Supporting Information for

Yeast Alcohol Dehydrogenase Structure and Catalysis

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This file contains text, tables and figures in the order that they are referred to the article.

Table 1S. Structural Comparisons of Domains and Subunits[a]

transformation, subunit(s)	coenzyme domain(s)	catalytic domain(s)	coenzyme and catalytic
B onto A	0.94	0.86	1.94
C onto A	0.27	0.32	0.30
D onto A	0.71	0.90	1.97
C onto B	0.96	0.86	1.97
D onto B	0.64	0.44	0.54
D onto C	0.77	0.93	2.01
CD onto AB	0.52	0.40	0.45
BA onto AB	1.02	1.50	1.04

[a]Root-mean-square deviation (Å) after superpositioning alpha carbon atoms of the residues in the subunit or dimer using the program O. The catalytic domain includes residues 1–154 and 294–347, and the coenzyme binding domain includes residues 155–293.

Table 2S. Accessible Surface Areas, Å^2, of Subunits and Oligomers[a]

subunit	area	dimer/ difference	area (buried)	tetramer/ difference	area (buried)
A	14900	AB	26900	AB:AB	49400
B	15500	A + B – AB	(3500)	2AB – AB:AB	(4400)
C	14900	CD	27200	CD:CD	50000
D	15800	C + D – CD	(3500)	2CD – CD:CD	(4400)
				AB:CD	52400
				AB + CD – AB:CD	(1700)

[a]Calculated with CCP4 ArealMol and PDBe-Pisa.

Figure 1S. Oligomeric Interactions in Yeast ADH1
Red, electrostatic; black, hydrogen bonds; brown, covalent; blue, hydrophobic. Distances in Å.

Intra-dimeric
A subunit to B subunit (interface 1515 Å^2)

101 Glu OE2, OE1 – 260 Arg NE, NH2 2.6, 2.8	260 Arg NH2 – 101 Glu OE1 3.1
279 Asp OD1 – 275 Lys NZ 2.8	240 His NE2 – 101 Glu OE2 3.2
102 Tyr OH – Wat – 261 Ala N 2.9, 2.6	261 Ala N – Wat – 102 Tyr OH 3.4, 2.6
107 Asn OD1 – Wat – 262 Asn OD1 2.6, 2.5	110 Asn OD1 – 262 Asn ND2 3.0
110 Asn ND2 – 287 Ser OG 2.8	110 Asn ND2 – 285 Val O 3.0
237 Gly O – Wat – 102 Tyr OH 3.0, 2.6	
274 Ala O – 280 Val N 2.9	280 Val N – 274 Ala O 3.0
274 Ala O – Wat – 281 Phe N 2.8, 2.6	274 Ala O – Wat – 279 Asp OD1 2.8, 2.7
276 Cys N – 278 Ser O 2.7	276 Cys O – 278 Ser N 2.9
277 Cys S – 277 Cys S 2.0	276 Cys O – Wat – 276 Cys O, 2.9, 2.7
278 Ser N – 276 Cys O 3.0	278 Ser O – 276 Cys N 2.9
283 Gln NE2 – 290 Ile O 2.9	290 Ile O – 283 Gln NE2 3.0
288 Ile O – 292 Gly N 3.0	292 Gly N – 288 Ile O 2.8
290 Ile N – 290 Ile O 2.8	290 Ile O – 290 Ile N 2.7
54 Trp CB, CG, CE3 – 281 Phe CE2 3.6, 3.6	281 Phe CE2 – 54 Trp CD1 4.0
55 Pro CG – 281 Phe CD1, CE1 3.6, 3.8	281 Phe CE2 – 270 Met CE 3.4
284 Val CG2 – 267 Leu CB 3.9	
267 Leu CD1 – 276 Cys CB 4.1	280 Val CG1 – 271 Pro CG, CD 3.2, 3.5
270 Met CB – 54 Trp CZ2 3.8	270 Met CA – 280 Val CG1 4.0
270 Met SD – 281 Phe CD2 3.7	270 Met CE – 284 Val CG1 3.7
274 Ala CB – 280 Val CB, CG2 3.9. 3.9	280 Val CG2 – 274 Ala CB 3.7
285 Val CA – 294 Tyr CG 3.9	294 Tyr CZ – 285 Val CG2 3.8

C subunit to D subunit (interface 1480 Å^2)

