

Molecular dynamics simulations of active site mutants of rat liver arginase

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By using molecular dynamics (MD) simulations and crystallographic data for rat liver arginase, the substrate positions in the active sites of native and mutant forms of the enzyme, were compared and correlated with known kinetic consequences of mutations. The mutants compared were His 141®Phe and His 141®Asn. The simulations show that mutation His141®Asn gives the greatest divergence from the atomic coordinates, when compared with the control native enzyme. The mutant Asp128®Asn does not show a change in atomic coordinates in the substrate, in agreement with the concept that a change in the metal coordination is responsible for the loss of catalytic activity in this mutant. Results obtained agree with reported kinetic consequences of mutations in arginase.

Arginase catalyzes the hydrolysis of arginine to urea and ornithine. The enzyme is highly distributed in living organisms, in which it serves several functions (Aminlari and Vaseghi, 1992; Carvajal et al. 1995), including a role in the regulation of nitric oxide signaling (Chang et al. 1998).

The crystal structure of rat liver arginase (Kanyo et al. 1996) shows a 15 Å deep active-site cleft in each monomer of the homotrimer, with two Mn²⁺ ions at the base of this cleft. Aspartate and histidine residues coordinate the metal ions and mutation of these residues has been shown to cause a lowering or loss of catalytic activity of the enzyme

(Cavalli et al. 1994; Perozich et al. 1998; Carvajal et al. 1999).

The mutation His 141→Asn leaves the enzyme with only 10% of the activity of the native enzyme (Cavalli et al. 1994) and determines a significant increase in the K_m value for arginine. In fact, the K_m value for wild type arginase is approximately 1.5 mM compared to > 40 mM for the mutant enzyme (Khangulov et al. 1998). On the other hand, binding of the competitive inhibitor lysine is about 10 times weaker in the mutant His 141→Asn than in the wild type enzyme (Khangulov et al. 1998). The mutation His 141→Phe also results in a loss of activity with a residual activity of about 10%, but in this case there is no change in the value of K_m (Carvajal et al. 1999).

For a better understanding of the kinetic consequences of replacement of His141 in arginase, we have now used molecular dynamics to compare substrate and inhibitor positions in the active sites of native and mutant forms of the enzyme.

Material and Methods

Structures used and data management

The crystal structure of rat liver arginase was used as the basis of this study. As rat liver arginase is a homotrimer and

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it is known that each monomer is active, all of the studies were done only with the chain A of IRLA (Kanyo et al. 1996). The identical B and C chains along with the water molecules that had been crystallized with them were removed. Water molecules in the active site, that obstructed the optimization of arginine but which were not coordinated with the metal ions, were also removed.

The substrate arginine and the inhibitor lysine were inserted in the enzyme using the Insight II program (Biosym, 1995). The structure of arginine was inserted in an extended configuration with the guanidine group close to the metal ions and the main chain in the opposite direction. The structure used for arginine was that generated by the HyperChem database (Hypercube, 1992). Coordinates for lysine were those of arginine in the active site, changing only the atoms necessary to make arginine into lysine. Insertions were accomplished by saving the monomer coordinates in PDB (protein database) format and then transferring them to the Insight II program. This program was used at this point because of the ease it provides for manually localizing the substrate in the active site. After making the insertion the enzyme-substrate complex was returned to HyperChem and saved as Hin format used by HyperChem. Optimizations were done with AMBER 4.1 force field using a cutoff of 8.0 Å. Residues were modeled as charged forms in accordance to the pH of enzyme activity and the dielectric constant was assumed to be 1.0. The conjugate gradient algorithm from Polak-Ribiere was used with 200 iterations or 0.01 kcal/(Å mol) as termination condition.

Substrate and inhibitor positions

Once inserted in the active site, the arginine was optimized to find the position of minimum energy for the substrate. In this process only arginine was allowed to move. The residues around the active site were held in position and the result of their forces on the substrate was studied. Since there was no charge available for Mn^{2+} at the time of this work, the metal ion was represented by the mass of Mn and its approximated van der Waals radius in the MD calculations. The same procedure was followed for lysine.

