Supplemental material

CONSERVED CATALYTIC RESIDUES OF THE ALDH1L1 ALDEHYDE DEHYDROGENASE DOMAIN CONTROL BINDING AND DISCHARGING OF THE COENZYME.

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1. Calculations of K_ds from fluorescent titration data.

K_d values for the $NADP^+$ binding were determined by non-linear fitting of experimental data points to the equation:

$$F = F_{\max} - (F_{\max} - F_{\min}) \cdot \frac{([P_{tot}] + [NADP_{tot}] + K_d - \sqrt{([P_{tot}] + [NADP_{tot}] + K_d)^2 - 4 \cdot P_{tot} \cdot [NADP_{tot}]})}{2 \cdot [P_{tot}]}$$

where F, F_{max} and F_{min} are observed, maximal and minimal fluorescence intensities at 330 nm, respectively, and $[P_{tot}]$ is the total protein concentration.

K_ds for the NADPH binding were determined by non-linear fitting of experimental data points to the equation:

$$\Delta F = \Delta F_{\min} + (\Delta F_{\max} - \Delta F_{\min}) \cdot \frac{([P_{tot}] + [NADPH_{tot}] + K_d - \sqrt{([P_{tot}] + [NADPH_{tot}] + K_d)^2 - 4 \cdot [P_{tot}] \cdot [NADPH_{tot}]})}{2 \cdot [P_{tot}]}$$

where ΔF, ΔF_{max} and ΔF_{min} are observed, maximal and minimal changes in NADPH fluorescence intensity upon binding to the protein, respectively.

All measurements were done in duplicates. The above equations were fit to the experimental data using non-linear regression procedure in Origin 8 (OriginLabs).

The above equations were derived as described below.

At equilibrium:

P + NADP ↔ P·NADP,

where P is free protein, NADP is free ligand and P·NADP is the complex between the two. Dissociation constant is given by:

$$K_d = \frac{[P]\cdot[NADP]}{[P\cdot NADP]} \quad (1)$$

Concentration of free protein (ligand) is the difference between the total concentration of protein (ligand) and the concentration of the complex:

$$[P] = [P_{tot}] - [P\cdot NADP] \quad (2)$$

$$[NADP] = [NADP_{tot}] - [P\cdot NADP] \quad (3)$$

Solving the system of equations 1-3 gives:

$$[P\cdot NADP] = \frac{[P_{tot}] + [NADP_{tot}] + K_d - \sqrt{([P_{tot}] + [NADP_{tot}] + K_d)^2 - 4\cdot[P_{tot}]\cdot[NADP_{tot}]})}{2} \quad (4)$$

Taking into account that binding of NADP quenches tryptophan fluorescence of C_t-FDH, the observed fluorescence signal F is related to the fraction of complex present in the following way:

$$F = F_{\max} - (F_{\max} - F_{\min})\cdot\frac{[P\cdot NADP]}{[P_{tot}]}$$

where F_{max} is the maximal signal (no NADP bound) and F_{min} is the minimal signal (all binding sites are saturated with NADP). Combining with equation 4 gives:

$$F = F_{\max} - (F_{\max} - F_{\min})\cdot\frac{([P_{tot}] + [NADP_{tot}] + K_d - \sqrt{([P_{tot}] + [NADP_{tot}] + K_d)^2 - 4\cdot P_{tot}\cdot[NADP_{tot}]})}{2\cdot[P_{tot}]}$$

Binding of NADPH leads to dequenching of its fluorescence. Thus, in this case

$$\Delta F = \Delta F_{\min} + (\Delta F_{\max} - \Delta F_{\min})\cdot\frac{[P\cdot NADPH]}{[P_{tot}]}$$

where ΔF is the difference between the fluorescence of NADPH in the presence of the protein and the same concentration of NADPH in the absence of the protein, ΔF_{max} is the maximal ΔF

(all protein binding sites are saturated with NADPH), and ΔF_{min} is the minimal ΔF (no NADPH is bound to the protein; $\Delta F=0$ in our case). Combining with equation 4 gives:

$$\Delta F = \Delta F_{\min} + (\Delta F_{\max} - \Delta F_{\min}) \cdot \frac{([P_{tot}] + [NADPH_{tot}] + K_d - \sqrt{([P_{tot}] + [NADPH_{tot}] + K_d)^2 - 4 \cdot [P_{tot}] \cdot [NADPH_{tot}]})}{2 \cdot [P_{tot}]}$$

