

## A molecular modeling study of the urease active site

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### Abstract

Urease catalyzes the decomposition of urea to ammonium and carbamate ions by its active site which contains two nickel(II) atoms. Here we report the results of a molecular dynamics investigation performed on a system constituted of the cavity of the enzyme and one hydroxamic acid molecule which acts as an inhibitor of the protein. The results agree with experimental data and represent a valid starting point for the design of more efficient urease inhibitors. © 1997 Elsevier Science B.V.

*Keywords:* urease inhibitors; molecular dynamics; hydroxamates

### 1. Introduction

Urease is an enzyme that is present in many plants and in soil. It catalyzes the hydrolysis of urea to ammonium and carbamate ions, which decompose to carbon dioxide and ammonia. The active site contains two nickel(II) atoms which, as shown by X-ray analysis, are linked by a carbamate bridge. Furthermore, two imidazole nitrogen atoms are bound to each nickel atom, and a carboxylate group and a water molecule fill the remaining coordination site of the metal ion [1]. Among the known inhibitors of urease, the most efficient are phosphorodiamides, phosphotriamides [2] and hydroxamic acid derivatives [3].

In order to discriminate among the inhibition capacities of various compounds, it is important to understand the coordination mechanism between the active site of the enzyme and the inhibitor. The molecular

modeling techniques constitute a valid and powerful tool for investigating such systems, as demonstrated by the large number of papers dealing with these subjects [4–6]. Alternative docking models for the urea on the urease active site have been studied [2] which provided information about the reaction mechanism of the inhibitors.

In order to achieve a better knowledge of the urease inhibition mechanism we performed a Molecular Dynamics (MD) study on the formation of hydroxamate complexes with urease. The results, compared with a recent model [7] proposed to explain the inhibition of urease, show satisfactory agreement.

### 2. Materials and methods

The urease active site and the hydroxamic acid molecule were used as a model inhibitor and are shown in Fig. 1. The conformation of the inhibitor was determined by a Molecular Mechanics search

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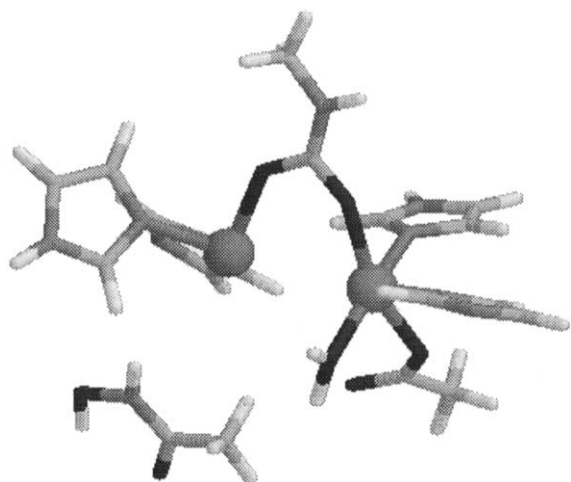


Fig. 1. The urease–hydroxamic acid system.

for the minimum energy conformation, while the coordinates of the urease active site were taken from the Brookhaven Protein Data Bank (PDB), ID = 1 kau [1]. As no information about the proton positions was given in the PDB structure, in order to keep the site total charge equal to zero, we suggested that the two imidazole rings of the binding site were in a protonate form, while the remaining two were in a  $-1$  charged deprotonated form. Each nickel atom coordinates to one protonated and one deprotonated imidazole ring. A water molecule completes the nickel coordination sphere. The coordination geometry of the first nickel

atom is pseudo-tetrahedral, while that of the second is roughly trigonal bipyramidal.

MD simulations were performed using the DLPOLY2 [8] program. The AMBER [9] and UFF [10] force fields were used with the necessary adaptations. The partial atomic charges were calculated by fitting the electrostatic potential computed by ab initio HF-SCF calculations at the 6-311G\*\* accuracy level with the MP2 perturbation treatment [11,12]. The Gamess program [13] was employed to perform both the ab initio and charge fitting computations. The values of the fitted charges are shown in Fig. 2.