101 Glu OE1,OE2 – 260 Arg NE,NH2 2.9,3.4	260 Arg NE,NH2 – 101 – Glu OE2,OE1 2.8,2.7
110 Asn OD1 – 262 Asn ND2 3.1	262 Asn ND2 – 110 Asn OD1 3.2
110 Asn ND2 – 285 Val O 3.1	110 Asn ND2 – 287 Ser OG 2.8
285 Val O – 110 Asn ND2 2.9	285 Val O – Wat – 110 Asn OD1 2.8, 3.2
237 Gly O – Wat – 102 Tyr OH 3.0, 2.8	236 Asp O – Wat – 102 Tyr OH 3.3, 2.7
261 Ala N – Wat – 102 Tyr OH 2.7, 2.8	
274 Ala O – 280 Val N 3.1	280 Val N – 274 Ala O 3.1
276 Cys N – 278 Ser O 2.8	276 Cys O – 278 Ser N 2.8
277 Cys S – 277 Cys S 2.0	
278 Ser N – 276 Cys O 3.3	278 Ser O – 276 Cys N 3.1
283 Gln NE2 – 290 Ile O 3.2	290 Ile O – 283 Gln NE2 3.2
288 Ile O – 292 Gly N 2.7	292 Gly N – 288 Ile O 2.8
290 Ile N – 290 Ile O 2.8	290 Ile O – 290 Ile N 2.6
54 Trp CB, CG, CD2 – 281 Phe CE1 3.6, 3.6, 3.7	281 Phe CE2 – 54 Trp CE2, CD2 4.1, 4.1
55 Pro CG – 281 Phe CD2, CE2 3.5, 3.5	270 Met SD – 281 Phe CD1 3.8
267 Leu CB – 284 Val CG2 3.7	284 Val CG2 – 267 Leu CB 3.9
270 Met CG – 284 Val CB, CG1 4.1, 4.1	284 Val CG1 – 270 Met CG, SD 3.7, 3.7

280 Val CG2 – 274 Ala CB 3.7
280 Val CG2 – 275 Lys CA 3.8
285 Val CG2 – 294 Tyr CE1, CZ 3.9, 3.8
294 Tyr CE1, CZ – 285 Val CG2 3.8, 3.8
287 Ser CA – 292 Gly C 3.9
292 Gly C – 287 Ser CA 3.9

Interactions in the A1B1:A2B2 Tetramer

A1 to A2 subunits (642 Å^2, additional symmetry-related A2 to A1 interactions implied)

104 Glu OE1,OE2 – 302 Arg NE,NH2 3.2, 2.8
78 Asn OD1 – 80 Lys NZ 3.0
98 Met O – 298 Arg NH1 2.9
104 Glu O – Wat – 108 Glu OE2 2.6, 2.8
78 Asn CG – 78 Asn CG 3.0
101 Glu CA – 302 Arg CZ 3.7
104 Glu CD – 298 Arg CB 3.8
105 Leu CD2 – 299 Ala CB 3.8

A1 to B2 subunits (454 Å^2, additional symmetry-related A2 to B1 interactions implied)

159 Tyr OH – Wat – 169 Ala O 2.7, 2.7
169 Ala O – Wat – 159 Tyr OH 2.8, 3.0
192 Ala O – Wat – 194 Gly O 3.2, 2.7
194 Gly O – Wat – 192 Ala O 2.7, 2.5
194 Gly O – Wat – 310 Arg NH2 2.7, 3.2
310 Arg NH2 – Wat – 194 Gly O 2.7, 2.7
163 Lys CD – 168 Met CE 3.5
168 Met CE – 163 Lys CE 3.3
169 Ala CB – 193 Met CA 3.6
193 Met CA – 169 Ala CB 3.8
169 Ala CB – 307 Phe CZ 3.8
307 Phe CZ – 169 Ala CB 4.2
196 Arg CD, CZ – 310 Arg CZ, CD 3.8, 3.9
310 Arg CD, CZ – 196 Arg CZ, CD 3.4, 3.8

B1 to B2 subunits (899 Å^2, additional symmetry-related B2 to B1 interactions implied)

104 Glu OE2 – 302 Arg NH1 3.0
98 Met O – Wat – 133 Ala O 2.9, 2.9
30 Ala CB – 30 Ala CB 4.2
78 Asn CG – 78 Asn CG 3.8
81 Gly CA – 99 Ala CB 3.8
98 Met CB – 134 Val CG2 4.1
105 Leu CD2 – 299 Ala CB 3.8

Interactions in the C1D1:C2D2 Tetramer

C1 to C2 subunits (660 Å^2, additional symmetry-related C2 to C1 interactions implied)

104 Glu OE1, OE2 – 302 Arg NE, NH2 2.9, 2.9
98 Met O – 298 Arg NH1 2.8
78 Asn CG – 78 Asn CG 3.2
98 Met CE – 98 Met CE 3.8
105 Leu CD2 – 299 Ala CB 3.8

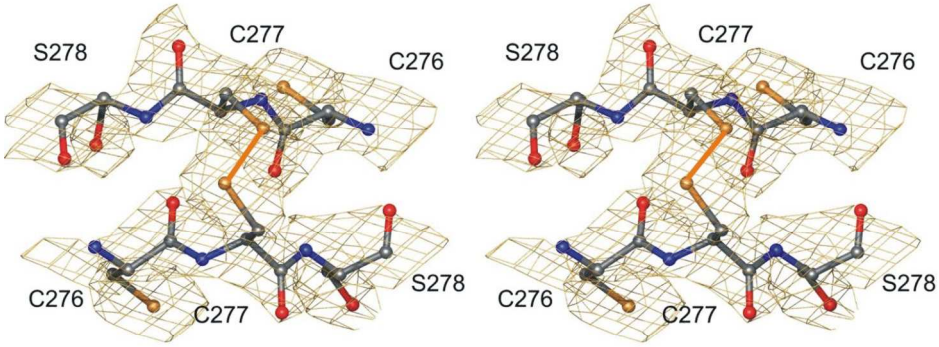
C1 to D2 subunits (442 Å^2, additional symmetry-related C2 to D1 interactions implied)

