Theoretical investigation on the restoring step of the carbonic anhydrase catalytic cycle for natural and promiscuous substrates

Paolo Piazzetta, Tiziana Marino, Nino Russo *

Dipartimento di Chimica e Tecnologie Chimiche, Università della Calabria, Via P. Bucci, cubo 14c, 87036 Rende, Italy

ARTICLE INFO

ABSTRACT

In the present study steered molecular dynamics simulations were applied to investigate the unbinding process of the complex of human carbonic anhydrase with the natural HCO_3^- and promiscuous H_2NCOHN^- products. This process is crucial for restoring the catalytic cycle of the enzyme. This investigation set out to give further insights on the release mechanism involved in the case of the promiscuous product believed suicide inhibitor for the hCAII against the natural final product. In particular, on the basis of the NPT molecular dynamics simulations performed on the bicarbonate, the penta-coordinated complex with the water is observed, while in the case of the ureate the same event does not take place. At this purpose the calculated potential of mean force based on the steered molecular dynamics (SMD) simulations shed light on an optimal pathway for the releasing of the products.

© 2015 Elsevier Inc. All rights reserved.

Introduction

The great and fascinating catalytic power of enzymes has stimulated an increasing interest in the scientific community with the final goal to obtain detailed understanding of their working mechanism [1–7].

In order to efficiently catalyze a chemical reaction, enzymes are required to maintain fast rates for the formation of the Michaelis complex (ES),[1] the chemical reaction (formation of EP from ES) and product release (E + P). The turnover number (k_{cat}) is the result of a series of microscopic rate constants related to the formation (k_1) and dissociation (k_2) of the Michaelis complex (ES), the chemical event (k_3), and product release (k_4), as shown in Eq. (1).

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \overset{k_3}{\rightarrow} EP \overset{k_4}{\rightarrow} E + P \tag{1}$$

In the major parts of enzyme catalysis the chemical reaction process (k_3) represents the rate determining step but, in some cases, the occurring conformational changes required in the product release make the last step governed by (k_4) that having the lowest rate [8–12].

Recently, the substrate promiscuity of human carbonic anhydrase against small molecules isoelectronic with the native CO_2 has been studied at both experimental and theoretical levels [13–16].

The generally accepted catalytic mechanism of carbonic anhydrase is constituted by two fundamental steps [17]: the nucleophilic attack of Zn—OH moiety on CO_2 with the formation of HCO_3^- (a) and the replacement of bicarbonate product by a water molecule in order to restore the catalytic cycle (b)

$$E—Zn—OH^- + CO_2 \rightleftarrows E—Zn—HCO_3^- \tag{a}$$

$$E—Zn—HCO_3^- + H_2O \rightleftarrows E—Zn—H_2O + HCO_3^- \tag{b}$$
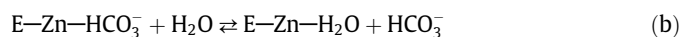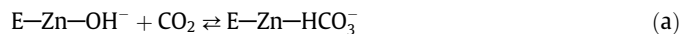
while the step (a) represented the main focus of different theoretical studies [15,18–20], the release of the product did not receive the same attention.

The carbodiimide molecule that binds to the active site and is catalyzed by hCA as proposed by experimental investigations [21–23] and recently confirmed by a theoretical study [15] gives an ureate product which results to be strongly bonded to the enzyme active site and consequently leads to the so-called "enzyme suicide" (i.e. the initially innocuous substrate is transformed in a chemical species unable to restore the catalytic cycle with consequent reduced turnover number). While the QM and QM/MM study of the chemical behavior of hCA towards carbodiimide [15] in comparison with the native CO_2 substrate was performed to gain insights into the main steps characterizing the catalyzed reaction, the physical process by which the final product is released to restore the catalytic cycle still remains unexplored.

---

* Corresponding author.

[1] *Abbreviations used:* SMD, steered molecular dynamics; MD, molecular dynamics; PME, Particle Mesh Ewald; PMF, potential of mean force; RMSD, root mean square deviation.

Starting from our previous mechanistic investigations on the hCAII activity towards carbon dioxide and carbodiimide [15], we have studied the final step of the reaction and the restoring of the catalytic cycle by using classical molecular dynamics (MD) and nonequilibrium steered molecular dynamics (SMD) simulation methods for the two products bicarbonate and ureate generated by the catalysis of CO_2 and carbodiimide substrates.

