Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary Information

Determination of the binding site of CDy2 with mitochondrial aldehyde dehydrogenase

Contents

Supplementary Table 1 . Interaction energies between CDy2 and ALDH2

Residues	type[a]	Interaction E (kcal/mol)
Phe459	π-π[b]	-14.564
Phe170	π-π	-7.792
Trp177	π-π	-7.555
Leu173	vdw[c]	-7.369
Cys302	vdw	-5.652
Phe465	π-π	-4.227
Met174	vdw	-3.206
Cyc303	vdw	-3.130
Asn169	vdw	-3.129
Val120	vdw	-2.196

[a] The major contribution of the interaction energy
[b] π-π interaction
[c] van der waals interaction

Supplementary Table 2. Calculated interaction energies of CDy2 with ALDH2 mutants.

ALDH2	Total Interaction Energy [a]	Reaction Distance [b]
WT	-69.78 Kcal/mol	5.26 Å
C301S	-70.54 Kcal/mol	4.79 Å
C302S	-69.90 Kcal/mol	4.55 Å [b*]
C303S	-68.77 Kcal/mol	4.69 Å

[a] The total interaction energy was calculated as stated in Supplementary Note 2, and each mutant structure was built with rotamer selection and side-chain dihedral adjustments.

[b] It indicates the distance between the chloro carbon of CDy2 and the thiol moiety of Cys302.

[b*] In the case of C302S, the distance indicates the distance between the reacting carbon atom of CDy2 and the hydroxyl moiety of C302S.

Supplementary Note 1. Site directed mutagenesis of ALDH2

GFP-tagged ORF-clone of Mus musculue ALDH2 (NM_009656) was purchased from OriGene Technologies, Inc. and site-directed mutagenesis was performed by using GeneTailor™ Site-directed Mutagenesis Kit (Invitrogen). Primer sequences are listed below.

Oligonucleotides used for Site-Directed-Mutagenesis

			301 302 303
SSS-F	5’	TTC AAC CAG GGC CAG TCC TCC TCC GCA GGC T	3’
		F N Q G Q S S S A G	
SCC-F	5’	TTC AAC CAG GGC CAG TCC TGC TGC GCA GGC T	3’
		F N Q G Q S C C A G	
CSC-F	5’	TTC AAC CAG GGC CAG TGC TCC TGC GCA GGC T	3’
		F N Q G Q C S C A G	
CCS-F	5’	TTC AAC CAG GGC CAG TGC TGC TCC GCA GGC T	3’
		F N Q G Q C C S A G	
SSS-R*	5’	A CTG GCC CTG GTT GAA GAA CAG GGC AAA GTG	3’
C49S-F	5’	ACA GGG GAG GTC ATC TCC CAG GTG GCC GAA	3’
		T G E V I S Q V A E	
C49S-R	5’	A GAT GAC CTC CCC TGT GGA AGG GTT GAC G	3’
C162S-F	5’	GAG CCT GTG GGC GTG TCT GGA CAG ATC ATT	3’
		E P V G V S G Q I I	
C162S-R	5’	A CAC GCC CAC AGG CTC ATG GCG GGT ATA	3’
C369S-F	5’	CG AAG CTG CTG TCT GGT GGG GGC GCT	3’
		K L L S G G G A	
C369S-R	5’	A CAG CAG CTT CGC CCC TTC TTG TTG TCC	3’
C455S-F	5’	ACT GTG TGG ATC AAC TCC TAC GAT GTG TTT	3’
		T V W I N S Y D V F	
C455S-R	5’	A GTT GAT CCA CAC AGT GCC AGC CTG	3’

‘F’ means forward primer and ‘R’ means reverse primer.
* The reverse primer (SSS-R*) was used for constructing SCC, CSC, CCS, and SSS mutants.

Supplementary Note 2. Docking of CDy2 to ALDH2

The crystal structure of ALDH2 was obtained from the RCSB Protein Data Bank (PDB code: 2VLE). The geometry of CDy2 was optimized at the Semi-empirical MO (AM1) level to adjust the initial structure. Then, CDy2 was docked with ALDH2 using Autodock 3.0.3[S2]. Lamarckian genetic algorithm (LGA) was applied to deal with ligand/protein interaction regarding the translational, rotational, and conformational change of CDy2. From the docking, the lowest energy structures were selected according to the criteria of interaction energy and the quality of geometrical matching. To refine the docked structures, the structures were further energy-minimized using 1000 steps of the steepest descents and 2000 steps of conjugate gradients. All calculations of molecular mechanics were carried out using the Discover 2.98 program (Accelrys Inc.)

(S1) Morris, G. M., Goodsell, D. S., Huey, R., Olson, A.J., Distributed automated docking of flexible ligands to proteins: parallel applications of AutoDock 2.4. *J. Comput.-Aided Mol. Des.* **1996**. 10, 293-304.

Experimental Procedure

Myogenic cell culture and differentiation. The C2C12 myoblast cell line was obtained from American Type Culture Collection (Rockville, MD). Undifferentiated myoblasts were grown in DMEM supplemented with 10% fetal bovine serum at 37°C in the presence of 5% CO_2. After 2 days, myoblasts were stimulated to differentiate by replacing the medium with DMEM supplemented with 2% heat-inactivated horse serum. Differentiation was allowed to continue for 5 days to prepare differentiated myotubes, with media replacement every two days.

Compounds: MitoTrackers were purchaced from Invitrogen Corporation and CDy2 was synthesized according to the previous report.

Dye labeling and in-gel fluorescence analysis. To label proteins in live cells, differentiated myotubes were incubated with CDy2 for 30 min, and washed three times with phosphate-buffered saline (DPBS, Sigma). Total cell lysates were prepared in mammalian cell lysis buffer (Sigma, C2978) containing protease inhibitor cocktail (Sigma, P8340). 10 μg of each lysate was run on a 10-14% SDS-PAGE gel. Then, fluorescent-labeled proteins on an SDS-gel were visualized by using Typhoon 9400 scanner (GE Healthcare).

Immunoblotting. Protein samples were separated by SDS-PAGE and transferred to a PVDF membrane. For tubulin immunohistochemistry, a mouse anti-α-tubulin antibody (GeneTex., Inc.) was used. For ALDH2 immunohistochemistry, goat anti-ALDH2-K15 (Santa Cruz Biotechnology, Inc.) was used.

Transfection of ALDH2 mutant constructs. For transfection, HEK293 were plated in 35-mm culture plate the day before transfection. The next day cells were transfected with ALDH2 constructs with Lipofectamin^{TM} 2000 (Invitrogen). Ater 48 hours of transfections, cells were labeled with CDy2 (1 μM) for 15 min and their lysates were subjected for in gel-fluorescence analysis.