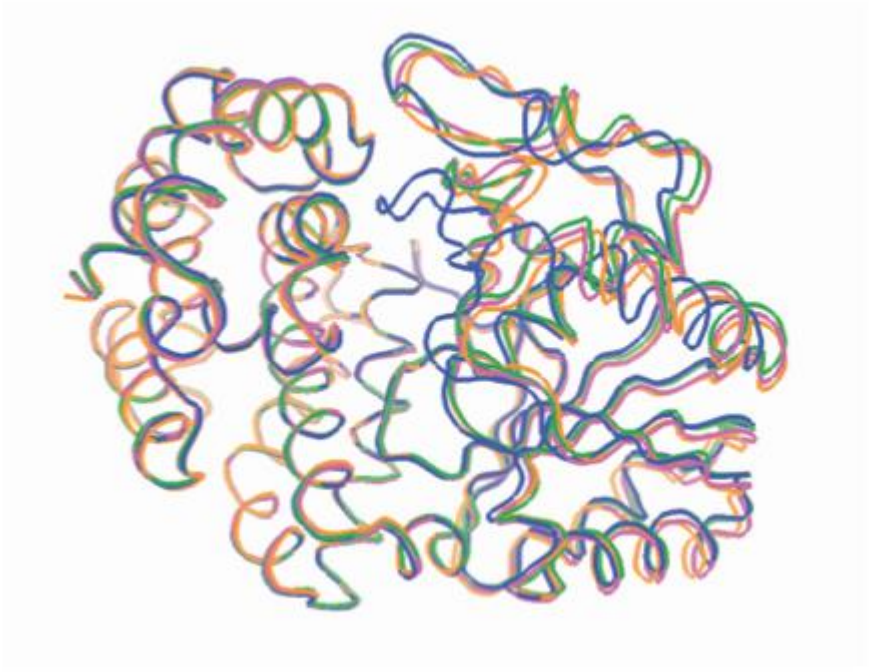

Figure S5 Shown are the (*a*) A chain and (*b*) B chain of the orthorhombic crystal form and (*c*) the enzyme of the tetragonal crystal form of FeADH from *T. thioreducens* illustrating the more open conformation of the non-NADP bound enzymes in (*b*) and (*c*) versus the NADP-bound enzyme in (*a*)

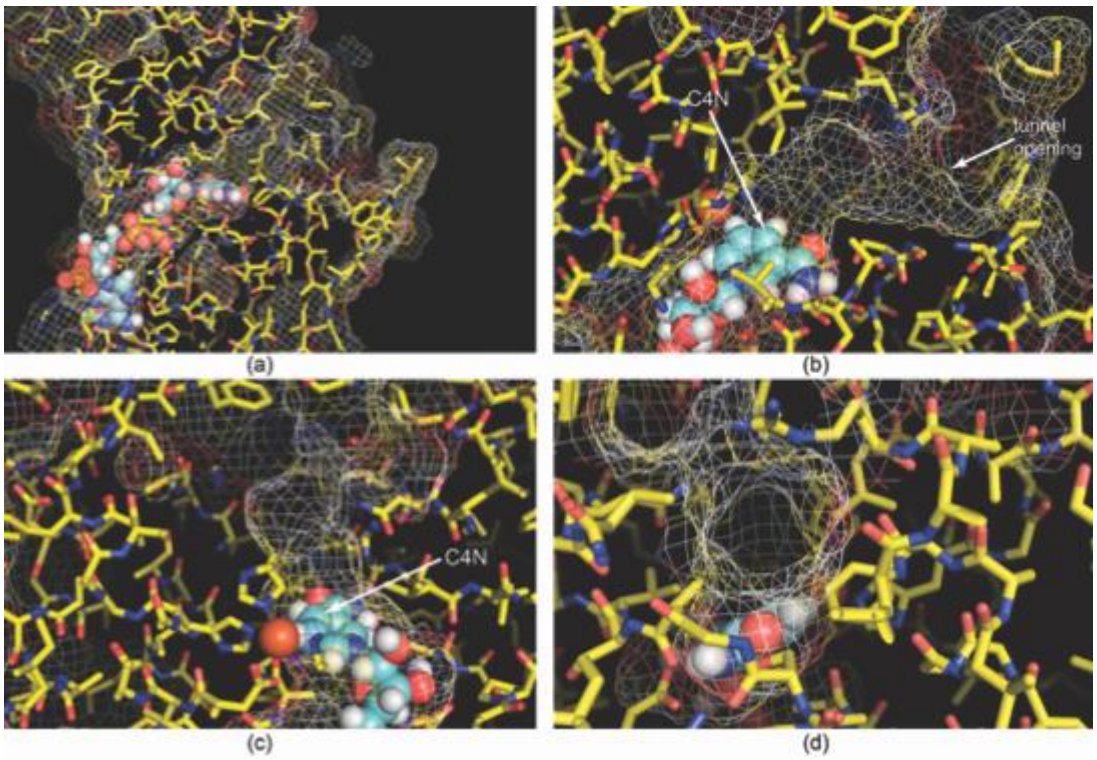

Figure S6 The surface of the A chain of the enzyme in the orthorhombic structure is displayed as a mesh for visualizing the interior of the active site tunnel. The iron and NADP are shown as space-filling spheres. In (*a*) the iron and NADP fill the tunnel from the adenine end to the nicotinamide ring

