

# Amino Acid Residues Critical for the Specificity for Betaine Aldehyde of Plant ALDH10 Enzymes Involved in the Response to Osmotic Stress<sup>1</sup>

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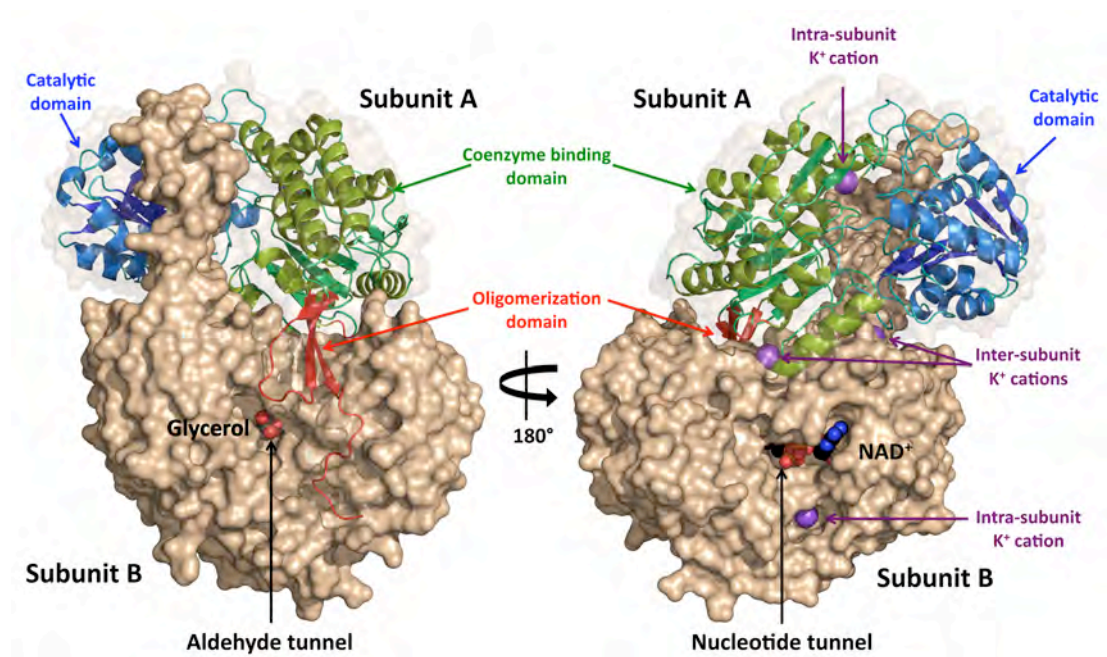
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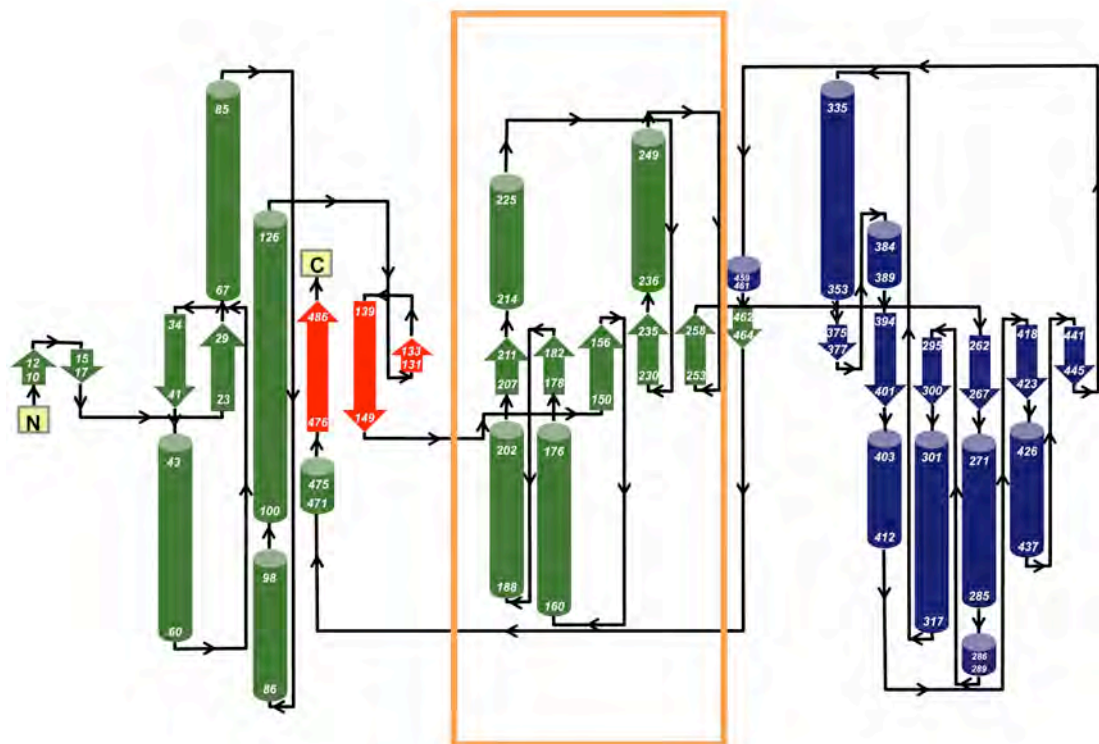
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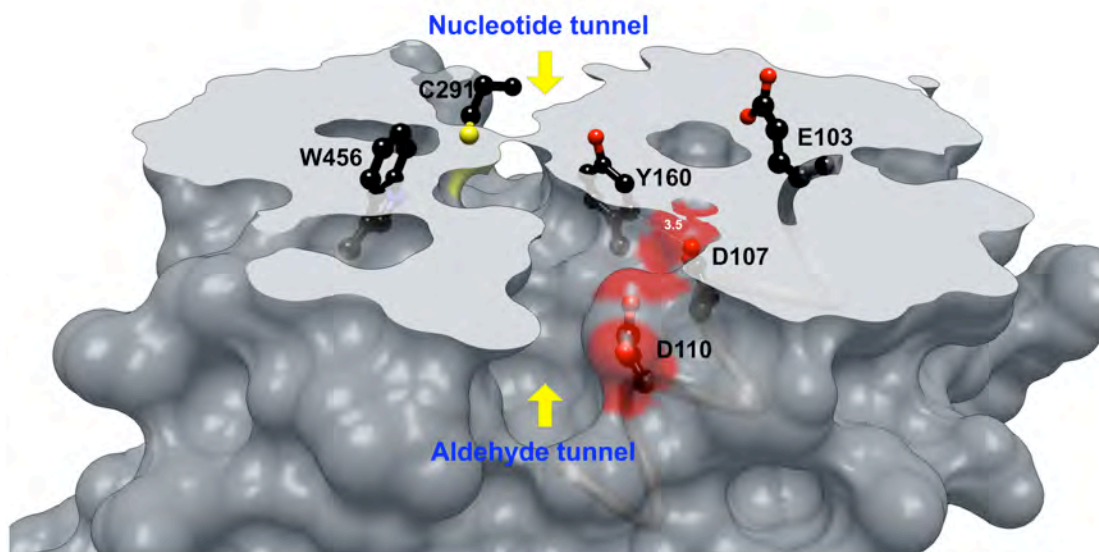
## SUPPLEMENTAL FIGURES



