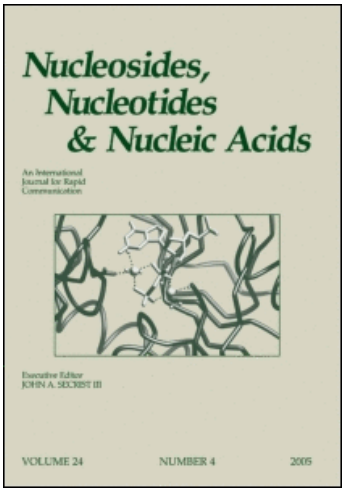

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Human Cytidine Deaminase: Understanding the Catalytic Mechanism

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Human Cytidine Deaminase: Understanding the Catalytic Mechanism

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ABSTRACT

In the absence of an experimentally elucidated three-dimensional structure of the human CDA, we built an homology model of this enzyme starting from the crystal structure of its *E. coli* homologous. Furthermore, we docked in the active site alternatively the substrate, the intermediate or the product. By means of molecular dynamics simulations, we determined the topology of the active site, identifying the amino acids involved in the catalytic mechanism, and outlining the central role played by E67.

Cytidine deaminase (CDA, EC 3.5.4.5) is an homotetrameric zinc-protein belonging to the pyrimidine salvage pathway, which catalyzes the deamination of cytidine and deoxycytidine. Furthermore, CDA deaminates also several cytosine nucleoside based drugs used as antineoplastic and antiviral agents causing the loss of their therapeutic efficiency.[1,2]

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Since no experimentally elucidated three-dimensional structure of the human CDA exists, in order to get an insight into the human CDA active site we built an homology model of this enzyme[3] starting from the crystal structure of its *E. coli* homologous.[4,5,6]

After the construction of the protein model, a cytidine molecule plus a water molecule, a deamination intermediate or a uridine molecule plus a leaving ammonia were alternatively docked into the four active sites of the enzyme, by superimposing the catalytic domain of the *E. coli* CDA to each of the four human monomers. With the same technique a zinc ion per monomer was added and coordinated with the sulfur atoms of C65, C99 and C102, as indicated by our mutagenesis experiments.[1,2]

The three complexes were then submitted to an energy minimization, followed by 50 ps of molecular dynamic simulation at 300°K and by a final energy minimization.

Homology modeling, energy minimizations and molecular dynamics simulations were carried out utilizing the Molecular Operating Environment (MOE).[7] For molecular mechanics calculations the AMBER 94[8] force field was utilized with a distance dependent dielectric constant scaled by a factor of 4.

All the energy minimizations were carried allowing to move first the hydrogen atoms, then the side chains, then everything but of the α-carbons and finally the whole protein. The truncated Newton method was always employed until an RMS of 0.001 kcal/mol/Å was reached.

RESULTS AND DISCUSSION

The human CDA monomer shows a β-sheet motif (two parallel and two antiparallel strands) surrounded by one α-helix on one side and two α-helices on the other side. Four identical monomers (A, B, C and D) are assembled together to form the biologically active homotetramer. The subunits A and B of the human CDA are related by a two fold symmetry and, with a rotation about a perpendicular 2-fold symmetry axis, can be superimposed to the subunits C and D. Thus the human CDA homotetramer shows overall 222 symmetry.

Analyzing the human CDA quaternary structure it seems evident that three different subunits concur to the formation of each of the four active sites (Fig. 1).

The nucleosides are held in position by a network of hydrogen bonds with amino acid residues in the active site: O-2 is hydrogen bonded with the backbone amide of A66 from subunit A; the OH-2′ group of the pentose forms hydrogen bonds with N54 and E56 of subunit A, while the OH-5′ is hydrogen bonded with the backbone amide of Y60 subunit D (Figure 1a,b). F137 residue coming from subunit C contributes to the generation of the uridine binding pocket. In this way a tetramer contains four independent active sites, while no complete active site is found within a single monomer. The carboxylate group of E67, corresponding to E104 in *E. coli*, plays a central role in the mechanism of catalysis. This residue, in fact, is involved in the ionization of a zinc-bound water molecule, in the