Molecular Dynamics

After obtaining the optimized structure for arginine in the active site, MD was used to find the most stable average substrate position. Collection of data was done only after systems were equilibrated and total energy remained constant. Two series of MD simulations were carried out in order to fix the atomic coordinates that would be used as the control for the substrate and to find the optimum times and temperatures to be used. These conditions remained constant in the rest of the simulations and constitute the basis for comparison. One simulation was done for 20 ps at 300 K and the other for the same time period but at 500 K. Each simulation was done in two 10 ps parts. In each case

the heating and cooling times were 0.1 ps and the step size was 0.001 ps. Data was collected every 5 time steps. Then, the snapshot files (data collected every 25 steps), were used to find the average positions. Since the two sets of simulations yield the same atomic coordinates for the substrate, the MD using 500 K was chosen and used to compare the atomic coordinates of the substrate or inhibitor in the wild type and mutants enzymes.

The arginine was fixed in the active site using coordinates of the average position and then optimized again. This was the structure that was used as the basis of all comparisons. To find the average atomic coordinates for arginine in the MD simulation, the coordinates from the snapshot file of MD were converted to a text file using a script file, was used to run a replay of the MD and write the coordinates to a text file. For the analysis of the data an MS Access database was created. This same process was repeated to find the average displacement of the substrate or inhibitor from the atomic coordinates established for the control.

MD Simulation of mutated arginase

Mutations examined were His 141→Asn (Cavalli et al. 1994); His 141→Phe (Carvajal et al. 1999). Insight II was again used to make the mutations. Then, the complex was returned to HyperChem and all the precautions mentioned above were followed to assure that the only changes were the mutation itself. An optimization of 50-150 cycles was used to minimize the energy of the switched residue and to find the most likely natural orientation of the residue. Atoms in a spherical area of 4 to 5 Å around the mutant residue were allowed to move. After the atomic coordinates of the mutant residue were secured, the substrate was again optimized and the molecular dynamics simulations repeated following the same protocol as in the wild type. The change in the average atomic coordinates of the substrate was noted and compared to the average position in the wild type.

Results and Discussion

To successfully prepare a control position for the substrate, it was necessary to remove the crystallized water molecules that were at the active site, while retaining the water molecule involved in the coordination of the metals ions. It was assumed that the exclusion of these crystallized water molecules from the active site occurs spontaneously in the active enzyme.

The two MD series at 300 K and 500 K yielded the same atomic coordinates for the arginine substrate. This was interpreted as confirmation of the probable position of the arginine in the active site during catalysis. To further confirm this position, the resulting structure was compared to that produced by the optimization of the substrate in *Bacillus caldovelox* (Bewley et al. 1999) followed by MD.

There was close agreement between the two complexes. When the two structures were superimposed the guanidine group and the amino and carboxyl groups occupied nearly the same position.

Since the MD series at 300 K and 500 K gave the same results, the model that resulted from the 500 K process was chosen as the basis for further studies. [Figure 1a](#) indicates the position that the substrate assumes in the active site during the MD. The substrate orients with the guanidinium group toward the metals with a distance of approximately 4.5 Å. The carbon chain of the arginine is approximately 4 Å from His141. The carboxyl group is about 3.6 Å from Asp 181 and 6.2 Å from Gly18.

After optimization, the substrate was seen to have taken a position slightly out of the active site of the H141F mutant enzyme, [Figure 1b](#). During MD the substrate maintained its attraction for the active site and repeatedly moved out of and then backs into the active site, while maintaining its extended structure. However, the substrate never completely returned to the optimized position that it had before the mutation. A much more drastic change was produced by mutation of His141 by asparagine. The substrate never assumed a fixed position and continuously travelled around the H141N enzyme molecule, [Figure 1c](#) and [Figure 1d](#).