**Supplemental Figure S1.** Fold and secondary structure elements of the dimeric SoBADH. The two views of the molecule, resulting from a rotation of 180°, show the aldehyde or nucleotide tunnel entrances, which are in the opposite face of each monomer. In the middle section of the aldehyde tunnel there is a glycerol molecule, whereas the NAD<sup>+</sup> molecule occupies its normal position in the nucleotide-binding site. Subunits are depicted as surface representation in light brown. Subunit A shows the secondary structure elements as cartoons with the coenzyme binding domain in two shades of green, the catalytic domain in two shades of blue, and the oligomerization domain in red. The K<sup>+</sup> ions found in the intra-subunit and inter-subunit monovalent-cation-binding sites are shown as purple balls. The glycerol and NAD<sup>+</sup> molecules are represented as spheres using atom-code colors (carbon black, oxygen red, and nitrogen blue). The figure was generated with PyMOL (<http://www.pymol.org/>).



**Supplemental Figure S2.** Topology diagram of the SoBADH subunit. Domains are shown using the same color code as in Supplemental Fig. S1. The Rossmann-type fold is enclosed in an orange rectangle. The figure was prepared using PDBsum (Laskowski RA, Chistyakov VV, Thornton JM (2005) PDBsum more: new summaries and analyses of the known 3D structures of proteins and nucleic acids. *Nucleic Acids Res* **33**: D266-D268).



**Supplemental Figure S3.** Section of a SoBADH monomer showing the aldehyde tunnel and the position of Glu-103, Asp-107, and Asp-110. The side-chain of Glu-103 is far from the tunnel. The side-chain of Asp-107 is at 3.5 Å from Tyr-160, as indicated by the dashed line. Amino acid side-chains are shown as balls and sticks with carbon atoms in black, oxygen in red, nitrogen in blue, and sulfur in yellow. Image was generated using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, CA (Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF Chimera—a visualization system for exploratory research and analysis. *Comput Chem* **13**: 1605-1612).