159 Tyr OH – Wat – 169 Ala O 2.5, 2.8
163 Lys CD – 168 Met CE 3.6
168 Met CE – 163 Lys CD 4.1
169 Ala CB – 193 Met CA 3.7
193 Met CA – 169 Ala CB 3.6
169 Ala CB – 307 Phe CZ 4.2
307 Phe CZ – 170 Gly CA 3.8
196 Arg CD, CZ – 310 Arg CZ, CD 4.0, 4.1
310 Arg CD, CZ – 196 Arg CZ, CD 3.3, 3.9

D1 to D2 subunits (914 Å^2, additional symmetry-related D2 to D1 interactions implied)

104 Glu OE2 – 302 Arg NH1 2.4
104 Glu OE2–Wat–Wat–91 Lys NZ 3.0,2.7,2.9
104 Glu OE2 – Wat – 298 Arg O 3.0, 2.6
104 Glu OE1–Wat–Wat–303 Glu OE2 2.7,2.7,2.9
31 Asn OD1 – 78 Asn ND2 3.1
78 Asn OD1 – Wat – 78 Asn OD1 2.9, 3.0
81 Gly O – Wat – 99 Ala O 2.8, 2.6
98 Met O – 298 Arg NH1 3.0
30 Ala CB – 30 Ala CB 4.0
81 Gly CA – 99 Ala CB 3.7
99 Ala CB – 138 His CE1 3.5
105 Leu CD2 – 299 Ala CB 4.1

Figure 2S. Disulfide bond between cysteine residues 277 in subunits A and B. The bond is also found between subunits C and D.



Sulfhydryl Groups and a Disulfide Bond in Yeast ADH1. Commercial yeast ADH is typically prepared from brewer's yeast, *Saccharomyces carlsbergensis*.[1] This ADH1 has four amino acid substitutions relative to the laboratory strain, *S. cerevisiae,* ADH1 (V58T, Q127E, Q147E and I151V), which account for the more negative charge of the commercial enzyme as compared to *S. cerevisiae* ADH1. The substitutions are not near the active site and do not affect catalytic activity significantly. A commercial preparation crystallized from ammonium sulfate, with purity (enzyme activity. active site titrations, and gel electrophoresis) similar to the preparations used for crystallography was studied for the presence of a disulfide bond.

Titration of free sulfhydryl groups with 5,5'-dithio-*bis*-(2-nitrobenzoic acid)[2] (with or without denaturation) showed about 6 free SH groups per subunit, and after treatment with dithiothreitol and desalting, 7 free SH groups were found. (The amino acid sequence contains 8 cysteine residues per subunit.) Evidence for a disulfide bond was obtained by a differential labeling protocol in which free cysteines were carboxymethylated in enzyme denatured with guanidinium chloride, followed by reducing the protein with dithiothreitol and labeling the newly formed cysteines with vinylpyridine. The modified protein had a molecular mass (Bruker Daltonics Biflex II MALDI-TOF) of 37,181 ± 14 Da, relative to an internal standard of horse liver alcohol dehydrogenase E isoenzyme (39,845 with an *N*-terminal acetyl group), which fits most closely with *S. carlsbergensis* ADH1 with 7 carboxymethylcysteines and 1 pyridylethylcysteine per molecule (predicted 37,194 Da). (The subunit mass of native *S. carlsbergenesis* ADH1 was estimated to be 36,682 Da, relative to the calculated value of 36,708, so it appears that any post-translational modifications, e.g., phosphorylation, are minimal.) Amino acid analysis found 6.1 carboxymethylcysteines per molecule, and about 1.5 pyridylethylcysteine (not determined accurately because it co-chromatographed with lysine and was estimated by difference).

The differentially-labeled protein was digested with trypsin and the peptides were separated by reverse phase HPLC and analyzed by MS. Masses for all 30 peptides expected were found, including the amino terminal *N*-acetylheptapeptide. (Sequences of peptides with masses of 288, 429, 614, 1009, 1369, and 1391 were not determined.) The peptide including residues 276 to 286 had a mass consistent with modification by one carboxymethyl group and one pyridylethyl group. Since cysteine residues 276 and 277 are in this peptide, it was not possible to determine which cysteine residue was in disulfide linkage, but the X-ray crystallography demonstrates that the bond is between Cys-277 of the two subunits that make up the dimer, labeled A and B, or C and D. It appears that the disulfide bond is present in purified and crystallized enzyme and is not affected by X-irradiation. The status of the cysteines in ADH in living yeast cells is not known. .