2. Covalent bond between Cys707 of the E673A mutant of C_t-FDH and the C4 atom of the nicotinamide ring of $NADP^+$.

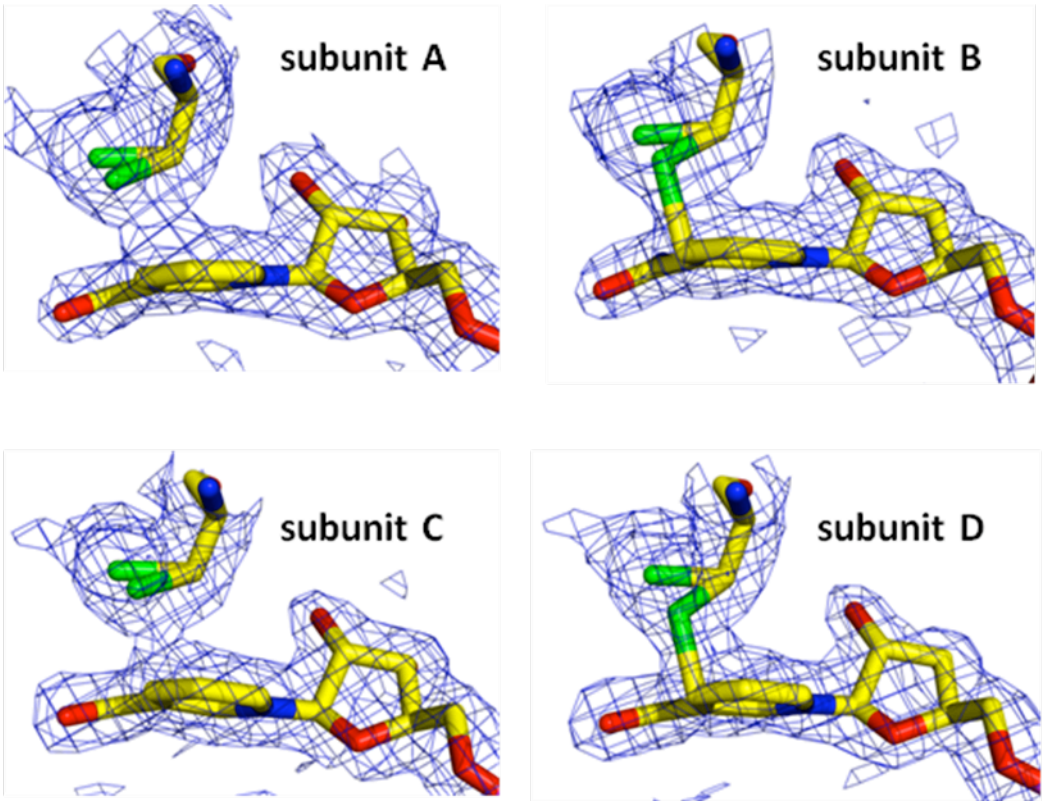

Fig. S1. Continuous electron density between one of the conformers of the sulfur atom (green) of Cys707 of the E673A mutant of C_t-FDH and the C4 atom of the nicotinamide ring of $NADP^+$ indicates the presence of a covalent bond. The $2|F_O|-|F_C|$ electron density map contoured at 1σ is shown in blue.

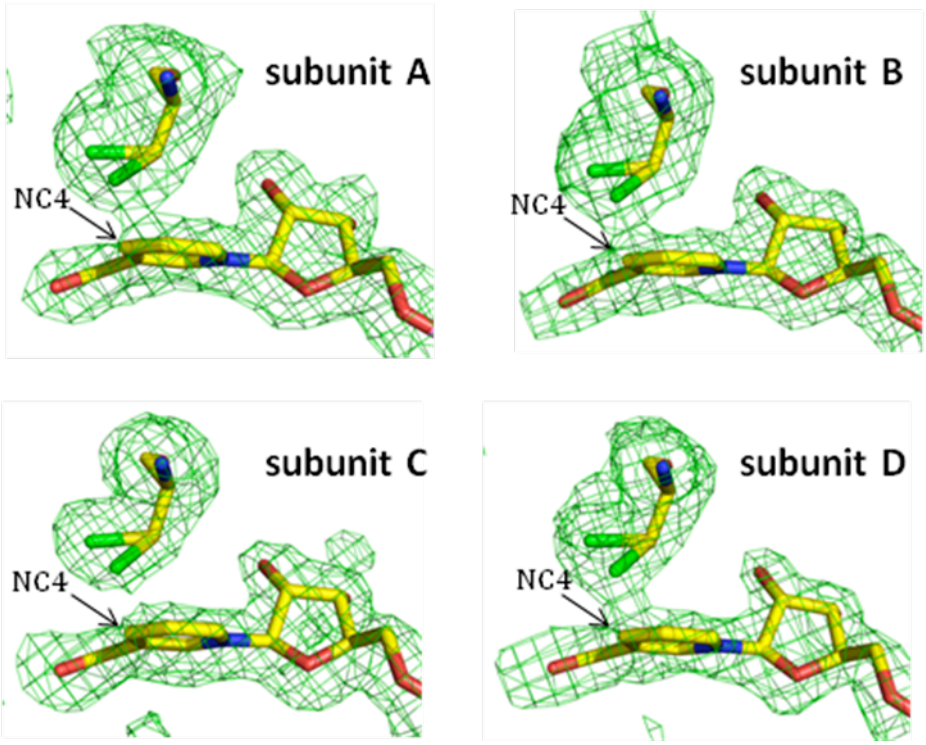

Fig. S2. Refinement of the E673A structure with the occupancies of all the atoms of Cys707 and $NADP^+$ set to zero resulted in continuous positive electron density that encompassed all atoms of Cys707 and $NADP^+$ in subunits A, B and D. The $|F_O|$-$|F_C|$ electron density map contoured at 3σ is shown in green. In subunits B and D the electron density directly connects the sulfur atom of Cys707 and the C4 atom of the coenzyme. Accordingly, we used a LINK statement to introduce covalent bonds between the cysteine and the NC4 atom of $NADP^+$ in these subunits.