Mutant Asp128→Asn had no effect on substrate position as shown [Figure 2a](#) and [Figure 2b](#) in spite of the small change in Asn position. Since lysine showed a broad spectrum of different positions into and outside the active site, seems not possible to find a direct relationship with its kinetic data ([Figure 3a](#), [Figure 3b](#) and [Figure 3c](#)). This inhibitor showed a high mobility but also a great deviation of its average position as is shown in [Figure 4](#).

In conclusion, theoretical MD simulations revealed significant differences in the average displacements of the substrate molecule in the active site of wild-type and mutant enzymes. Interestingly, the more drastic differences were noted for the H141N mutant. This is interesting if one considers the kinetic consequences of replacement of His141 by phenylalanine or asparagine. Although both H141F and H141N exhibit about 10% of wild-type activity, only the His141→Asn mutation is accompanied by a change in the K_m value for arginine (Khangulov et al. 1998; Carvajal et al. 1999). The fact that mutation to Phe involves a change in charge more than a change in shape, favors a role for His141 in the positioning of the substrate in the active site, as suggested by Bewley et al. 1999.

Theoretical MD simulations are of necessity very short and, for this reason; it is impossible to see all of the possible positions that may be occupied by a molecule. In any case, some conclusions may be drawn about the position of the substrate in the enzyme molecule. On the other hand, the

usefulness of the comparative approach used here and the validity of the conclusion that mutation of His141 by asparagine is significantly more drastic than a change by phenylalanine is reinforced by the observation of nearly the same position for the substrate in wild-type and D128N enzymes. Asp-128 is known to interact with the metal ion and not with the substrate in arginase (Kanyo et al. 1996; Perozich et al. 1998).