The effects of dithiothreitol on ADH1 are complicated. Storage of crystalline yeast ADH in ammonium sulfate leads to loss of activity, oxidation of sulfhydryl groups and dissociation to monomers, and treatment of aged enzyme with 2-mercaptoethanol or dithiothreitol can partially restore activity.[3] We find that treatment of fresh samples of *S. cerevisiae* ADH1 or older, crystallized *S. carlsbergensis* ADH1 with 4 or 18 mM dithiothreitol at pH 8 and 25 °C can increase activity transiently (in a few minutes) by 10–20%, apparently due to reduction of a disulfide bond, but activity decreases after 20 h to less than 10% of the initial activity. Perhaps dithiothreitol slowly destabilizes the enzyme by chelating zinc. ADH1 is also very susceptible to thiol reagents, as we find that 0.1 mM 5,5'-dithio-*bis*-(2-nitrobenzoic acid) or 1 mM sodium tetrathionate inactivate the enzyme in 1 day.

Structural Homology of Medium-Chain ADHs. The medium-chain ADHs are homologous, and it is of interest to identify the common core elements among the three-dimensional structures. Sequence alignments of 13 diverse dimeric and tetrameric ADHs for which three-dimensional structures have been determined (Table 3S) suggest 54–63% identities among the dimeric horse, cod, mouse and human ADHs, 37–64% identity among the tetrameric *E. coli* (BADH[9] or 4gkv.pdb), *Bacillus stearothermophilus*, *Pseudomonas aeruginosa*, and yeast ADHs, whereas five other ADHs (PDB entries 1qor, 1ped, 1e3j, 1jvb and 1h2b) were less than 39% identical to any of the other ADHs.

The structural core was determined by initial automated superpositioning followed by multiple, manual alignments, using program O[4] with the criterion that the α-carbon atoms of the structural elements were in similar conformations. Subunit A, or its two domains, of yeast ADH1 was compared to one subunit of the other structures The alignments identified 16 common core elements (15 gaps), comprising 248 amino acid residues (Table 4S). (A structural alignment of 6 tetrameric ADHs and the dimeric horse ADH identified 190 structurally equivalent residues.[5]) These common elements include the β-sheet and 4 flanking α-helices of the coenzyme binding domain, except for some connecting loops. Such similarity is expected, as the Rossmann fold is common to many divergent dehydrogenases and other enzymes,[6] even though only 4 residues (3 Gly and 1 Asp in NAD-dependent dehydrogenases) are conserved.[7] The "catalytic domains" are not conserved among all enzymes with the Rossmann fold, but for these 13 diverse, medium-chain alcohol dehydrogenases, the catalytic domains are very similar, with the notable difference that the deletion of about 21 residues in the tetrameric enzymes and rearrangements near the structural zinc site (including β-sheet (III)) have altered the structure. (See Ref. 8 for location of structural elements.) There is also a solvent-exposed β-strand on the top of a major β-sheet (I) and a short strand near the amino terminal that vary. Otherwise the differences are associated with connecting loops.

Yeast ADH1 has closest structural homology with the *E. coli* inducible (BADH[9] or 4gkv.pdb) and *P. aeruginosa* ADH structures. Superpositioning of 271 alpha carbon atoms (core regions) of the apoenzyme subunits (no coenzyme bound) of the *E. coli* ADH structure onto the apoenzyme subunits (B and D) of yeast ADH1 gives rmsd values from 1.4–1.5 Å and onto holo-subunits (A and C) of yeast ADH1 gives rmsd values from 1.6–2.1 Å. As compared to *E. coli* ADH, yeast ADH1 has an additional 5 residues at the amino terminus, an insertion at position 14, two inserted residues at position 54, one insertion each at positions 178, 225, 237 and 274, and a deletion at residue 192. As compared to *Pseudomonas* ADH, yeast ADH1 has insertions at positions 178, 225, 237, 273, 346 and 347. Although the structures of the medium chain ADHs are very similar, evolution has led to many differences that affect substrate specificity and catalysis.

Table 3S. Percent Identity of Diverse Alcohol Dehydrogenases by Sequence Alignments[a]

PDB	4dxh	1cdo	1e3i	1teh	1qor	1ped	1e3j	1jvb	1h2b	4gvk	1rjw	1llu	4w6z
4dxh	100	58	55	62	22	28	22	26	24	24	29	26	22
1cdo	58	100	55	64	18	29	23	26	26	24	29	26	24
1e3i	55	56	100	55	19	30	24	25	26	26	29	26	23
1teh	62	64	55	100	23	27	22	27	26	28	30	26	25
1qor	22	18	19	23	100	18	21	24	23	22	23	22	24
1ped	27	29	30	27	18	100	25	23	23	20	23	23	20
1e3j	22	23	24	22	21	25	100	26	22	20	27	25	27
1jvb	26	26	25	27	24	23	26	100	38	31	34	30	31
1h2b	24	26	26	26	23	23	22	38	100	32	39	38	30
4gkv	24	24	26	28	22	21	21	31	32	100	52	45	37
1rjw	29	29	29	30	23	23	27	34	39	52	100	64	43
1llu	26	27	26	26	22	23	25	30	38	45	64	100	44
4w6z	22	23	23	25	24	20	27	31	30	37	43	44	100

[a]Sequence alignments suggest 55–64% identities among the dimeric horse, cod, mouse and human ADHs (4dxh, 1cdo, 1e3i, 1teh); and 37–64% identity among tetrameric ADHs (BADH[9] or 4gkv, 1rjw, 1llu, 4w6z); whereas the other ADHs (1qor, 1ped, 1e3j, 1jvb, 1h2b) were 18–39% identical to any of the other ADHs.