The MD simulations were performed at  $T = 298$  K, with no applied periodic condition, as we were only concerned with studying the docking of the acid to the enzyme. The atoms of the active site were held rigid, while those of the inhibitor molecule and the water molecule were free to move. The cut-off for Coulomb forces was set to  $20 \text{ \AA}$ , so that all the interactions were included. Several trajectories were generated allowing the hydroxamic acid molecule to start from different positions, with the carbonyl oxygen, the oxime oxygen and the methyl group oriented towards the axis joining the nickel atoms,  $20 \text{ \AA}$  further away. Each trajectory was equilibrated for 50 ps using time steps of 0.001 ps, then a 2 ns run was performed on accumulated data, even though the final configurations were reached after 100 ps, and took about 30 000 sec on an IBM RS 6000 Mod. 355.

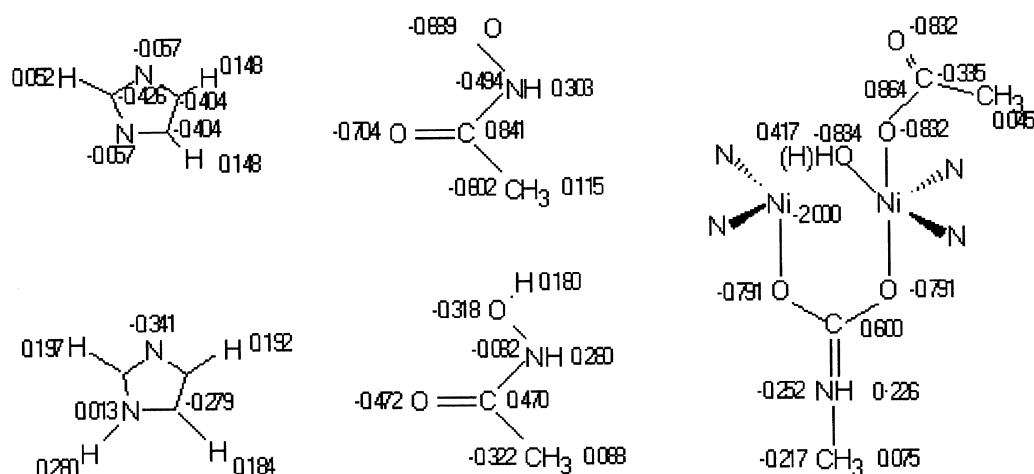


Fig. 2. Partial atomic charges of urease, hydroxamic acid and hydroxamate anion.

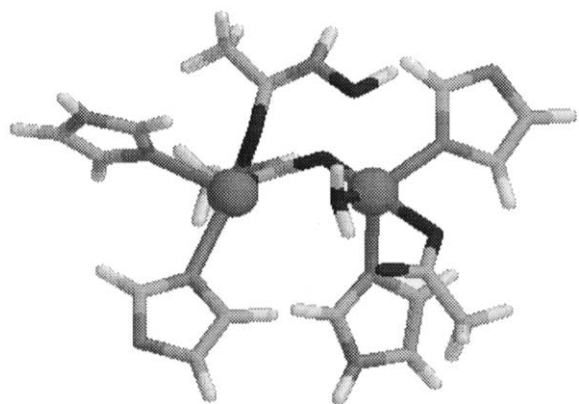


Fig. 3. A snapshot of the conformation of the urease–hydroxamic acid system after 2 ns trajectory.

