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A computational integrating kinetic study on the flexible active site of human acetaldehyde dehydrogenase 1

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ABSTRACT

In order to gain more insight into the relation between the structure of acetaldehyde dehydrogenase 1 (ALDH1) and its catalytic and regional active site properties, the denaturant guanidine hydrochloride (GdnHCl) was employed in this study. The effects of GdnHCl on ALDH1 conformational and functional changes were evaluated by kinetic analysis and by performing computational molecular dynamics (MD) simulations. We found that direct binding of GdnHCl to ALDH1 induced complete inactivation prior to conspicuous changes in its tertiary structure or hydrophobic exposure, indicating that the active site is flexible compared to the overall structure. Kinetic experimental results and computational simulations indicated that there are specific sites on ALDH1 to which guanidine binds, resulting in blocking of catalytic function without a large degree of structural disruption. These sites may lay specifically in a cofactor-binding region, which was suggested by the observation of mixed-type inhibition. Our study provides insight into the flexibility of the ALDH1 active site through the use of GdnHCl denaturant and computational simulations to suggest possible binding mechanisms of inhibitors for the clinical applications.

1. Introduction

Acetaldehyde dehydrogenase 1 (EC 1.2.1.10; ALDH1) belongs to the aldehyde dehydrogenase superfamily. The gene encoding human ALDH1 is located at 9q21.13 (Gene ID: 216), and an ALDH1 crystallography study [1] has recently determined the tertiary structure of ALDH1 as an apo-enzyme at 1.75 Å resolution (PDB ID: 4WJ9) and in a complex with NADH at 2.1 Å resolution (PDB ID: 4WB9). A high degree of similarity between the cofactor binding sites of ALDH1 and those of other closely related superfamily enzymes was found in structural comparisons. In a previous study, structural analysis by molecular dynamics (MD) simulations of ALDH1 revealed that interactions with Asn169 and Glu399 were preserved [2].

It has been reported that several divalent ions can directly affect the catalytic role of ALDH1. Mg^{2+} may play an important role in prolonging the residence time of the cofactor NADH in the active site pocket. A previous study found that as the Mg^{2+} concentration was increased, there was a consistent decrease in ALDH1 activity [3]. The direct binding of Zn^{2+} to ALDH1 induces structural changes and inhibits ALDH1 activity, and Zn^{2+}-mediated inactivation of ALDH1 is associated with structural changes. The crystallographic structure of ALDH1 was applied to computational docking as well as molecular dynamics simulations wherein the beta regions of ALDH1 were found to be exposed upon binding of Zn^{2+}, and to undergo significant conformational changes, including loss of beta secondary structure [4].

ALDH1 was found to be significantly downregulated in proteomic studies in the atopic dermatitis (AD) proteome obtained from patient-derived primary cultured fibroblasts. The measurement of transcriptional levels of ALDH1 by RT-PCR and real-time

Abbreviations: ALDH1, acetaldehyde dehydrogenase 1; GdnHCl, guanidine hydrochloride; ANS, 1-anilinonaphthalene-8-sulfonate; MD, molecular dynamics.

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PCR further confirmed the downregulation of ALDH1 in all of the examined AD-fibroblasts ($n = 20$). The expression levels of ALDH1 in whole skin tissue samples were further supported by results from primary cultured samples. These findings demonstrate that ALDH1 could be a dermal biomarker for AD disease [5].

In early work, it was reported to be an important enzyme downstream of alcohol dehydrogenase in the major pathway of alcohol metabolism; however, the exact function of ALDH1 is in dispute since another isozyme found in mitochondria, ALDH2, plays a more important role in the detoxification of acetaldehyde with respect to kinetic properties; that is, mitochondrial ALDH2 has a higher affinity for acetaldehyde and acts predominantly in the mitochondrial matrix, unlike the cytosolic ALDH1 [6,7]. Therefore, ALDH2 is believed to be crucial for alcohol metabolism as the key enzyme that oxidizes acetaldehyde for detoxification. Some studies have indicated that measurement of ALDH1 activity shows potential as a universal marker for the identification and isolation of stem cells and cancer stem cells isolated from mammary glands and breast cancer [8] as well as lung cancer [9]. ALDH1 is also known to be associated with progression of gynecological malignancies [10]. In particular, ALDH1 was analyzed as a biomarker to predict tumor progression and poor survival in breast cancer, and it was suggested that ALDH1 should be taken into consideration in the development of new diagnostic and therapeutic programs for breast cancer [11].