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Appendix

Figures

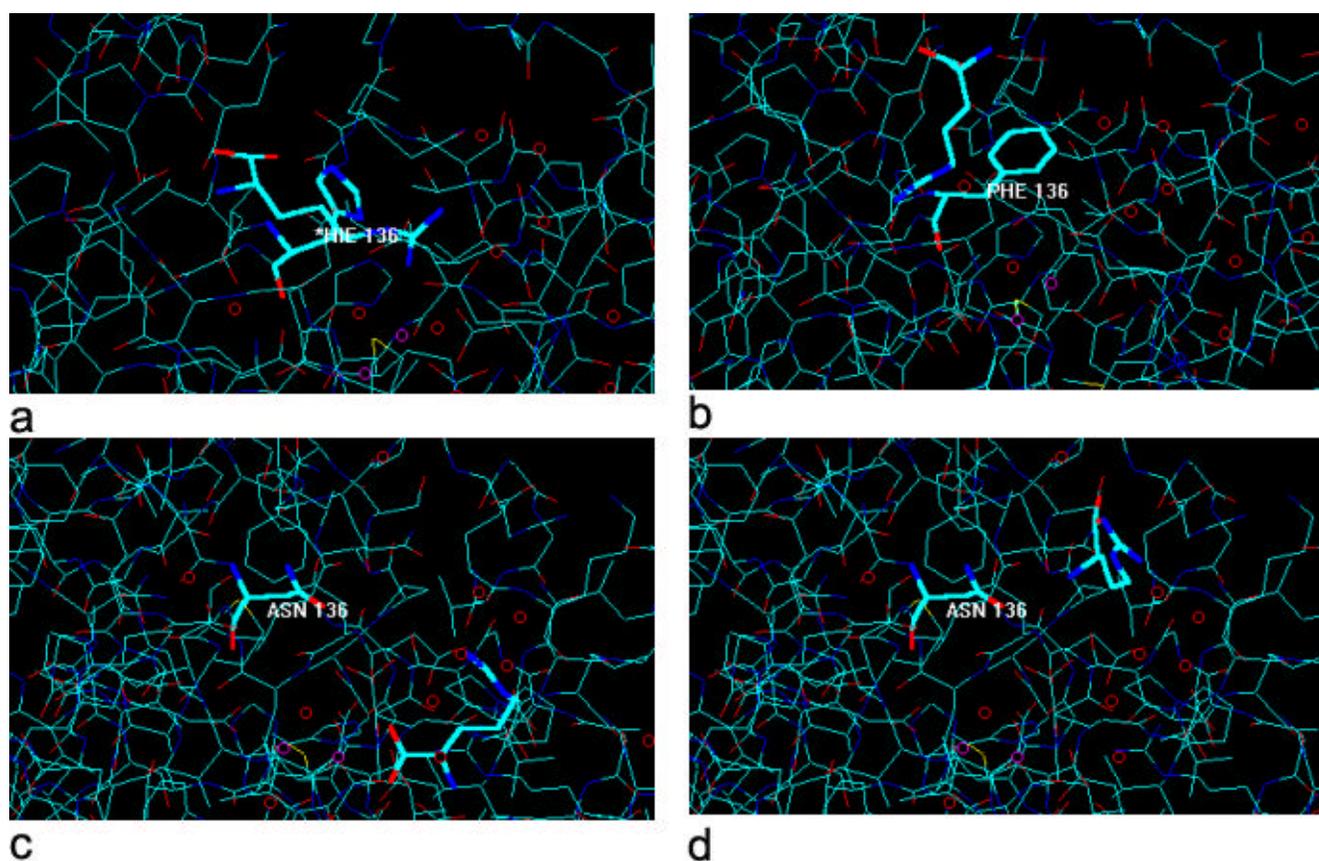


Figure 1. Positions of substrate and residue 141 (residue 136 according PDB file 1RLA) after 20 picoseconds of MD at 500 K.

- Position of substrate arginine in the wild type enzyme.
- Position of arginine in mutant His 141→Phe.
- and d) Positions of arginine in His 141→Asn mutant which are only two of the many and broadly extended positions during the MD simulation. The orientation of the protein is approximately the same in all figures. Mn²⁺ ions are shown in purple for reference.

