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Reaction mechanism of matrix metalloproteinases with a catalytically active zinc ion studied by the QM(DFTB)/MM simulations

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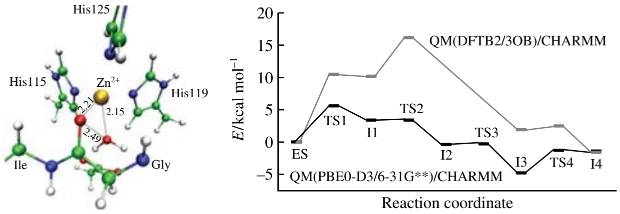
The simulations of oligopeptide hydrolysis by the matrix metalloproteinase MMP-2 using the density functional tight binding (DFTB) quantum chemistry method and the QM/MM methodology partly reproduce the qualitative features of the reaction mechanism but show deviations of the computed patterns from those obtained with the conventional DFT-based approaches.



Matrix metalloproteinases (MMPs) are the zinc-dependent enzymes regulating proteolysis in an extracellular matrix whose malfunction is associated with severe diseases. Knowledge on the reaction mechanism of oligopeptide hydrolysis by a particular type of MMPs is essential to propose efficient inhibitors capable to control the enzyme activity.[1] Considerable efforts have been undertaken to reveal the details of chemical transformations at the active sites of MMPs using the quantum mechanics–molecular mechanics (QM/MM) theory.[2–4] The choice of the QM approaches, which provide the accurate but inexpensive calculations of energies and forces in relevant model systems, is an important issue in computational enzymology.[5] This problem is especially important for metalloenzymes containing catalytically active metal ions.

The method of self-consistent charge density functional tight binding (SCC-DFTB),[6] or DFTB in short, has evolved from the conventional density functional theory (DFT) by a second-order expansion of the total energy around a reference density. This approach promises great savings in computer simulations of complex chemical processes due to a number of approximations in the electronic structure framework. The DFTB parameters are fitted to the reference data computed independently within the DFT technique. Like any parameterized simulation tool, the DFTB method should be carefully tested to guarantee its correct use in applications. The initial second-order DFTB approach (DFTB2) has recently been extended to the third-order DFTB3 method aiming to improve the description of systems with hydrogen bonds and localized charges.[7] The 3OB parameterization for the most common atoms (H, C, N, O, P and S)[8,9] and metal ions[10] including Zn^{2+} promises an application of DFTB to a wide range of important systems including metalloenzymes.

In this work, we compare the performance of the DFTB2 and DFTB3 approaches with the 3OB parameter set to that of the conventional DFT treatment in simulations of a proteolysis reaction catalyzed by a zinc-containing metalloproteinase type 2 (MMP-2) at the QM/MM level. The initial coordinates of the model system for the QM/MM calculations were taken from the crystal structure of the catalytic domain of MMP-2 from the Protein Data Bank (PDB ID: 1QIB[11]). The oligopeptide Ace-Gln-Gly~Ile-Ala-Gly-Nme was used as a substrate (the tilde specifies the scissile peptide bond). Construction of the system and details of the QM-MM partitioning were described previously.[4] The QM region included the Zn^{2+} ion, its coordination sphere, a part of the substrate and two water molecules (108 atoms in total). The energy and energy gradients in the QM region were computed at the DFTB2 and DFTB3 levels of theory with the

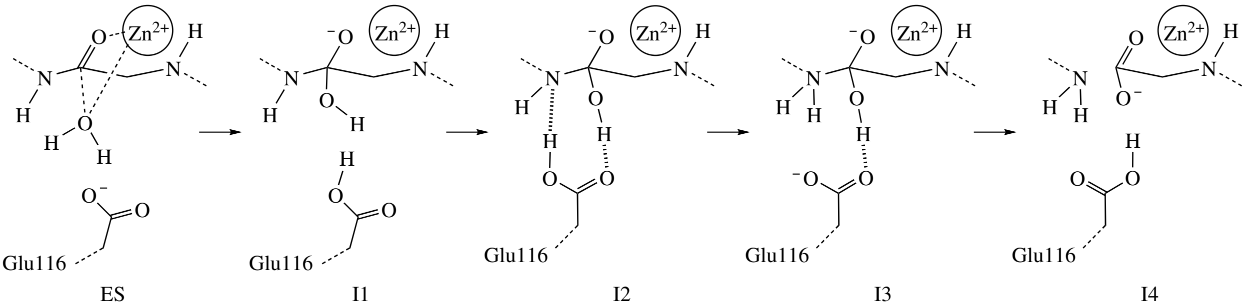


Figure 1 Reaction mechanism of peptide bond hydrolysis in the active site of MMP-2.[4]

Table 1 Key interatomic distances (Å) at the stationary points along the reaction path from the QM(PBE0-D3/6-31G**)/MM (denoted here as PBE0), QM(DFTB2/3OB)/MM (DFTB2) and QM(DFTB3/3OB)/MM (DFTB3) calculations.

