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Cryo-EM reveals architecture and domain interactions of putative tumor suppressor ALDH1L1, a product of natural fusion of three unrelated genes

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ALDH1L1 (10-formyltetrahydrofolate dehydrogenase; also abbreviated as FDH) is an enzyme of folate metabolism catalyzing the NADP-dependent conversion of 10-formyltetrahydrofolate to tetrahydrofolate and CO_2 (1). This reaction regulates the overall flux of folate-bound one-carbon groups. The *ALDH1L1* gene represents a natural fusion of three unrelated genes. Accordingly, the ALDH1L1 protomer consists of distinct N-terminal (Nt), intermediate, and C-terminal (Ct) domains. The Nt domain, which binds 10-formyltetrahydrofolate and cleaves off the formyl group, has sequence and structural similarity to methionyl-tRNA formyltransferase. The Ct domain, the site for the oxidation of formyl group to CO_2, belongs to the family of aldehyde dehydrogenases, enzymes catalyzing the conversion of various aldehydes to corresponding acids. As most aldehyde dehydrogenases, Ct forms a tetramer, which brings the total domain count of ALDH1L1 to twelve and the molecular mass to ~400 kDa (Fig. 1a). Curiously, the intermediate domain is a close homolog of acyl carrier proteins (ACPs), which are normally employed in fatty acid biosynthesis, and carries a 4'-phosphopantetheine (4'-PP) prosthetic group (2). The fact that the typical role of ACPs is to shuttle reaction components between catalytic centers of complex enzymes implies their high mobility, suggesting a dynamic structural organization of ALDH1L1. Though the structures of the Nt and Ct domains expressed separately were resolved by X-ray crystallography (3, 4), attempts to determine the X-ray structure of full-length ALDH1L1 were unsuccessful.

We used a combination of negative-stain EM and cryo-EM to determine the architecture of full-length ALDH1L1 and resolve its domain interactions. Negative-stain EM resolved a stable core of ALDH1L1, consisting of the Ct tetramer and four intermediate domains, and revealed that the four Nt domains are highly mobile (Fig. 1b). The cryo-EM structures of ligand-free ALDH1L1 and ALDH1L1 in complex with NADP, determined to resolutions of 3.9 Å and 2.9 Å, provided detailed information about the stable core of ALDH1L1. Each of the four intermediate domains was found to interact with the structural elements forming the outer part of the substrate entrance tunnel of one of the four Ct protomers. Interestingly, the intermediate domain of chain A was docked into the substrate entrance tunnel of the Ct domain of chain D, whereas the intermediate domain of chain D was paired to the Ct domain of chain A. An identical arrangement was found for protomers B and C. We found that the intermediate and Ct domains formed a relatively large contact interface. The 4'-PP group of the intermediate domain penetrated deeply into the substrate entrance tunnel of the Ct domain, placing the sulfhydryl group of 4'-PP close to the active site cysteine of the Ct domain. Such an arrangement should enable the positioning of the formyl group carried by the 4'PP arm suitable for the nucleophilic attack by the active site cysteine. We used 3D classification to isolate two ALDH1L1 conformations with defined Nt domains. In one structure, the Nt domains interacted with the region of the Ct domain responsible for binding of NADP. We infer that this conformation of ALDH1L1 may serve to regulate binding of the coenzyme. In another map, the amino-terminal sub-domain of Nt interacted with the linker connecting the intermediate and Ct domains. We hypothesize that the role of this interaction may be to extract the intermediate domain from the substrate entrance tunnel of the Ct core.

In summary, ALDH1L1 is organized as a stable core containing the C-terminal tetramer with ACP-like intermediate domains docked into the substrate entrance tunnels. The N-terminal domains are highly dynamic, but they form transient complexes with the core, which may facilitate various stages of the catalysis.

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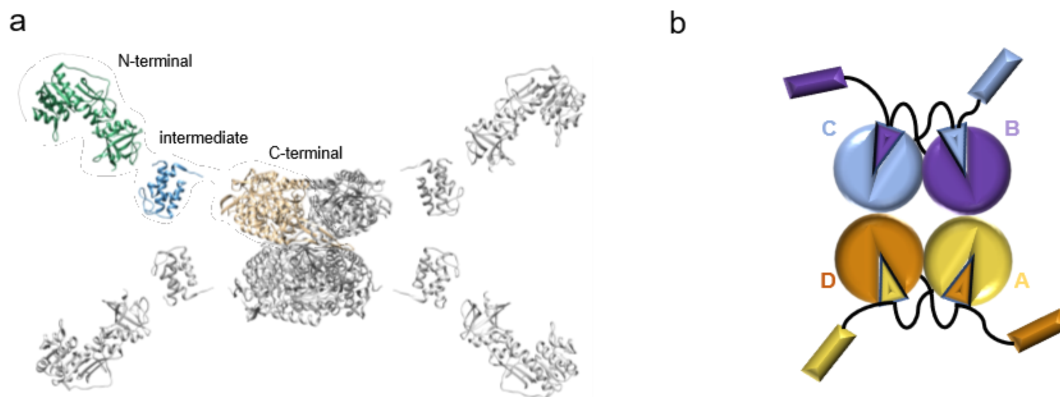

Figure 1. The architecture of 10-formyl tetrahydrofolate dehydrogenase (ALDH1L1). a: A schematic of the spatial organization of FDH based on available atomic structures of its individual domains. PDB structures 1s3i, 2cq8, and 2o2p, respectively, were used to depict the N-terminal, intermediate, and C-terminal domains. For one protomer of the tetrameric enzyme, the domains are labeled and colored. b: A schematic depicting the architecture of ALDH1L1 determined by cryo-EM. Each intermediate domain interacts with the C-terminal domain of the second protomer within the same dimer, whereas the N-terminal domains remain mobile.

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