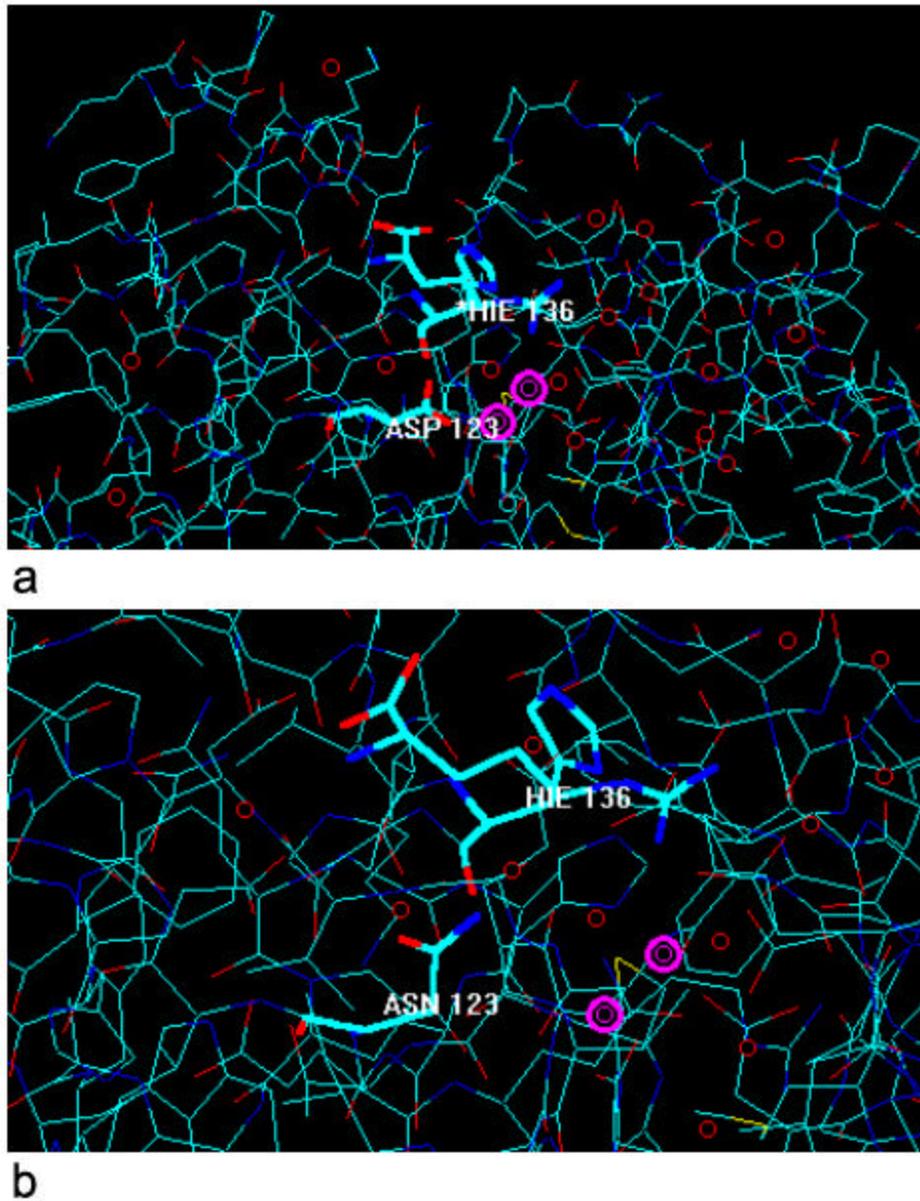


Figure 2.

- Position of arginine and Asp 128 (123 according PDB file 1RLA) in wild type arginase.
- Position of substrate and Asn 128 in mutant Asp→Asn. Note the position of the amide group assumed in Asn 128 in relation to carboxyl group of Asp 128. Mn²⁺ ions are shown in purple for reference.