Table 4S. Common Structural Elements of Diverse Medium-Chain Alcohol Dehydrogenases[a]

PDB	Domain, #residues	Cat 34	Cat 33	Cat 9	Cat 18	Coe 10
4dxh	*Equus caballus* E	21-54	61-93	144-152	155-172	174-183
1cdo	*Gadus callarias*, cod	21-54	62-94	145-153	156-173	175-184
1teh	*Homo sapiens* ADH3	21-54	62-94	145-153	156-173	175-184
1e3i	*Mus musculus* ADH2	21-54	61-93	148-156	159-176	178-187
1qor	*E. coli* quinone	16-49	57-89	93-101	104-121	123-132
1ped	*C. beijerinkii*	12-45	53-85	118-126	131-148	150-159
1e3j	*B. argentifolii* sorbitol	16-49	60-92	123-131	134-151	152-161
1jvb	*S. solfataricus*	13-46	62-94	124-132	136-152[b]	154-163
1h2b	*Aeropyrum pernix*	28-62[c]	73-105	135-143	146-166[d]	168-177
BADH	*E. coli,* inducible	12-45	52-84	115-123	126-143	145-154
1rjw	*B. stearothermophilus*	13-46	55-87	118-126	129-146	148-157
1llu	*P. aeruginosa*	19-52	61-93	124-132	135-152	154-163
4w6z	*S. cerevisiae*, ADH1	18-51	60-92	123-131	134-151	153-162

PDB	Coe 14	Coe 12	Coe 24	Coe 9	Coe 23	Coe 9	Coe 9
4dxh	186-199	201-212	219-242	250-258	261-283	287-295	300-308
1cdo	187-200	202-213	220-243	251-259	262-284	288-296	300-308
1teh	187-200	202-213	220-243	251-259	262-284	288-296	301-309
1e3i	190-203	205-216	223-246	254-262	265-287	291-299	302-310
1qor	135-148	151-162	168-191	197-205	209-230[f]	233-241	245-257[g]
1ped	161-174	176-187	194-217	223-231	235-257	260-268	273-281
1e3j	163-176	178-189	195-218	229-237	240-262	265-273	276-284
1jvb	165-178	181-192	199-222	228-236	240-262	265-273	276-284
1h2b	179-194[e]	196-207	214-237	242-250	254-276	279-287	289-297
BADH	156-169	171-182	189-212	218-226	228-250	253-261	264-272
1rjw	159-172	174-185	191-214	220-228	230-252	255-263	266-274
1llu	165-178	180-191	197-220	226-234	236-258	261-269	272-280
4w6z	164-177	180-191	197-220	227-235	238-260	263-271	275-283

PDB	Coe 9	Cat 16	Cat 13	Cat 6	% Ident	Residues in subunit aligned
4dxh	311-319	326-341	351-363	369-374	22	374, dimer
1cdo	311-319	326-341	351-363	369-374	24	374, dimer
1teh	312-320	327-342	352-364	370-375	25	373, dimer A3-A375
1e3i	313-321	328-343	353-365	371-376	23	376, dimer
1qor	258-266	278-293	303-315	322-327	24	326, dimer A2-A327
1ped	287-295	300-315	326-338	346-351	20	351, tetramer
1e3j	288-296	299-314	324-336	344-349	26	348, tetramer A4-A351
1jvb	288-296	300-315	323-335	342-347	31	347, tetramer
1h2b	301-309	313-328	335-347	354-359	30	343, tetramer, A17-A359
BADH	276-284	288-303	310-322	329-334	37	336, tetramer
1rjw	278-286	290-305	312-324	331-336	43	339, tetramer
1llu	284-292	296-311	318-330	337-342	44	341, tetramer A2-A342
4w6z	287-295	299-314	321-333	340-345	100	347, tetramer

[a]The number of residues in elements of the catalytic ("Cat") and coenzyme-binding ("Coe") domains is indicated at the top of the columns. In some cases, the domains were superimposed separately because the conformational change differs among crystal forms. The residue numbers across from the Protein Data Bank entries correspond to the residue numbers for that particular structure that are in the common elements. The alignments are based on inspection of superimposed structures, with the criterion that superimposed Cα atoms were in similar conformations. Automated alignments were used for initial superpositioning.[10] The structure for BADH was obtained from Hans Eklund.[9] "% Ident" refers to sequence alignment with yeast ADH1. (See also Table 3S.) The program PDBeFold[11] identified 15 common structural elements for 275 amino acid residues, approximately agreeing with those identified here. [b]Shorter sequence due to deletion at residue 142. [c]Insertion of one residue at position 43. [d]Addition of three residues at 156. [e]Insertion of two residues at position 181. [f]Deletion of one residue at position 218. [g]Insertion of four residues at position 246.