near the iron. In (*b*) it can be seen that there is a cavity extending from above the nicotinamide ring to the tunnel opening. Atom C4N of the nicotinamide ring is identified in (*b*) and (*c*). This atom is proposed as the atom that accepts the hydride H atom from the substrate and it lies at the bottom of the cavity. In (*c*) and (*d*) are two more views of the cavity, (*d*) being a view into the cavity from outside the tunnel opening identified in (*b*) which is about 4 Å in diameter.

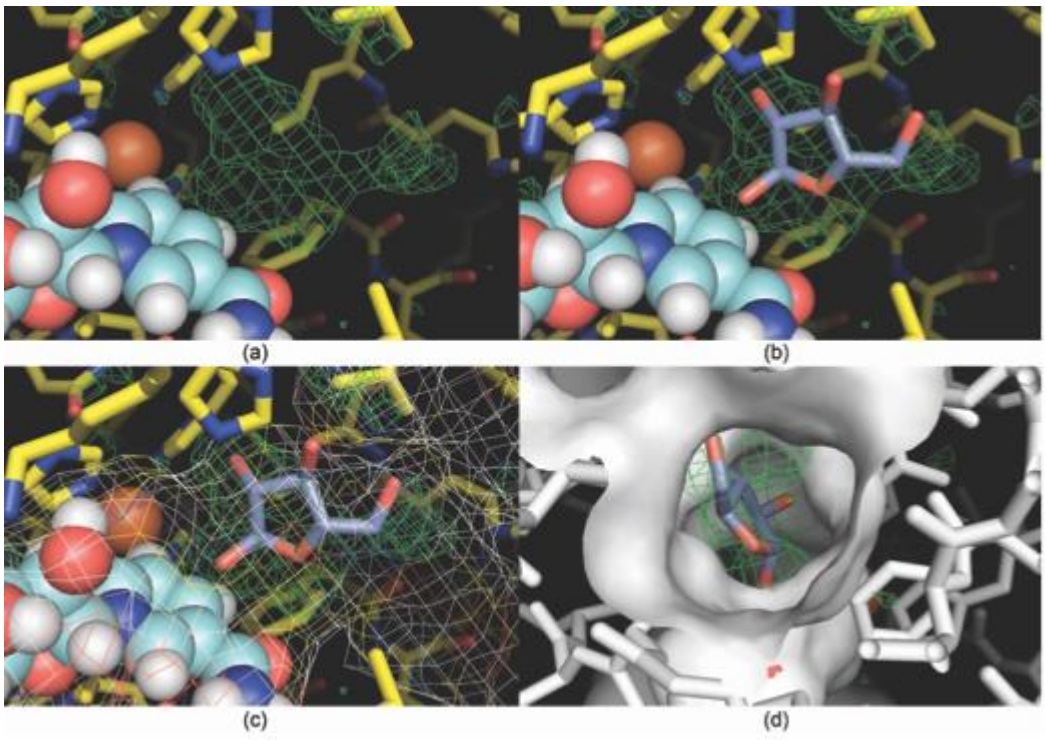

Figure S7 Some residual F_o-F_c density is located above C4N of the nicotinamide ring in the orthorhombic structure as seen in (*a*). Since it is not known what this density is, it was not modeled. However, a ribose and a glucose were loosely fitted into the density which appears to be a ringed structure. The ribose is shown in (*b*)-(*d*) along with the density. In (*c*), the surface mesh is added to show that the density and the ribose molecule fit well within the cavity of the tunnel between the nicotinamide ring and the end of the tunnel. In (*d*) the ribose is in the cavity in the same view as Figure S6(*d*). In each part, the difference map is contoured at 2.5 σ.

References

Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54,** 905-921.

Emsley, P. & Cowtan, K. (2004). *Acta Cryst.* D**60**, 2126-2132.