Stationary point	Key distance	PBE0	DFTB2	DFTB3
ES	Zn···O-Gly	2.22	2.21	2.24
	Zn···O-Wat	2.02	2.15	2.23
	Gly-C···O-Wat	2.43	2.49	2.51
I1	Zn···O-Gly	1.93	1.92	
	Zn···O-Wat	2.41	2.68	
	Gly-C···O-Wat	1.49	1.56	
I3	Zn···O-Gly	2.01	1.99	2.03
	Zn···O-Wat	2.18	2.65	2.62
	Gly-C···O-Wat	1.38	1.34	1.36
I4	Zn···O-Gly	2.01	1.94	1.98
	Zn···O-Wat	2.28	2.85	2.78
	Gly-C···O-Wat	1.28	1.27	1.29

3OB parameters. The MM part was modeled by the CHARMM force field.[12] All QM/MM calculations were performed using the CHARMM program package.[13]

According to the QM(PBE0-D3/6-31G**)/MM (DFT/MM in short) simulations,[4] the reaction mechanism consists of four steps (Figure 1): a nucleophilic attack of the lytic water molecule on the carbonyl carbon of the scissile bond, hydrogen bond rearrangement, proton transfer, and C–N bond cleavage in the substrate. These DFT/MM calculations consistently yield rather low overall barriers for the chemical steps, in a range of 5–10 kcal mol^{-1}. As described below, the results of the QM(DFTB2/3OB)/MM (DFTB2/MM) and QM(DFTB3/3OB)/MM (DFTB3/MM) calculations are partly consistent with this mechanism. Table 1 collects the data on the key distances along the reaction pathway.

The DFTB2/MM geometry of the ES complex is fairly close to the DFT/MM reference structure (Table 1); however, the energy barrier (TS1) is about 5 kcal mol^{-1} higher than that of the DFT/MM approach. The first intermediate I1 is barely stable on the DFTB2/MM potential energy surface; the Zn···O-Wat distance is 0.27 Å larger than that in DFT/MM (Table 1). Discouragingly, the DFTB3/MM potential energy surface shows no minimum energy structure corresponding to intermediate I1 [Figure 2(*b*)]. Variations of newly introduced ζ and Hubbard derivatives parameters in DFTB3 do not improve the results.

Potential energy profiles of the second and third steps of the reaction are shown in Figure 2(*c*). They correspond to hydrogen bond rearrangement and proton transfer to the nitrogen atom of the substrate (Ile-N). The DFTB2/MM potential energy surface shows a single transition state (with a barrier of 6 kcal mol^{-1}), which directly leads to intermediate I3 skipping I2. There is a substantial barrier in the DFTB2/MM profile, while these steps are nearly barrierless at the DFT/MM level [Figure 2(*c*)]. The Zn···O-Wat distance in I3 differs from the DFT/MM reference values by 0.47 and 0.44 Å for DFTB2/MM and DFTB3/MM, respectively. In the DFTB2/MM and DFTB3/MM structures of I3, the Ile-N···C-Gly bond is already cleaved, and the C–N distances are 2.04 and 1.96 Å, as compared to 1.60 Å in the DFT/MM reference structure.

The fourth step in the DFT/MM protocol corresponds to the Ile-N···C-Gly rupture and concomitant proton transfer from Gly to the side chain of Glu116, which corresponds to an energy increase. When using DFTB2/MM, this step mainly involves proton transfer because the Ile-N···C-Gly bond has already been cleaved in the preceding step, and there is a drop in the energy profile. In the structure of intermediate I4, the Zn···O-Wat distance is overestimated at the DFTB2/MM and DFTB3/MM levels (by 0.57 and 0.50 Å, respectively).