Zinc Content and Coordination. The determination of total zinc in yeast ADH1 has produced differing results. One per subunit (four per tetramer) was reported early on,[12,13] but another study found two per subunit.[14] Later studies with the recombinant D49N or E67Q enzymes found two zincs per subunit.[15] Treatment of commercial ADH containing 2 zincs per subunit with dithiothreitol decreases zinc content by half, presumed to be loss of the structural zinc, making the enzyme labile to heat treatment at 50 °C, but without decreasing the initial enzyme activity; incubation with 10 μM $ZnCl_2$ restores the zinc content to 2 per subunit and also increases activity of the commercial preparation, perhaps by zinc binding to the active site.[16] The differing results from various laboratories could be due to the purification and treatment of the enzyme, but preparations from two different procedures gave nearly identical results.[17] We purified recombinant yeast ADH1 by a new procedure that avoids the 5 day autolysis at room temperature and crystallization with high concentrations of ammonium sulfate, and instead uses a bead beater for disruption of wet cells, ion exchange chromatography on DEAE-Sepharose and hydrophobic interaction chromatography on Octyl-Sepharose. Specific enzyme activities are generally comparable to those for the best commercial preparations. In any case, the crystallography confirms that two zincs per subunit are present in the structure.

The coordination of the structural zincs with four cysteine residues is similar to that in dimeric horse liver ADH and some tetrameric ADHs (*E. coli*, *P. aeruginosa*, *B. stearothermophilus,* and *B. argentifolii*), but the hyperthermophilic archaeon tetrameric ADHs from *S. sofataricus* and *A. pernix* have the zinc coordinated to an aspartic acid instead of Cys-97. Two tetrameric NADP-dependent bacterial ADHs (*T. brockii* and *C. beijerinkii)* do not have structural zinc in their monomers, but the structural peptide lobe is maintained by an electrostatic interaction and a hydrogen bond.[18] Apparently the "structural zinc" lobe does not require a zinc, but the functional role of this zinc is not yet clear. The dimeric quinone oxidoreductase has no zincs, but the enzyme does bind NADPH and catalyzes reduction of some quinones.[19]

Table 5S. Coordination Distances for the Zincs

Zinc site/atoms		subunit		
catalytic	A	B	C	D
Cys-43 SG	2.3	2.5	2.3	2.3
Cys-153 SG	2.4	2.6	2.5	2.7
His-66 NE2	2.0	2.2	2.1	2.2
CF_3CH_2OH O	1.8	3.2	1.9	2.9
Glu-67 OE2	4.8	2.3	5.0	2.0
structural	A	B	C	D
Cys-97 SG	2.3	2.2	2.2	2.3
Cys-100 SG	2.3	2.3	2.2	2.3
Cys-103 SG	2.4	2.3	2.4	2.4
Cys-111 SG	2.4	2.3	2.3	2.6

Determination of the Concentration of Yeast ADH, its Molecular Weight and Stoichiometry of Coenzyme Binding. We have routinely used the extinction coefficient based on dry weight (ε_{280} = 1.26 $cm^{-1}mg^{-1}ml$)[20] and a calculated subunit molecular weight of 36,849 for a subunit with an *N*-terminal acetyl group (the initial methionine is removed in processing) and 2 zinc atoms, or a molar extinction coefficient of 185,720 $M^{-1}cm^{-1}$ and a tetramer molecular weight of 147,396. Preparations of the purified enzyme have an A_{280}/A_{260} ratio of 1.82, indicating that extraneous nucleotide is not present. We determined the protein concentration by amino acid analysis (using norleucine as an internal standard) and obtained the same ε_{280} of 1.27 $cm^{-1}mg^{-1}ml$. However, values of 1.46[3] and 1.23 ± 0.01[13] were also reported. Using the estimated extinction coefficients for tyrosine, tryptophan, and cystine in a folded protein[21], we calculate a value of 1.31. We also used the UV spectrum of the protein in 0.1 N NaOH and extinction coefficients for tyrosine and tryptophan[22] to determine the protein concentration (14 tyrosine and 5 tryptophan residues) and a calculated ε_{280} of 1.36 $cm^{-1}mg^{-1}ml$. Thus, the values vary by up to 15 %.

Molecular weights of 150,000 and 141,000 were determined by sedimentation velocity and diffusion experiments, but aged preparations also contained perhaps 10% of inactive monomeric enzyme.[3,20] These various considerations lead to uncertainties of 20% or more in determination of the concentration of active sites of the protein.