Hammes-Schiffer, S. & Benkovic, S. (2006). *Annu. Rev. Biochem.* **75**, 519-531.

iXpressGenes

Iron-containing Alcohol Dehydrogenase

Catalogue:186
Lot number: 20130319
Source organism: *Thermococcus thioreducens*
Recombinant protein expressed in *E. coli.*

Number amino acids: 378
Amino Acid Sequence:
MFWLKTRIIEGEGSLSRLSREVKGHERVLILASGSMKRHGFLSEAEDYVKEAGAEVFSIA
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EPKPEEIKELYLKAYRGE
Molecular weight: 44.5 KDa
Theoretical pI: 6.36
Extinction coefficient: 0.740

Form: Liquid
Buffer: 50mM HEPES, pH 7.5, 50mM NaCl
Amount: 1X 7mL (conc= 7.8mg/mL)
Total: 55mg

Biological Description:

Alcohol dehydrogenases facilitate oxidation/reduction reactions to interconvert alcohols to the related aldehyde or ketone. The redox reaction is balanced by coenzyme NAD^+ or $NADP^+$. In multicellular organisms, alcohol dehydrogenase functions mainly to oxidize toxic alcohols for removal. However, in unicellular organisms, the alcohol dehydrogenase reduce aldehydes or ketones in a process known as fermentation to provide sources NAD^+ or $NADP^+$ for energy production. Iron-containing alcohol dehydrogenases (Fe-ADH) are typically oxygen-sensitive and are prevalent in the *Thermococcus* family of anaerobic hyperthermophilic archaeons. A single point mutation in oxygen-sensitive *E.coli* proteins has been shown to reduce oxygen-sensitivity. However, oxygen resistance seems to be associated with loss of thermal stability. Currently it is unknown how oxygen inactivates the enzyme or how point mutations confer oxygen resistance. Closely related Fe-ADH from *Thermococcus* strain ES1 showed the highest activity against 1-pentanol and then 1-butanol using $NADP^+$ as the coenzyme.

Iron-containing Alcohol Dehydrogenase
Lab Code: 186
Last Update: 2012-10-03
Source organism: *Thermococcus thioreducens*
Gene identifier code (Artemis): COG1454

BLAST (standard protein) Top three:

Max identity	Species name	Accession code	Protein name
91%	*Thermococcus sp. 4557*	YP 004761647.1	Fe-containing ADH
88%	*Thermococcus onnurimeus*	YP 00230731.1	Fe-containing ADH
85%	*Thermococcus kodakarensis*	YP 183421.1	Fe-containing ADH

Properties from Protparam http://web.expasy.org/protparam/

Number amino acids: 378
Molecular weight: 41.52 kD
Theoretical pI: 6.36
Abs (0.1%): 0.740

Biological Relevance:

Alcohol dehydrogenases facilitate oxidation/reduction reactions to interconvert alcohols to the related aldehyde or ketone. The redox reaction is balanced by coenzyme NAD^+ or $NADP^+$. In multicellular organisms, alcohol dehydrogenase functions mainly to oxidize toxic alcohols for removal. However, in unicellular organisms, the alcohol dehydrogenase reduce aldehydes or ketones in a process known as fermentation to provide sources NAD^+ or $NADP^+$ for energy production. Iron-containing alcohol dehydrogenases (Fe-ADH) are typically oxygen-sensitive and are prevalent in the *Thermococcus* family of anaerobic hyperthermophilic archaeons. A single point mutation in oxygen-sensitive *E.coli* proteins has been shown to reduce oxygen-sensitivity (Holland-Staley et al. 2000). However, oxygen resistance seems to be associated with loss of thermal stability (Lu et al. 1998). Currently it is unknown how oxygen inactivates the enzyme or how point mutations confer oxygen resistance. Closely related Fe-ADH from *Thermococcus* strain ES1 showed the highest activity against 1-pentanol and then 1-butanol using $NADP^+$ as the coenzyme (Ying et al. 2009).

Sources:
Holland-Staley CA, Lee K, Clark DP, Cunningham PR (2000) Aerobic activity of *Excherichia coli* alcohol dehydrogenase is determined by a single amino acid. J Bacteriol 182:6049-6054

Lu Z, Cabiscol E, Obradors N, Tamarit J, Ros J, Aguilar J Lin ECC (1998) Evolution of an *Escherichia coli* protein with increased resistance to oxidative stress. J Biol Chem 273:8308-8316

Ying X, Grunden AM, Nie L, Adams MWW, Ma K (2009) Molecular characterization of the recombinant iron-containing alcohol dehydrogenase from the hyperthermophilic Archaeon *Thermococus* strain ES1. Extremophiles 13:299-311.

GENE SEQUENCE

LOCUS CP015105 2065932 bp DNA circular BCT 05-JUL-2017
DEFINITION Thermococcus thioreducens strain OGL-20P, complete genome.
ACCESSION CP015105
VERSION CP015105.1
DBLINK BioProject: PRJNA274230
BioSample: SAMN03324168
KEYWORDS .
SOURCE Thermococcus thioreducens
ORGANISM Thermococcus thioreducens
Archaea; Euryarchaeota; Thermococci; Thermococcales;
Thermococcaceae; Thermococcus.
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