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Modeling of interactions between functional domains of ALDH1L1

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ABSTRACT

ALDH1L1, a member of the aldehyde dehydrogenase superfamily of enzymes, catalyzes the conversion of 10-formyltetrahydrofolate to tetrahydrofolate and CO_2. The enzyme is a tetramer of identical subunits, with each subunit consisting of three functional domains that originated from unrelated genes. The N- and C-terminal domains are catalytic, while the intermediate domain transfers the reaction intermediate from the N- to the C-terminal domain. The intermediate domain is an acyl carrier protein, possessing the covalently attached 4'-phosphopantetheine (4-PP) prosthetic group. This prosthetic group is known to function as a swinging arm transferring intermediates between enzymes in complex biosynthetic reactions. Here we have applied computer modeling using available structures of the three functional domains of ALDH1L1 to evaluate the extent of flexibility within the full-length protein. This approach allowed us to define positions of the 4-PP arm within the two catalytic domains and to predict N-terminal:intermediate and intermediate:C-terminal domain interfaces. Our models further suggested high degree of flexibility within the full-length enzyme.

1. Introduction

ALDH1L1 (aldehyde dehydrogenase 1 family member L1; 10-formyltetrahydrofolate dehydrogenase) is a member of the aldehyde dehydrogenase (ALDH) superfamily of enzymes [1,2]. This enzyme plays a key role in the regulation of folate-mediated one-carbon metabolism and downstream cellular processes [3–9]. ALDH1L1 catalyzes oxidative decarboxylation of 10-formyltetrahydrofolate (10-fTHF) to tetrahydrofolate with concomitant reduction of $NADP^+$ to NADPH (Fig. 1) [10]. The two-step reaction is carried out by structurally and functionally distinct catalytic domains linked by a physical transfer of a formyl group from the first reaction center to the second [11–13]. The need to couple two discrete reaction steps led to the fusion of three unrelated primordial genes, yielding a complex three-domain protein [1]. Full-length ALDH1L1 is a homotetramer of 902 amino acid subunits, with each monomeric subunit having a modular organization (Fig. 1). The 500-residue C-terminal core domain includes the tetramer interface [14]. This domain is a structural and functional homolog of aldehyde dehydrogenases and is responsible for the original assignment of the protein to the ALDH superfamily. The 310-residue N-terminal domain resembles methionine-tRNA formyltransferase, binds the folate substrate, and can function (at least *in vitro*) as a 10-fTHF hydrolase [12,15]. The two catalytic domains are connected by and communicate *via* the 90-residue intermediate domain, which is a structural and functional homolog of acyl carrier proteins (ACP) [13]. Another example of such a carrier protein in humans is the fatty acid synthase complex, where it functions in transferring of growing acyl chain [16]. The distinctive feature of acyl-carrier proteins is the presence of a covalently linked 4'-phosphopantetheine (4-PP) prosthetic group which functions as a long (~20 Å), flexible arm that can reach into buried catalytic centers and carrying the reaction intermediate from one center to another [16].

The two-step conversion of 10-fTHF to tetrahydrofolate and CO_2 catalyzed by ALDH1L1 requires a concerted action of three enzyme domains (Fig. 1) [1]. In the first catalytic step, which takes place in the N-terminal formyltransferase domain, the formyl group is transferred to the 4-PP arm of the intermediate ACP domain (Fig. 1). In the second step, catalyzed by the C-terminal dehydrogenase domain, the formyl group delivered by 4-PP is oxidized to CO_2 through an $NADP^+$-dependent aldehyde dehydrogenase-like mechanism (Fig. 1). *In vitro*, both the full-length enzyme as well

Abbreviations: ALDH, aldehyde dehydrogenase; ACP, acyl carrier protein; 4-PP, 4'-phosphopantetheine; 10-fTHF, 10-formyltetrahydrofolate; 10-fDDF, 10-formyl-5,8-dideazafolate; 2-ME, 2-mercaptoethanol.