Binding of NADH gave linear Scatchard plots with 3.6 NADH binding per tetramer, and enzyme kinetics also had a linear dependence with NAD^+ or NADH (Eadie-Hofstee-Scatchard plots), providing no evidence for cooperativity in binding.[20] Later it was found that the stoichiometry was 3.0 NADH per tetramer.[17] Further work found that the tetramer could bind 2 NADH and be inactivated by reaction with 2 iodoacetate molecules, but these preparations had 4.3–5.0 zinc atoms per tetramer.[23] The stoichiometry of NADH binding was also determined by circular dichroism and a gel filtration method to be 4 per tetramer, for enzyme with 4 zinc atoms per tetramer.[13] Binding of coenzyme in binary or ternary complexes estimated by fluorescence changes gave linear Hill plots with slopes of 1.0, but stoichiometry was not determined.[24]

Classical and Alternative Coordination of the Catalytic Zinc in Alcohol Dehydrogenases. (Refer to Table 6S, following). The classical coordination was described for horse liver ADH in studies with the apoenzyme, which has a water molecule ligated to the zinc, as well as with ternary complexes with coenzyme and a substrate analogue.[8,25–27] Dimeric human ADH3 apoenzyme also has the classical coordination (1m6h.pdb), as does the abortive ternary complex with NADH and the alcohol substrate (1mc5.pdb). The classical coordination is found in ternary holoenzyme complexes of *S. solfataricus* (1r37.pdb), *A. pernix* (1h2b.pdb), and *P. aeruginosa* (1llu.pdb) ADHs. In contrast, and similar to *S. cerevisiae* ADH1, the alternative coordination is found in dimeric and tetrameric apoenzymes from *C. beijerinkii* (1ped.pdb), *S. solfataricus* (1jvb.pdb), *B. argentifolii* (1e3j.pdb), and *H. sapiens* (1p17.pdb, sorbitol dehydrogenase). Note that the coordinating glutamate residue is the next residue in the amino acid sequence after the conserved histidine that ligates the zinc. For other structures, however, the picture is more complicated. Complexes of *C. beijerinkii* (1kev.pdb), *T. brockii* (1ykf.pdb), and *E. coli* (4gkv.pdb or Ref. 9) ADHs with NAD(P) have both forms of coordination (perhaps a consequence of partial occupancy by coenzyme). In the complex of *B. stearothermophilus* ADH complexed with TFE (1rjw.pdb), two of the subunits have the classical coordination, but the other two may have low occupancy zinc in the alternative coordination ligated to Glu-62.[5]

Coordination with other metals may also be flexible, as shown in various studies. In one subunit of horse ADH where Cd^{2+} has replaced the catalytic zinc, OE2 of the carboxyl group Glu-68 is positioned 3.0 Å from the Cd^{2+} and a water is 2.3 Å from the Cd^{2+} in a bipyramidal coordination, but the electron density is elongated, which would be consistent with alternative positions for the metal.[28] EPR studies on horse enzyme with Co^{2+} replacing the catalytic zinc suggested, on the basis of comparison of zero field splitting constants with model compounds and calculations, that the Co^{2+} was pentacoordinate in complexes with NAD^+ or NAD^+-pyrazole,[29] but the x-ray structures clearly show the zinc is tetracoordinate.[30]

The homologous *C. beijerinkii* and *T. brockii* ADHs are interesting because Asp-150 replaces the Cys-153 found in yeast ADH1, and the apoenzyme form is tetracoordinated with Cys-37, His-59, Glu-60 and Asp-150 (1ped.pdb), similar to the "alternative" position in yeast ADH1 subunits B and D, whereas the binary complexes with NADPH lose the inner-sphere coordination with Glu-60 (1kev.pdb, 1ykf.pdb). X-Ray absorption data on the *T. brockii* apoenzyme or complexes with $NADP^+$ or NADPH were consistent with the tetracoordination, but data for ternary complexes with $NADP^+$ or NADPH with the product analog dimethylsulfoxide were best described by penta- or hexa-coordination.[31] A three-dimensional model was built that could explain the results. However, no three-dimensional structures are available for ternary complexes, and X-ray absorption studies with the Co^{2+}-substituted *T. brockii* ADH suggested that the cobalt formed an octahedral complex, even when $NADP^+$ and dimethylsulfoxide were bound.[32] (In these experiments, the concentrations of ligands were not specified, and it is expected that dimethylsulfoxide binds best to ADHs with the reduced coenzyme.)

Binary or ternary complexes of *Haloferax mediterranei* glucose dehydrogenase have three different tetracoordinated structures for the catalytic zinc, all of which ligate His-63 and a water, but differ in the relative positions of the zinc, the water, and ligation to other groups.[33] The binary complexes with NADP(H) have zinc ligated also to Glu-64 and Glu-150, whereas a

ternary complex formed with $NADP^+$ and glucose has Asp-38 and O1 of glucose as ligands. (In these structures, the oxidation state of $NADP^+$ is not established by the crystallography, and partial occupancies for catalytic zincs complicate the interpretations of the electron density maps.) A mechanism is proposed in which the adjacent bound water (as hydroxide) deprotonates the O1 hydroxyl group of glucose when hydride is transferred from C1 of glucose to the nicotinamide ring; the authors suggest that the "text book" mechanism for medium chain ADHs requires modification. However, glucose dehydrogenase has Asp-38 and Glu-150 instead of the two cysteine residues found in liver or yeast ADH and the chemistry of the mechanism does not need to be the same as for other ADHs. Moreover, no text book has suggested how the water ligated to zinc in horse or yeast ADHs is replaced by the oxygen of the substrate. Such mechanisms have been discussed in the literature and are considered in the main article.

