Structure of Human Cytidine Deaminase Bound to a Potent Inhibitor

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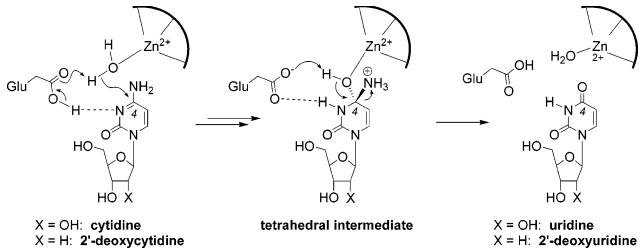
Abstract: Human cytidine deaminase (CDA) is an enzyme prominent for its role in catalyzing metabolic processing of nucleoside-type anticancer and antiviral agents. It is thus a promising target for the development of small molecule therapeutic adjuvants. We report the first crystal structure of human CDA as a complex with a tight-binding inhibitor, diazepinone riboside **1**. The structure reveals that inhibitor **1** is able to establish a canonical π/π-interaction with a key active site residue, Phe 137.

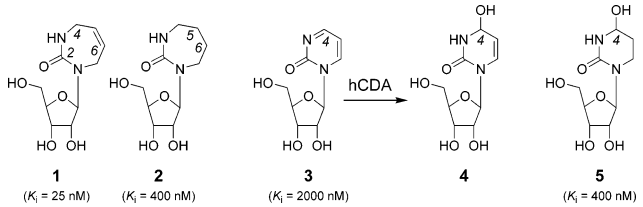
Cytidine deaminase (CDA, EC 3.5.4.5), an evolutionarily conserved enzyme of the pyrimidine salvage pathway, catalyzes the hydrolytic deamination of cytidine and deoxycytidine to form uridine and deoxyuridine, respectively.[1−3] In addition to these natural substrates, human CDA (hCDA) deaminates a number of synthetic cytidine analogues, including several agents used in the clinical treatment of cancer and viral diseases.[4−7] Processing of drugs by hCDA typically results either in loss of pharmacologic activity or acquisition of undesirable side-effects. This, together with the observation that hCDA is often overexpressed in cancers having acquired resistance to cytidine analogues,[5,8] has stimulated interest in the development of hCDA inhibitors for use as therapeutic adjuvants.[7,9−11] Efforts to design such agents on the basis of the enzyme mechanism has shown some promise,[12−14] but complementary approaches using structure-based design have been held back by the unavailability of a high-resolution structure for hCDA. Here we report the first crystal structure of hCDA complexed with the tight-binding diazepinone riboside **1**.

Biochemical and structural studies on bacterial CDAs have suggested the reaction mechanism shown in Scheme 1.[15−18] The enzyme contains an active site zinc atom to which is coordinated a nucleophilic water/hydroxide. A conserved active site glutamate (E67 in hCDA) is envisaged to promote the initial attack at C4 of the cytosine ring by protonating the adjacent N3-position and deprotonating the nucleophilic water, then again using general acid/base chemistry to facilitate breakdown of the tetrahedral intermediate. The proposed reaction scheme is supported, among other things, by studies on the interaction of the enzyme with zebularine, **3**.[12,19] CDAs bind **3** weakly, but catalyze the

Scheme 1. Proposed Reaction Mechanism for HCDA



formation of the hydration product **4**, to which the enzyme binds tightly. X-ray structures of *E. coli* CDA bound to **4** reveal that that the C4-OH is indeed coordinated to zinc, thus the hydration product serves as a stable analogue of the tetrahedral intermediate (as in Scheme 1, but $NH_3^+ = H$).[17] A similar mode of interaction via zinc coordination has been observed for the tetrahydrouridine analogue **5** bound to *B. subtilis*



CDA.[18] More mysterious, especially in light of this common binding theme, is the reason for the tight binding of **1**.[20−22] The seven-membered ring of **1** contains no functionality at or near C4 that would be conducive to hydration, nor any other functional group suitably disposed to coordinate the active site zinc. To gain insight into this problem, we cocrystallized hCDA with **1** to determine the structure.