Enzyme denaturation studies using the denaturant guanidine hydrochloride (GdnHCl) have provided insights into enzyme structures related to catalysis, including the forces required to maintain tertiary structures, folding behaviors, and conformational flexibility. The ions arising from GdnHCl modulate electrostatic interactions, and in conjunction with their hydrogen bond-breaking effects and modulation of hydrophobicity, provide the driving force for enzyme unfolding as well as destabilization of enzymes. It has been reported that inactivation occurs before noticeable conformational changes of the whole enzyme molecule, indicating that some active sites of enzymes situated in a limited number of regions of the enzyme molecules are very flexible [12–14]. In this study, we aimed to probe the active site properties of ALDH1 to determine whether it is more flexible than the enzyme molecule as a whole, and to examine unfolding behavior during GdnHCl-mediated denaturation. We prepared recombinant human ALDH1 and evaluated its structural properties by applying kinetic measurements integrated with computational molecular dynamics (MD) simulations. The kinetic evaluation revealed flexible active site characteristics of ALDH1, and the MD simulation provided insights suggesting that the residues that bind GdnHCl act to trigger unfolding in the initial stage. The stability of ALDH1 and the functional role might be relevant to various important roles in various diseases. This study increases our understanding of ALDH1 and provides a scientific basis via the application of kinetic analysis and computational simulation to understand enzyme mechanisms that are relevant to catalytic function.

2. Materials and methods

2.1. Materials

Guanidine hydrochloride, acetaldehyde, β-nicotinamide adenine dinucleotide (β-NAD), and 1-anilinonaphthalene-8-sulfonate (ANS) were purchased from Sigma-Aldrich (USA). Other chemicals were obtained locally and were of the highest analytical grade. Hiprep 26/60 Sephacryl S-200HRand HiTrap Q HP were obtained from GE Healthcare Life Sciences (USA).

2.2. Recombinant ALDH1 purification and assay

Protein expression and purification of the recombinant ALDH1 enzyme were performed following a protocol based on a previous report [6]. For purification, the target protein was sequentially applied to a Hiprep 26/60 Sephacryl S-200HR column and a HiTrap Q HP column (1.6 cm × 2.5 cm) using an AKTA FPLC System (GE Healthcare, USA). Purified ALDH1 migrated as a homogenous band in SDS-PAGE analysis; protein concentration was determined using the Bradford method.

ALDH1 activity (v) was determined by measuring the change in absorbance at 340 nm, as per a previous study [6]. The reactions contained a substrate mixture (22.2 mM acetaldehyde, 2.22 mM NAD^+, and 0.2 M KCl) and 1.5 μM enzyme in 0.5 ml of 50 mM phosphate buffer (pH 7.0); they were monitored using a Hitachi U3900 UV–vis Spectrophotometer (Hitachi, Japan). ALDH1 was inactivated by incubation with varying concentrations of GdnHCl in 50 mM phosphate buffer (pH 7.0) at 25 °C.

2.3. Kinetic analysis

For general analysis of mixed-type inactivation, the Lineweaver-Burk equation can be written in double reciprocal form:

$$\frac{1}{v} = \frac{K_m}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)\frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_i}\right) \quad (1)$$

Secondary plots can be constructed from

$$Slope = \frac{K_m}{V_{max}} + \frac{K_m[I]}{V_{max}K_i} \quad (2)$$

$$K_m^{app} = \frac{K_m[I]}{K_i} + K_m \quad (3)$$

and K_i, K_m, and V_{max} values can be derived from these two equations. The secondary replot is linearly fitted assuming a single inhibition site or a single class of inhibition sites.

2.4. Spectrofluorometry: intrinsic and ANS-binding fluorescence studies

Fluorescence emission spectra of ALDH1 in both the absence and presence of GdnHCl were measured with a Hitachi F-4500 fluorescence spectrophotometer (Hitachi, Japan) using a 1-cm path-length cuvette. An excitation wavelength of 280 nm was used for detecting tryptophan side chain, and emission wavelengths ranged between 300 and 400 nm after the enzyme was incubated with various concentrations of GdnHCl. To probe hydrophobic surface changes, fluorescence spectra changes were studied by labeling with 60 μM ANS for 45 min prior to measurement. An excitation wavelength of 380 nm was used for ANS-binding fluorescence, and the emission wavelength ranged from 400 to 600 nm. All spectra were collected at 25 °C in 50 mM phosphate buffer (pH 7.0).

2.5. Computational MD simulations with guanidine

Since the three-dimensional structure of human ALDH1 has recently been solved (PDB ID: 4WJ9) [3], we used the PDB structure for docking and MD simulations. The chemical reaction region for ALDH1 was found using the Dockable Pocket Site Prediction (DPSP) method, which was executed in three ways: geometric pocket search, plausible ligand search, and DPSP score measurement. In the first step, binding pocket residues were calculated based on the 3D structure of ALDH1 via the Pck pocket detection program (http://schwarz.benjamin.free.fr/Work/Pck/home.htm). We collected a pocket list with the volumes of pockets and the residues comprising them. Pockets with volume less than 100 $Å^3$ were excluded. A possible ligand search was performed based on sequence alignment