## SUPPLEMENTAL TABLES

**Supplemental Table S1.** *Data collection and refinement statistics for the SoBADH crystal*

Enzyme	SoBADH-NAD <sup>+</sup>
<u>Data collection<sup>a</sup></u>	
Space group	P1
Unit cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	69.4, 80.9, 85.5
$\alpha$ , $\beta$ , $\gamma$ (°)	79.1, 84.9, 77.9
Wavelength	0.9795
Asymmetric unit	two dimers
Resolution range (Å)	30.0-2.3 (2.4-2.3)
<i>R</i> <sub>merge</sub> (%)	4.1 (38.6)
<i>I</i> / $\sigma$ ( <i>I</i> )	10.8 (2.2)
Completeness (%)	93.2 (81.1)
Redundancy	2.3 (2.2)
Unique reflections	76,619 (2,428)
<u>Refinement</u>	
Resolution range (Å)	30.0 – 2.3
<i>R</i> <sub>work</sub> / <i>R</i> <sub>free</sub> (%)	21.3/24.6
Number of atoms	
Protein	15,382
Ligand (NAD <sup>+</sup> /K <sup>+</sup> )	176/8
Water	524
Glycerol	42
Average <i>B</i> -values (Å <sup>2</sup> )	
Protein atoms	55.5
Water atoms	47.7
Ligand atoms	51.7
Root mean square deviation from ideal stereochemistry	
Bond lengths (Å)	0.012
Bond angles (°)	1.35
Ramachandran plot	
Favored (%)	91.8
Additional allowed (%)	5.6
Disallowed (%)	2.6

<sup>a</sup>The values within parentheses correspond to the highest resolution shell.

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**Supplemental Table S2.** *Kinetic parameters of wild-type (WT) and mutant SoBADH enzymes using NAD<sup>+</sup> as variable substrate*

Initial velocities were obtained at 30 °C in 50 mM HEPES-KOH buffer, pH 8.0, containing 1 mM EDTA. Betaine aldehyde (BAL) was kept fixed at the given concentrations (mM), which are at least 4-times the  $K_m$ (BAL) value for each enzyme. The kinetic parameters were estimated by non-linear regression of the experimental data to the Hill equation. S.E values were around 10 % of the estimated kinetic parameters.  $V_{max}$  and  $K_m$  values are expressed as U/mg protein and  $\mu$ M, respectively.

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ENZYMES	WT	Y160A	W285A	W167A	W456A	A441I	A441C
[BAL]	0.5	20	4	2	1	5	0.5
$V_{max}$	3.9	2.0	1.8	1.0	2.0	0.4	2.0
$K_m$	32	12	9	55	47	3	7
<i>Hill number</i>	1.0	1.0	1.0	1.8	2.1	1.0	1.0

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**Supplemental Table S3. Plant isoenzymes of the ALDH10 family**