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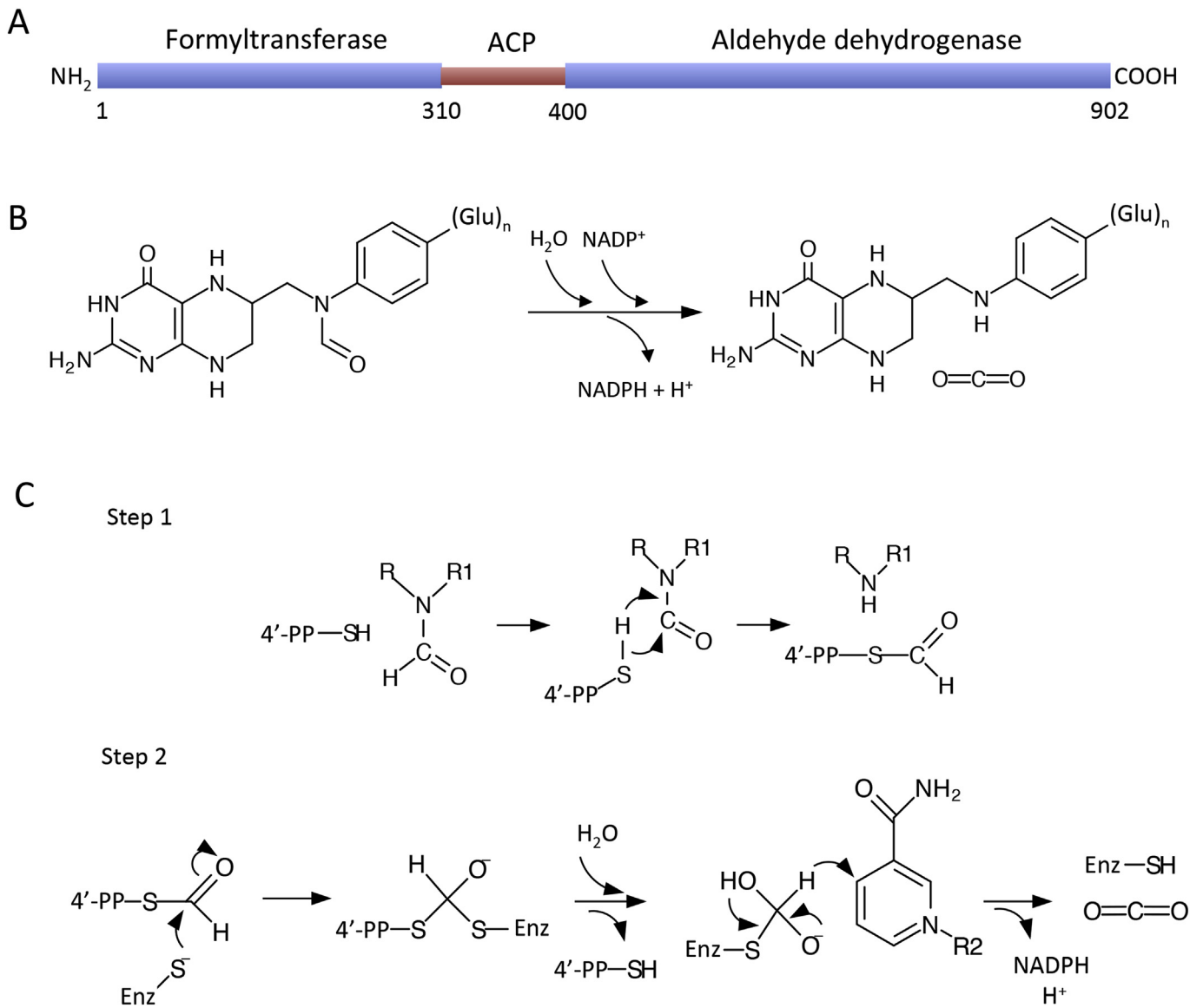

Fig. 1. Diagram depicting domain organization of ALDH1L1 and the catalyzed reaction. (A) The ALDH1L1 subunit consists of three domains: N-terminal fornyltransferase; intermediate ACP; and C-terminal aldehyde dehydrogenase. Numbers indicate amino acid residues at domain boundaries. (B) The overall 10-fTHF dehydrogenase reaction. (C) Proposed steps of the ALDH1L1 catalysis (step 1, formyltransferase catalysis taking place in the N-terminal domain; step 2, dehydrogenase catalysis taking place in the C-terminal domain; the transfer of formyl group covalently attached to the 4-PP moiety connects the two catalytic steps).

as the exogenously expressed formyltransferase domain are capable of the hydrolytic cleavage of 10-fTHF, releasing formate [10,12]. The requirement of millimolar concentrations of 2-mercaptoethanol (2-ME) for this hydrolysis [10,12] suggests a mechanism where the formyl group is likely transferred first to the sulfhydryl of 2-ME, which in this case plays the role of the 4-PP sulfhydryl, before being released.

Although the mechanisms of the two catalytic steps were proposed [14,17,18], it remains unclear how the formyl product from the first reaction in the formyltransferase domain is physically transported to the catalytic center of the dehydrogenase domain. An NMR solution structure of the ALDH1L1 ACP domain has been solved (PDB 2CQ8), and several crystal structures of the dehydrogenase domain of the rat enzyme and the formyltransferase domain from several species have been reported [14,18–22]. The structure of the full-length protein, however, is still not available. Our attempts to crystallize the full-length ALDH1L1 yielded crystals, which did not diffract beyond 7.8 Å at a synchrotron source. While an interpretable electron density was seen for the dehydrogenase domain, and it was possible to model this domain using its high-resolution crystal structure, we were unable to assign the ACP or formyltransferase domains. The lack of defined electron density for either the formyltransferase or ACP domains is likely the consequence of these domains adopting multiple orientations within the crystal lattice relative to the C-terminal domain. The existence of multiple domain-domain arrangements is consistent with the movement of the ACP domain between the catalytic domains as a mechanism of the formyl group transport. Furthermore, ALDH1L1 is a tetramer and there is no evidence that catalysis or domain rearrangements are synchronized between subunits. In the absence of additional experimental data, we have here applied a molecular modeling approach, using existing structures of the individual ALDH1L1 domains, to predict interactions between the ACP and the two catalytic domains of the enzyme consistent with

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