Table 6S. Coordination of Catalytic Zinc in Alcohol Dehydrogenases

species	PDB	form	substrate ligands	subunit: zinc ligands, amino acid residues	comments
Equus caballus	1ye3	apo		C46, H67, C174, H_2O	structural Zn, 4 Cys
ADH1E	4dxh	holo	NAD, tri-fluoroethanol	C46, H67, C174, TFE O	ternary complex
"	1p1r	holo	NADH, methylhexyl-formamide	C46, H67, C174, formamide O	ternary complex
Saccharoymyces cerevisiae ADH1	4w6z	~apo	TFE, 2nd sphere	B, D: no NAD, binary C43, H66, C153, E67	structural Zn, 4 Cys
"	4w6z	holo	NAD, TFE	A, C: C43, H66, C153, TFE O	ternary complex
Homo sapiens ADH3	1m6h	apo		C44, H66, C173, H_2O	structural Zn, 4 Cys
"	1m6w	apo	12HDDA, binary	C44, H66, C173, OR	mix of H_2O, OH of hydroxydodecanoate
"	1teh	holo	NAD(H)	A: C44, H66, C173, E67, H_2O	~ bipyramidal, binary
"	1teh	holo	NAD(H)	B:C44,H66,C173,E67	binary
"	2fze	apo	ADP-ribose	75% A, B: C44, H66, C173, H_2O	A, B subunits have alternative Zn sites
"	2fze	apo	ADP-ribose	25% A, B: C44, H66, C173, E67	Each zinc is tetracoordinate
"	1mao	holo	NAD, DDA inhibited	C44, H66, C173, E67	-CH_3 of dodecanoate close to Zn
"	1mc5	holo	NADH, HMGSH	A: C44, H66, C173, OH of HMGSH	hydroxymethylgluta-thione, abortive
"	1mc5	holo	NADH	B: C44, H66, C173, E67, H_2O	~ bipyramidal, binary
Clostridium beijerinkii	1ped	apo		C37, H59, D150, E60	no structural Zn (Trp, Leu, Gln, Asn)
"	1kev	holo	NADP(H)	A, C, D: C37, H59, D150, S39 2nd sphere	low NADP(H) occupancy
"	1kev			B: C37, H59, D150, E60	low NADP(H) occupancy
Thermoanaerobacter brockii	1ykf	holo	NADP(H)	A, B: C37, H59, D150, S39, E60 2nd sphere	low NADP(H) occupancy, no structural Zn
"	1ykf	holo	NADP(H)	C, D: C37, H59, D150, S39 2nd sphere	
Sulfolobus solfataricus	1jvb	apo		C38, H68, C154, E69	structural Zn 3Cys, Glu
"	1r37	holo	NADH ethoxyethanol	C38, H68, C154, EtOEtO	ternary

Aeropyrum pernix	1h2b	holo	NADH, octanoate	C54, H79, D168, octanoate carboxyl	structural Zn, 3 Cys, Asp; or disulfide
E. coli, inducible ADH	none	apo		A: C37, H58, C145, H_2O B, C, D: C37, H58, C145, E59	structural Zn, 4 Cys
"	none	holo	NAD	A: C37, H58, C145, E59 B, C, D: C37, H58, C145, H_2O	no NAD in subunit A, binary complex
"	4gkv	holo	NAD	C37, H58, C145, H_2O	binary
Bacillus stearothermophilius	1rjw	apo	TFE	C38, H61, C148, TFE, but A and C subunits may have low occupancy Zn ligated to E62	structural Zn, 4 Cys 55% ident to *E. coli,* binary
Pseudomonas aeruginosa	1llu	holo	NADH, ethyleneglycol	C44, H67, C154, glycol, abortive complex	structural Zn, 4 Cys
Bemisia argentifolii ketose reductase	1e3j	apo		C41, H66, E67, H_2O	structural Zn, 4 Cys
Homo sapiens sorbitol dehydrogenase	1pl7	apo		C44, H69, E70, H_2O	Glu70 is displaced by inhibitor ligated to Zn
"	1pl8	holo	NAD	C44, H69, E70, H_2O	
"	1pl6	holo	NAD, SDI-158 inhibitor	C44, H69, H_2O, N1 and O30 of SDI-158	SDI-158 chelates Zn in pentacoordination
Haloferax mediterranei glucose dehydrogenase	2vwp 2vwq	holo	NADP(H) binary	H63, E64, E150, H_2O, D38 nearby	
"	2vwh	holo	NADP, glucose	D38, H63, H_2O, Glucose O1	E64 and E150 ligate water on zinc
"	2vwg	holo	$NADP^+$,gluconolactone	D38, H63, E64, H_2O	abortive complex

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