Plant family	Plant species	Accession number	Chromosome mapping	Position441	BAL affinity	Peroxisomal signal	GB-accumulator
<b>DICOTS</b>							
<b>Amaranthaceae</b>	<i>Amaranthus hypochondriacus</i>	GI2811031	—	A	High <sup>a</sup>	No	Yes <sup>a</sup>
	<i>Atriplex centralasiatica</i>	GI2388710	—	I	—	No	Yes <sup>j</sup>
	<i>A. hortensis</i>	GI104303705	—	A	—	No	Yes <sup>k</sup>
	<i>A. micrantha</i>	GI124264139	—	A	—	No	—
	<i>A. prostrata</i>	GI20135572	—	A	—	No	Yes <sup>l</sup>
	<i>A. tatarica</i>	GI146289946	—	A	—	No	Yes <sup>m</sup>
	<i>Beta vulgaris</i>	GI118490	—	A	—	No	Yes <sup>n</sup>
	<i>Halostachys caspica</i>	GI134034500	—	A	—	No	Yes <sup>o</sup>
	<i>Haloxylon ammodendron</i>	GI247421572	—	A	—	No	—
	<i>Spinacia oleracea</i>	GI1813538	—	A	High <sup>b</sup>	No	Yes <sup>p</sup>
	<i>Suaeda liaotungensis</i>	GI17026368	—	A	—	No	Yes <sup>q</sup>
	<i>Kalidium foliatum</i>	GI115353973	—	I	—	No	Yes <sup>o</sup>
	<b>Acanthaceae</b>	<i>Avicennia marina</i>	GI11414908	—	A	High <sup>c</sup>	No
		GI11414910	—	I	High <sup>c</sup>	SKL	—
<b>Aizoaceae</b>	<i>Sesuvium portulacastrum</i>	GI341868781	—	I	—	No	—
<b>Solanaceae</b>	<i>Lycium barbarum</i>	GI237506885	—	I	—	No	No <sup>p</sup>
	<i>Solanum lycopersicum</i>	GI113205159	—	I	—	No	No <sup>s</sup>
	<i>Solanum tuberosum</i>	PGSC000DMT400063205	Chromosome III	V	—	SKL	No <sup>s</sup>
		PGSC000DMT400083025	Chromosome VI	I	—	SRN	—
<b>Asteraceae</b>	<i>Chrysanthemum lavandulifolium</i>	GI63145792	—	I	—	No	—
		GI63145794	—	I	—	No	—
	<i>Helianthus annuus</i>	GI256260278	—	I	—	SKL	No <sup>p</sup>
<b>Betulaceae</b>	<i>Corylus heterophylla</i>	GI321272538	—	I	—	No	—
<b>Araliaceae</b>	<i>Panax ginseng</i>	GI34595979	—	I	—	SKL	—
<b>Vitaceae</b>	<i>Vitis vinifera</i>	GI225452652	—	I	—	No	—
<b>Brassicaceae</b>	<i>Arabidopsis lyrata</i>	GI297819432	—	I	—	SKL	—
		GI297842237	—	I	—	No	—
	<i>A. thaliana</i>	GI15228346	Chromosome III	I	—	SKL	No <sup>s</sup>
		GI334183914	Chromosome I	I	Low <sup>d</sup>	No	No <sup>t</sup>
	<i>Brassica napus</i>	GI33943628	—	I	—	SKL	No <sup>t</sup>
	<i>Chorispora bungeana</i>	GI119336424	—	I	—	No	—
<b>Jatrophaeae</b>	<i>Jatropha curcas</i>	GI134285028	—	I	—	SKL	Yes <sup>u</sup>
<b>Malvaceae</b>	<i>Gossypium hirsutum</i>	GI40850676	—	I	—	SKL	No <sup>v</sup>
<b>Eupobiaceae</b>	<i>Ricinus communis</i>	GI255540797	—	I	—	SKL	—
<b>Saliceae</b>	<i>Populus trichocarpa</i>	GI224121626	Chromosome XIV	I	—	SKL	—
		GI224135733	Chromosome XV	I	—	AKL	No <sup>w</sup>
<b>Fabaceae</b>	<i>Glycine max</i>	GI167962545	—	I	—	SKL	—
		GI100170731	—	I	—	SRL	—
	<i>Medicago truncatula</i>	GBABE82378	—	I	—	AKL	No <sup>x</sup>
	<i>Pisum sativum</i>	GI284055630	—	I	Low <sup>e</sup>	AKL	No <sup>y</sup>
	GI284055632	—	—	I	Low <sup>e</sup>	SKL	—
<b>Cucurbitaceae</b>	<i>Cucumis melo</i>	GI341579690	—	I	—	SKL	No <sup>z</sup>

**Supplemental Table S3. Plant isoenzymes of the ALDH10 family**

Plant family	Plant species	Accession number	Chromosome mapping	Position441	BAL affinity	Peroxisomal signal	GB-accumulator	
<b>MONOCOTS</b>								
<b>Poaceae</b>	<i>Zoysia tenuifolia</i>	GI50950081	—	A	—	SKI	Yes <sup>ab</sup>	
		GI50950101	—	I	Moderate <sup>f</sup>	SKL		
	<i>Hordeum vulgare</i>	GI144228152	—	—	C	High <sup>g</sup>	No	Yes <sup>ac,s</sup>
		GI15147873	—	—	I	Low <sup>g</sup>	SKL	
	<i>H. brevisubulatum</i>	GI45479172	—	—	I	—	SKL	—
	<i>Leymus chinensis</i>	GI57635840	—	—	I	—	SKL	Yes <sup>ad</sup>
		GI57635842	—	—	C	—	No	
	<i>Sorghum bicolor</i>	GI242079177	—	Chromosome VII	C	—	SKL	Yes <sup>ae</sup>
		GI520546	—	Chromosome VI	I	—	SKL	
	<i>Triticum aestivum</i>	GI21747870	—	—	C	—	No	Yes <sup>af</sup>
	<i>Zea mays</i>	GI256355113	—	Chromosome I	C	High <sup>h</sup>	SKL	Yes <sup>ag</sup>
		GI194707306	—	Chromosome X	I	—	SKL	
	<i>Agropyron cristatum</i>	GI270282575	—	—	I	—	SKL	—
		—	—	—	—	—	—	
	<i>Oryza sativa</i>	GI125590656	—	Chromosome VIII	I	Moderate <sup>i</sup>	SKL	No <sup>ah</sup>
GI115458832		—	Chromosome IV	I	Low <sup>i</sup>	SKL		
<b>CONIPHEROPHYTA</b>								
<b>Pinaceae</b>	<i>Picea sitchensis</i>	GI116786844	—	I	—	SKL	—	