Thus, the structure of the E673A mutant of C_t-FDH suggests covalent interactions between the active site cysteine and $NADP^+$, and these interactions were confirmed spectroscopically (see Results and Fig. 1B). However, this covalent adduct is apparently weaker than that observed earlier in the structure of wt C_t-FDH (*1*). We suggest that this is because of the smaller probability of forming such an adduct reflected by the averaging among a large number of molecules in the protein crystal. This agrees well with the proposed role of the active site glutamate as the proton acceptor but also suggests that the cysteine can be activated through other mechanisms.

3. $NADP^+$ is trapped in the E673A mutant of C_t-FDH but can be removed with extensive dialysis at acidic/high salt conditions.

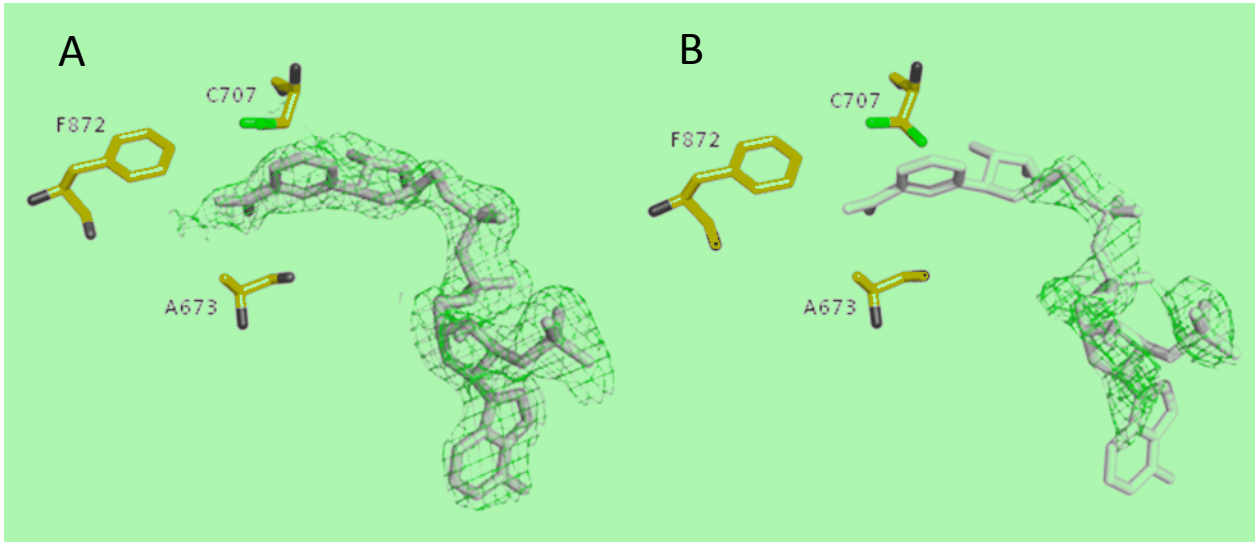

Fig. S3. *A*, the E673A mutant crystallized after extensive dialysis at physiological conditions to remove $NADP^+$ (see Experimental Procedures and Results) still shows strong electron density for the co-purified coenzyme (structure resolution: 2.7 Å). *B*, $NADP^+$ can be removed by extensive dialysis at pH 5.6 in the presence of 0.5 M NaCl (structure resolution: 2.0 Å). Only traces of electron density are visible around the phosphate groups of the coenzyme. The absence of the nicotinamide ring in the active site is confirmed by the position of the sulfur atom of Cys707 typical of coenzyme-free C_t-FDH structures (*1*). Shown in green and contoured at 3 σ is the $|F_O|-|F_C|$ electron density for $NADP^+$ obtained by refinement in the absence of the coenzyme in the models. The $NADP^+$ molecule from the holo-structure of wt C_t-FDH (PDB code 2O2Q) is displayed in both panels in grey for reference.

REFERENCES

1. Tsybovsky, Y., Donato, H., Krupenko, N.I., Davies, C., and Krupenko S. A. (2007) Crystal structures of the carboxyl terminal domain of rat 10-formyltetrahydrofolate dehydrogenase: inplications for the catalytic mechanism of aldehyde dehydrogenases. *Biochemistry, 46*, 2917-2929.