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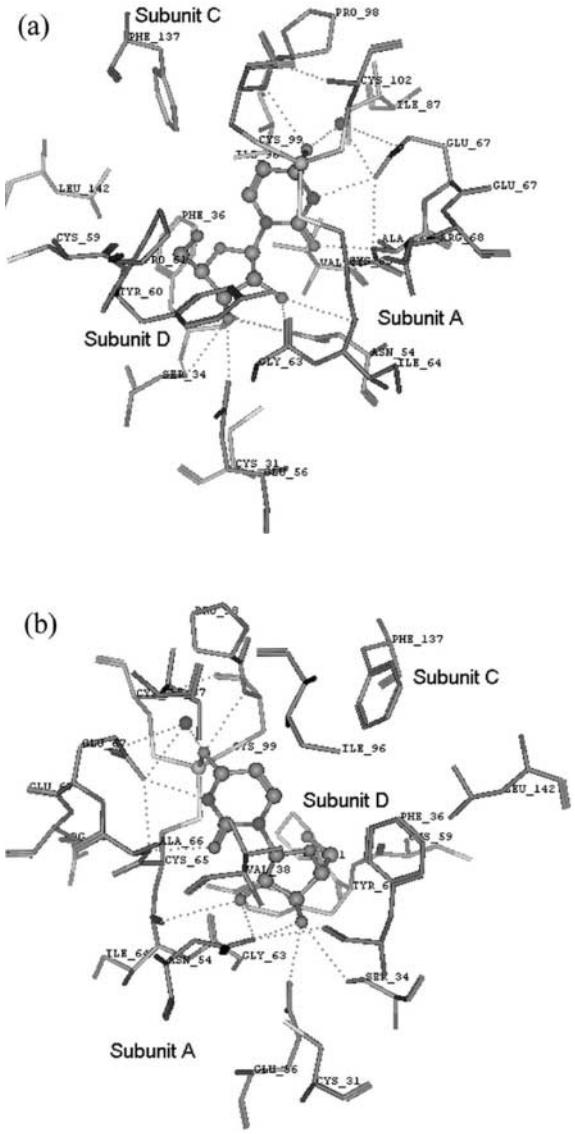

Figure 1. Two different views of the human CDA active site.

polarization of the resulting hydroxide ion and, finally, in the protonation of cytidine N-3 (Fig. 2).

CONCLUSIONS

Despite of the structural differences and the low homology of the amino acid sequences between *E. coli* and human CDA (about 30% of identity), our model appears very reliable according to the experimental data. Furthermore, its soundness

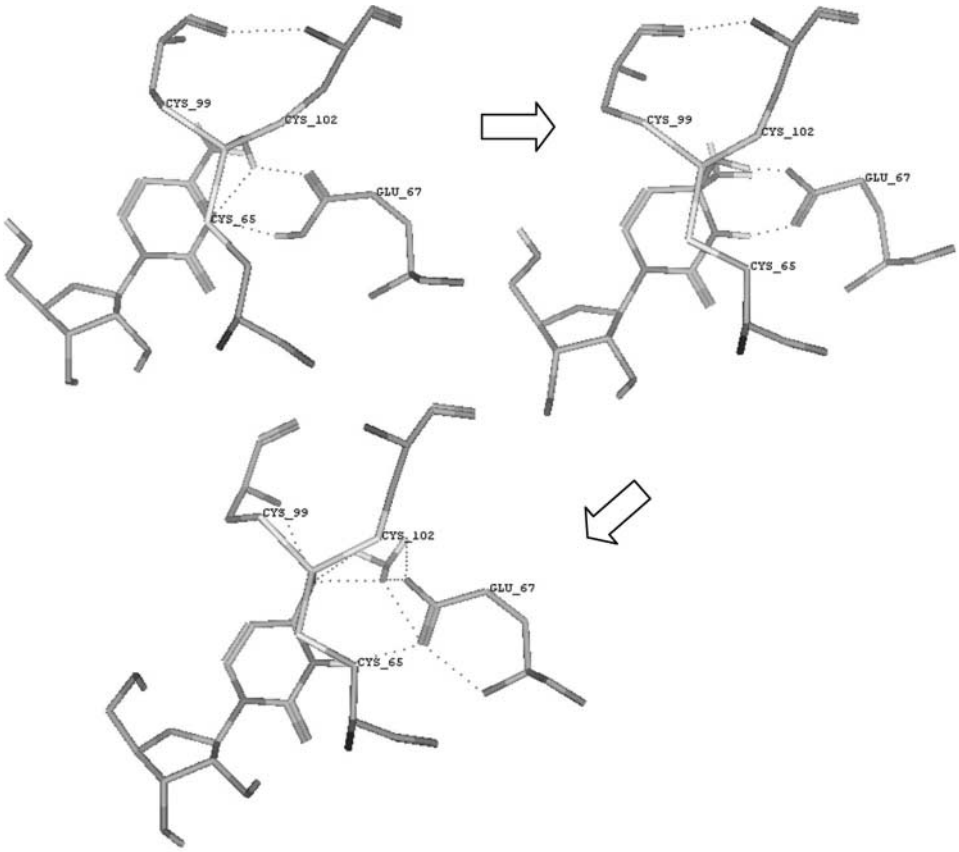

Figure 2. Human CDA catalytic mechanism.

is strengthened by the recent publication reporting the crystal structure of CDA from *Bacillus subtilis*,[9] which is much more strictly related to the human enzyme.

Therefore, with this model we gained a good knowledge of the human CDA structure. This will be the starting point for a structure-based drug design aimed at the discovery of new inhibitors to be used in association with those antiviral or antitumoral drugs that are inactivated by deamination.

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