The symbol — indicates that, to the best of our knowledge, information is not available.

<sup>a</sup>Valenzuela-Soto and Muñoz-Clares, 1994. *J Plant Physiol* **143**: 145-152. <sup>b</sup>Weretilnyk and Hanson, 1989. *Arch Biochem Biophys* **271**: 56-63. <sup>c</sup>Hibino et al., 2001. *Plant Mol Biol* **45**: 353-363. <sup>d</sup>Missihoun et al., 2011. *Planta* **233**: 369-382. <sup>e</sup>Tylichová et al., 2010. *J Mol Biol* **396**: 870-882. <sup>f</sup>Oishi and Ebina, 2005. *J Plant Physiol* **162**: 1077-1086. <sup>g</sup>Fujiwara et al., 2008. *Physiol Plantarum* **134**: 22-30. <sup>h</sup>Kopěčný et al., 2011. *FEBS J* **278**: 3130-3139. <sup>i</sup>Mitsuya et al., 2009. *FEBS Lett* **583**: 3625-3629. <sup>j</sup>Yin et al., 2002. *Biochim Biophys Acta* **1577**: 452-456. <sup>k</sup>Shen et al., 2002. *Theor Appl Genet* **105**: 815-821. <sup>l</sup>Yancey et al., 1982. *Science* **217**: 1214-1222. <sup>m</sup>Tipirdamaz et al., 2006. *Environ Exp Bot* **57**: 139-153. <sup>n</sup>Hanson and Wyse, 1982. *Plant Physiol* **170**: 1191-1198. <sup>o</sup>Wang et al., 2007. *Act Bot Bor-Occ Sinica*. DOI : CNKI:SUN:DNYX.0.2007-03-015. <sup>p</sup>Weretilnyk et al., 1989. *Planta* **178**: 342-352. <sup>q</sup>Li et al., 2003. *Biotechnol Lett* **25**: 1431-1436. <sup>r</sup>Poljakoff-Mayber et al., 1987. *Aust J Plant Physiol* **14**: 341-350. <sup>s</sup>Wyn Jones and Storey, 1981. Betaines. In: Paleg LG, Aspinal D, eds, *The Physiology and Biochemistry of Drought Resistance in Plants*, Academic Press, New York, pp 171-204. <sup>t</sup>Selvaraj et al., 1995. *Phytochemistry* **38**: 1143-1146. <sup>u</sup>Zhang et al., 2008. *Plant Sci* **174**: 510-518. <sup>v</sup>DeRidder and Crafts-Brandner, 2008. *Physiol Plantarum* **134**: 430-439. <sup>w</sup>Bray et al., 1991. *Physiol Plantarum* **83**: 136-143. <sup>x</sup>Naidu et al., 1992. *Phytochemistry* **31**: 1195-1197. <sup>y</sup>Charlton et al., 2008. *Metabolomics* **4**: 312-327. <sup>z</sup>Itai and Paleg, 1982. *Plant Sci Lett* **25**: 329-335. <sup>aa</sup>Liu et al., 2010. *Open Biotechnol J* **4**: 18-25. <sup>ab</sup>Marcum, 1999. *Crop Sci* **39**: 1153-1160. <sup>ac</sup>Hitz et al., 1982. *Crop Sci* **22**: 47-54. <sup>ad</sup>Cui et al., 2008. *Chem Res Chin Univ* **24**: 204-209. <sup>ae</sup>Grieve and Maas, 1984. *Physiol Plantarum* **61**: 167-171. <sup>af</sup>McDonnell and Wyn Jones, 1988. *J Exp Bot* **39**: 421-430. <sup>ag</sup>Hitz and Hanson, 1980. *Phytochemistry* **19**: 2371-2374. <sup>ah</sup>Rathinasabapathi et al., 1993. *Crop Sci* **33**: 534-538.