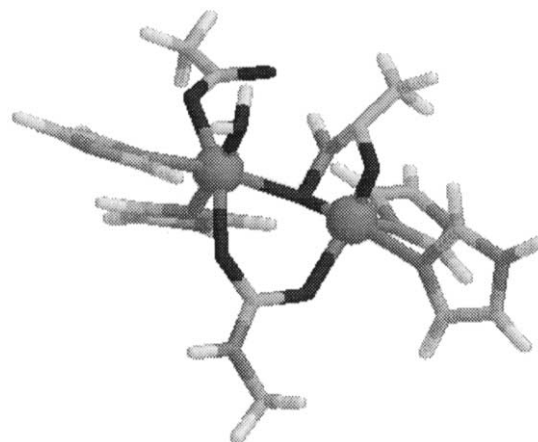


Fig. 4. A snapshot of the conformation of the urease–hydroxamate anion system after 2 ns trajectory.

### 3. Results and discussion

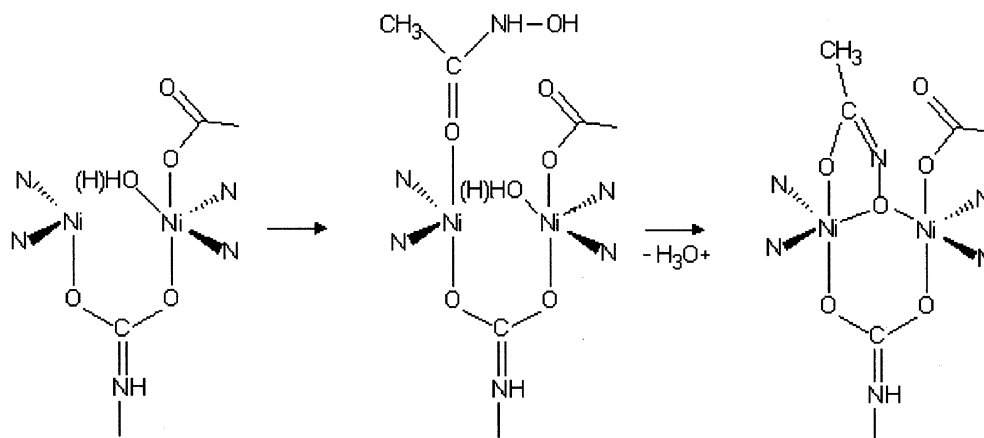
A snapshot of the trajectory of the urease–hydroxamic acid couple after 2 ns is shown in Fig. 3. The hydroxamic acid acts as a monodentate ligand, being bound to the nickel atom in a pseudo-tetrahedral coordination geometry with the carbonyl oxygen. This conformation agrees completely with the models proposed by Stemmler [6] and Lippard [14] for urea bound to nickel (see Scheme 1). In both cases the hydroxamic acid binds the nickel atom in the same way.

We performed several MD experiments changing the initial position and orientation of the hydroxamic

acid. In all cases we obtained the final conformation shown in Fig. 3.

The second step in Scheme 1 involves a deprotonated hydroxamate bridge between the nickel atoms which acts as a chelate on the first nickel atom. In order to reproduce these alkaline conditions, we removed a hydrogen atom from the hydroxamic acid and calculated the partial atom charges again (see Fig. 2). The initial conformation for this new set of MD simulations is shown in Fig. 3. After a 2 ns run we observed the final conformation represented in Fig. 4.

The agreement with the Stemmler model is satisfactory. The oxime oxygen acts as a bidentate ligand,



Scheme 1.

bridging two metal atoms and forming, by the carbonyl oxygen, a chelate with the first nickel. However, we did not observe the dehydration of the enzyme proposed by Stemmler [6].

#### 4. Conclusions

The results reported here indicate a good qualitative agreement with the experimental data and confirm the ability of molecular dynamics to describe the inhibition of urease and the reaction mechanism. The comparison with the model suggested by various other experimental data confirms that our approach reproduces the principal characteristics of the inhibition mechanism and may be used to design more efficient urease inhibitors. However, we have to point out that, owing to the reduced dimensions of the binding site adopted in our model, these results should be considered as only the first stage of a more extensive investigation on the urease inhibition process. Further development should consider atoms at larger distances from the nickel atoms (e.g. 10–15 Å) and also an explicit treatment of the solvent.

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