The three-dimensional structure was determined using molecular replacement, and the model was refined at a final resolution of 2.4 Å. The protein self-associates to form a tetramer, which is best viewed as a dimer of dimers. Pairs of monomers assemble to form two pseudo-2-fold symmetric dimers, which actually comprise the asymmetric unit (magenta and dark blue dimer or green and light blue dimer); these dimers are in turn related in the tetramer by a strict crystallographic 2-fold symmetry axis. The tetramer, which has 2-fold but not 4-fold symmetry, is known biochemically to be the functional unit of hCDA.[1] A high level of structural conservation is observed between the human and *B. subtilis* enzymes (Figure 1B, backbone RMSD = 1.3 Å), despite their having only 45% identity at the amino acid level. Each subunit is bound to one molecule of the inhibitor **1** (Figure 1A, gold), which is completely engulfed by the protein structure and is inaccessible to solvent. Interestingly, each active site is made up of residues from three of the four hCDA protamers, and all three contribute directly to recognition of the inhibitor **1** (Figure 2). This explains why the functional hCDA is a tetramer. The tetramer contains two slightly different types of inhibitor recognition pockets, with those located across from each other in the tetramer being identical. One of these has a water molecule

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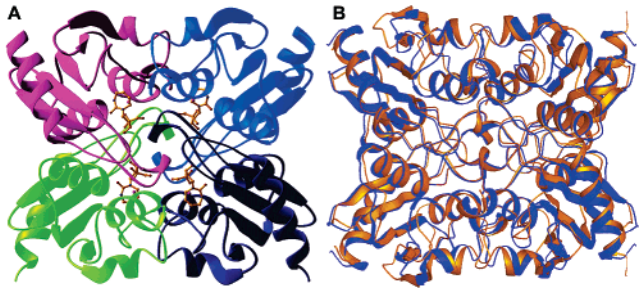

Figure 1. A. Ribbon diagram of hCDA bound to **1**. The four protamers that make up the homotetramer are each colored individually. The inhibitor **1** is shown in gold. B. Least-squares backbone superposition of the human (gold) and *B. subtilis* (blue) orthologs of CDA.

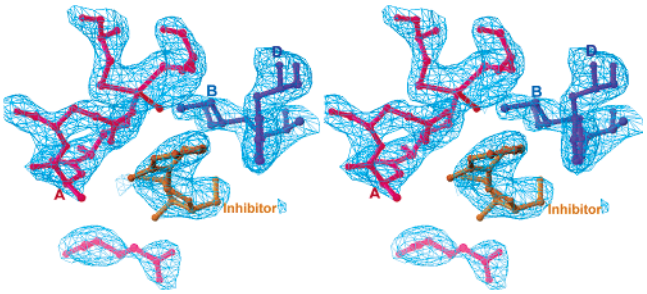

Figure 2. Superposition of the hCDA active site on a $F_o - F_c$ simulated annealing omit-electron density map contoured at 2.5 σ. (Red: subunit A; magenta, subunit B; blue, subunit D (Phe 137); gold, inhibitor).

coordinated to the active site zinc, whereas the other is only partially occupied. In the latter, electron density for some parts of **1** is weaker than for other parts, suggesting either multiple closely related binding modes or conformational flexibility. Owing to these local ambiguities in the latter type of binding pocket, we will focus our interpretation on the more well-defined of the two.

Inspection of the hCDA active site (Figure 3) reveals that the zinc ion is coordinated to three active site cysteine residues, Cys 65, Cys 99, and Cys 102. The fourth coordination site on the metal is not occupied by the inhibitor, but instead is bound by a solvent water molecule. Thus, **1** is unique among all structurally characterized CDA inhibitors in its lack of reliance on metal coordination to target the enzyme active site. This observation begs the question as to how **1** acquires its high affinity for the CDA active site, without taking advantage of the strong coordinate interaction so gainfully employed by other inhibitors. Is there some interaction available to **1** that compensates for the loss of metal coordination?