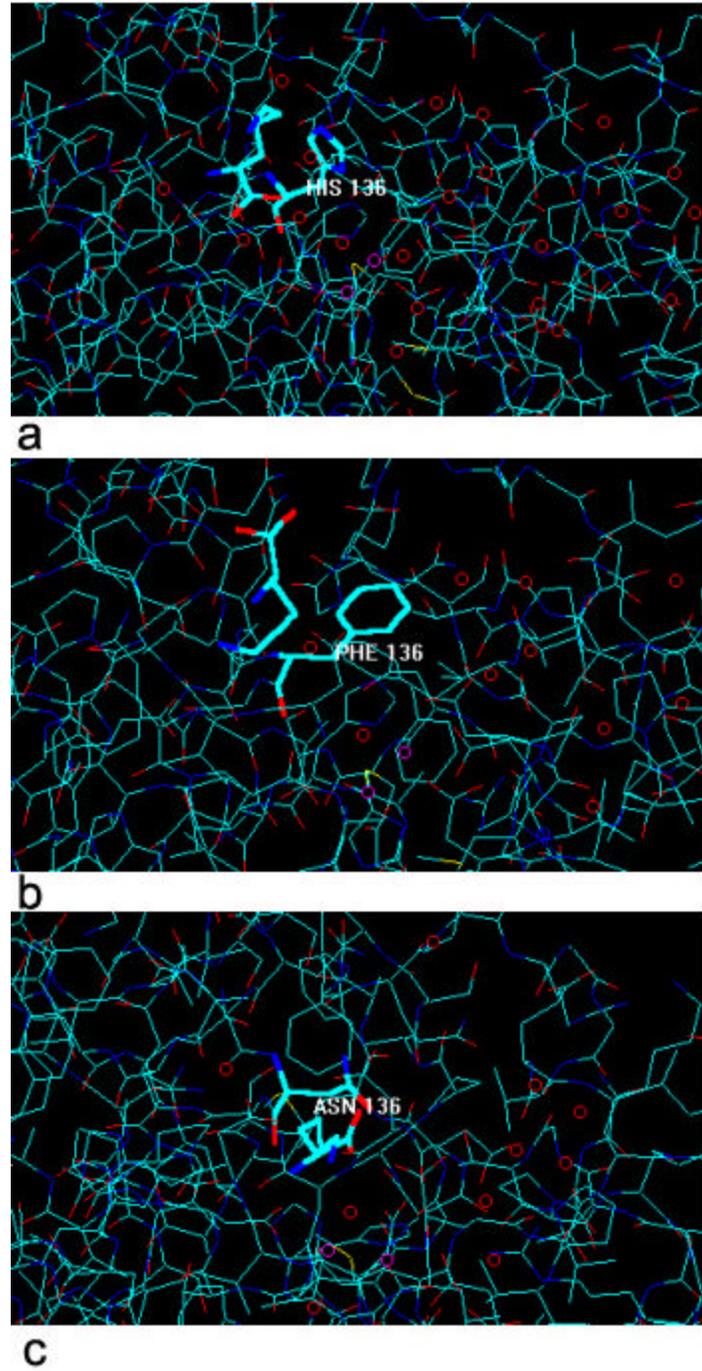


Figure 3. Positions of Inhibitor lysine in arginase. Positions assumed after 20 ps of MD at 500 K.

- a) Position in wild type arginase.
- b) Position in His 141→Phe mutant.
- c) Position in His 141→Asn mutant.

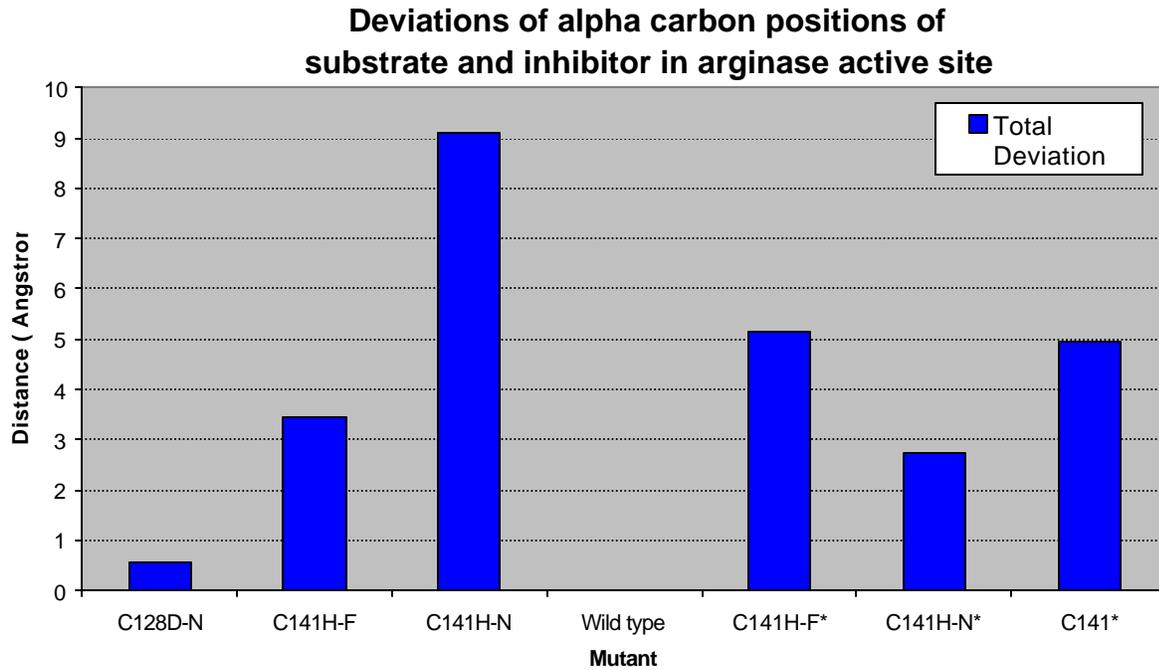


Figure 4. Average displacements of the substrate and inhibitor compared with position of substrate in wild-type enzyme.

C128D-N, arginine in Asp 128→Asn mutant;

C141H-F, arginine His 141→Phe mutant;

C141H-N, arginine in His 141→Asn mutant;

C141H-F*, lysine in His 141→Phe mutant;

C141H-N*, lysine in His 141→Asn mutant;

C141*, lysine in wild type enzyme.