Computational details

MD simulations were carried out with NAMD 2.9 [24] using CHARMM [25] force field. The native binding poses for both HCO_3^-–hCAII and H_2NCOHN^-–hCAII complexes obtained by X-ray data (PDB Code 2VVB [26] and 1BV3 [21] respectively) were used as starting structures. In Fig. 1 is illustrated the active site for both considered systems. Both ligands required custom parameters for the force field. In the case of bicarbonate they were previously obtained [16] while for ureate ligand the parametrization procedure was necessary in order to create the topology and the input files required by CHARMM [25]. In Table 1 are reported the used parameters for the zinc coordination in the ureate–hCAII complex. The obtained models were built up combining bonded and non-bonded terms. Constraints on the zinc binding site were introduced using the Colvars module of NAMD with values of bonds, angles and dihedral arising from QM/MM calculations [15], which were restrained with harmonic constraints [27]. The charges were set using the NBO values arising from DFT calculations [15] and reported in Table 2. The constraints of the angles defined by substrate – Zn-histidine ligands were used during the MD simulation and removed in the subsequent SMD simulations. A water layer of 16 Å was built around each studied enzyme–product complex by creating a periodic water box of $80 \times 78 \times 89$ Å^3 for HCO_3^-–hCAII and $81 \times 78 \times 88$ Å^3 for H_2NCOHN^-–hCAII. The resulting number of atoms was to 52,101 for HCO_3^-–hCAII and 51,054 for H_2NCOHN^-–hCAII. Due to the neutral charge of the studied systems no further neutralization was needed. SHAKE [28] algorithm was practiced in order to constrain all bonds involving hydrogen atoms. The long-range electrostatic interactions were evaluated by Particle Mesh Ewald (PME) method [29]. The obtained supramolecular systems including protein, water molecules and the ligand (HCO_3^- or ureate) were minimized using the conjugate gradient algorithm. A time step of $\Delta t = 2$ fs was used for both the investigated systems. A cutoff of 12 Å for non-bonded interactions was applied and a switching scheme was used. Scaled 1–4 parameters were enabled for 1–4 interactions.

The final MD equilibration state was used as initial point for ten SMD simulations of 1 ns for both ligands. The external force was applied to the carbonyl carbon of each substrate which coincides approximately with the center of mass. In the adopted constant velocity SMD, the value of the spring force varies significantly depending on what pulling velocity and spring constant were applied to the system. We carried out 5 different pulling velocity simulations (ranging from 0.0125 Å/ps to 2.5 Å/ps) to identify the suitable velocity value for monitoring the detachment step of the two examined ligands. The "rupture" force was defined as the highest peak value of the pulling force. A pulling velocity of 0.025 Å/ps was chosen since better reproduces the behavior of both ligands. In order to compare the binding properties of the two complexes, the SMD simulations were carried out using the same velocity as previously suggested [30,31]. An important variable to be set is represented by the spring constant (k) whose value is strictly related to the background noise that could take place [32,33].

Table 1
Parameters for the zinc coordination in the ureate–hCAII complex.

Atoms	Force constant	Equilibrium
Bonds	k	r_e
Zn N$_{119}$	30 kcal/mol/Å^2	2.10 Å
Zn N$_{94}$	30 kcal/mol/Å^2	2.04 Å
Zn N$_{96}$	30 kcal/mol/Å^2	2.05 Å
Angles	K	θ_e
N$_{119}$ Zn N$_{96}$	23 kcal/mol/rad^2	114.0°
N$_{96}$ Zn N$_{94}$	23 kcal/mol/rad^2	100.0°
N$_{94}$ Zn N$_{119}$	23 kcal/mol/rad^2	95.0°
O$_{ureate}$ Zn N$_{119}$	0.05 kcal/mol/rad^2	111.5°
O$_{ureate}$ Zn N$_{94}$	0.05 kcal/mol/rad^2	121.0°
O$_{ureate}$ Zn N$_{96}$	0.05 kcal/mol/rad^2	110.0°
Improper dihedrals	K	ϕ_0
Zn N$_{119}$ N$_{96}$ N$_{94}$	25 kcal/mol/rad^2	0

Table 2
NBO charges values (in e) for the indicated atoms of the two investigated substrates.

HCO_3^-–hCAII		H_2NCOHN^-–hCAII	
Zn^{2+}	1.635	Zn^{2+}	1.597
Zn^{2+}—**O**—C	−0.966	Zn^{2+}—**N**H1—C	−1.147
O—**C**=O	1.031	Zn^{2+}—N**H1**—C	0.430
O—C=**O**	−0.733	NH1—**C**=O	0.798
O—C—**O**H	−0.760	NH1—C=**O**	−0.820
O—C—O**H**	0.542	NH1—C=H2**N**H3	−0.916
		NH1—C=H2N**H3**	0.430
		NH1—C=H2N**H3**	0.438

Fig. 1. The reported active site residues correspond to His94, His96, His119 belonging to the inner coordination shell of the metal ion and Glu106, Thr199, Thr200 belonging to the outer one.