The majority of the interactions made by **1** to the active site of hCDA are quite similar to those made by **4** and **5** to the active sites of bacterial CDAs.[17,18] Namely, the sugar 3′-OH is hydrogen bonded to a conserved glutamate (Glu 56) and the 5′-OH is hydrogen bonded to the main-chain carbonyl and NH of Ala 58 and Tyr 60, respectively. In addition, the "nucleobase" C2=O and N3-H, which **1** shares in common with **4** and **5**, are engaged in conserved interactions with the hCDA active site. Namely, N3-H is hydrogen bonded to the side chain carboxylate of the essential residue Glu 67, as would the substrate be during the catalytic cycle.

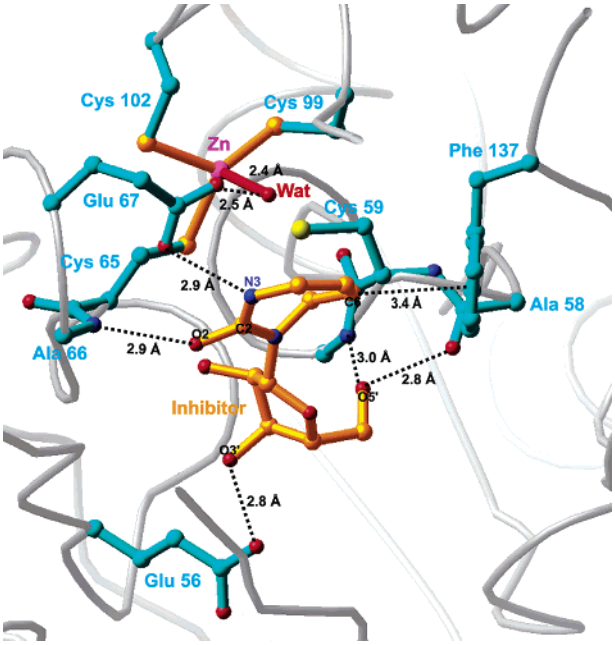

Figure 3. Recognition of **1** by hCDA. The inhibitor **1** makes direct contacts with three subunits of tetrameric hCDA: subunit A (Ala 58, Tyr 60), subunit B (Glu 56, Cys 65, Ala 66, Glu 67, Cys 99, and Cys 102), subunit D (Phe 137). Interestingly, Cys 59 is conserved in mammalian CDAs, but not in bacterial or yeast CDAs.

Furthermore, the O^2 atom of **1** is hydrogen bonded to the main chain NH of Ala 66.

In addition to its lack of suitable functionality to coordinate the zinc, **1** does contain one other major point of structural divergence from other CDA inhibitors. Whereas **4** and **5** contain relatively flat six-membered rings, the seven-membered diazepinone ring of **1** bears a pronounced pucker. As a result, the C5,C6 olefin juts out from the plane of the ring at an angle that would be impossible to attain with any six-membered ring. The edge of the C5,C6 olefin impinges directly on the π-face of Phe 137 to establish a virtually canonical π/π-interaction. This interaction, unique to **1**, provides the most likely origin for the ability of this inhibitor to bind tightly to hCDA without making use of metal coordination. Consistent with this notion, reduction of the double bond to the corresponding saturated system (**2**) results in a 16-fold loss in binding affinity.[22]

Amino acid variations (polymorphisms) at several amino acid positions of hCDA have been identified. One of these variations, namely substitution of threonine for alanine at position 70 (A70T hCDA), results in a greater than 50% reduction in catalytic activity toward Ara-C and cytidine than prototype hCDA,[23] leading patients with this polymorphism to exhibit increased sensitivity toward nucleoside chemotherapy. The present crystal structure reveals that Ala 70 is located on the same face of an α-helix as Glu 67 and Ala 68, one turn distal from the active site. Thr 70 may destabilize this helix or compete for hydrogen bonding with the side-chain carboxylate of Glu 67, thereby decreasing its availability to function in activating the nucleophilic water.

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Supporting Information Available: The X-ray crystal structure of hCDA in complex with **1** has been deposited with the PDB, accession number 1MQ0. A table of the data collection and model statistics and experimental details are available free of charge via Internet at http://pubs.acs.org.

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