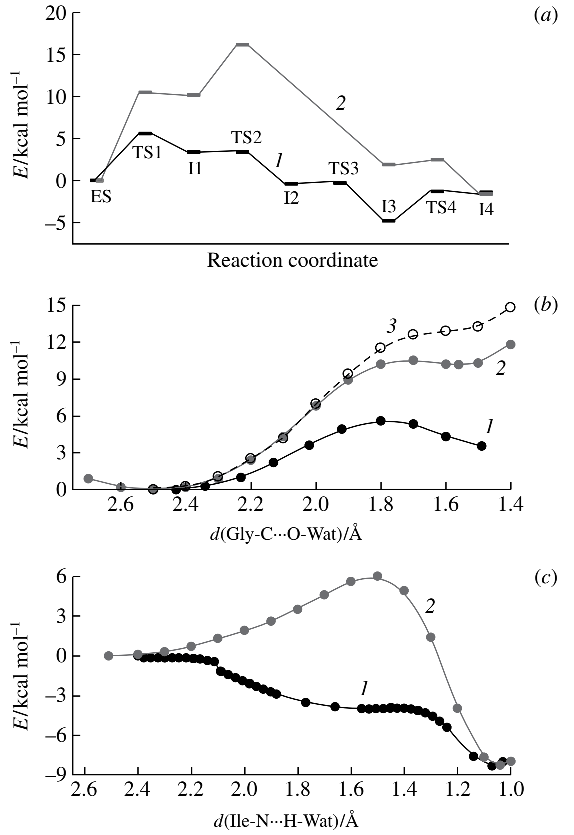


Figure 2 Potential energy profiles for (*a*) the overall reaction, (*b*) the first step of the reaction and (*c*) the second and third steps of the reaction. (*1*) QM(PBE0-D3/6-31G**)/CHARMM, (*2*) QM(DFTB2/3OB)/CHARMM and (*3*) QM(DFTB3/3OB)/CHARMM simulations.

The overall potential energy diagram for the catalytic reaction [Figure 2(*a*)] shows two high energy barriers of 10.5 (step 1) and 6 kcal mol^{-1} (steps 2, 3) for DFTB2/MM compared with a single step (step 1) with a high barrier of 5.5 kcal mol^{-1} for DFT/MM.

We conclude that the DFTB-based methods modestly reproduce the qualitative features of the reaction mechanism of oligopeptide hydrolysis by the enzyme with the catalytically active zinc ion. However, the description of a zinc coordination shell at the reaction intermediates lacks of the proper accuracy. The present calculations show that DFTB2/3OB performs better than DFTB3/3OB by describing the first step of the reaction in a qualitatively correct manner. The following steps of the reaction involving the H-bond rearrangements and proton transfer events are described rather poorly by DFTB2, which yields energy profiles that differ qualitatively from the reference DFT counterparts. The DFTB-based computational protocols are fast enough to be used in QM/MM molecular dynamics and free energy calculations of enzymatic reactions; however, from the quantitative side these applications are not warranted.

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References

1 R. E. Vandenbroucke and C. Libert, *Nat. Rev. Drug Discov.*, 2014, **13**, 904.
2 V. Pelmenschikov and P. E. Siegbahn, *Inorg. Chem.*, 2002, **41**, 5659.
3 N. Díaz and D. Suárez, *J. Phys. Chem. B*, 2008, **112**, 8412.
4 T. Vasilevskaya, M. G. Khrenova, A. V. Nemukhin and W. Thiel, *J. Comput. Chem.*, 2015, **36**, 1621.

5 H. M. Senn and W. Thiel, *Angew. Chem. Int. Ed.*, 2009, **48**, 1198.

6 M. Elstner, D. Porezag, G. Jungnickel, J. Elsner, M. Haugk, T. Frauenheim, S. Suhai and G. Seifert, *Phys. Rev. B*, 1998, **58**, 7260.

7 M. Gaus, Q. Cui and M. Elstner, *J. Chem. Theory Comput.*, 2011, **7**, 931.

8 M. Gaus, A. Goez and M. Elstner, *J. Chem. Theory Comput.*, 2013, **9**, 338.

9 M. Gaus, X. Lu, M. Elstner and Q. Cui, *J. Chem. Theory Comput*., 2014, **10**, 1518.

10 X. Lu, M. Gaus, M. Elstner and Q. Cui, *J. Phys. Chem. B*, 2015, **119**, 1062.

11 V. Dhanaraj, Q.-Z. Ye, L. L. Johnson, D. J. Hupe, D. F. Ortwine, J. B. Dunbar Jr., J. R. Rubin, A. Pavlovsky, C. Humblet and T. L. Blundell, *Structure*, 1996, **4**, 375.

12 A. D. MacKerell Jr., D. Bashford, M. Bellott, R. L. Dunbrack Jr., J. D. Evanseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F. T. K. Lau, C. Mattos, S. Michnick, T. Ngo, D. T. Nguyen, B. Prodhom, W. E. Reiher, B. Roux, M. Schlenkrich, J. C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiórkiewicz-Kuczera, D. Yin and M. Karplus, *J. Phys. Chem. B*, 1998, **102**, 3586.

13 B. R. Brooks, C. L. Brooks III, A. D. Mackerell Jr., L. Nilsson, R. J. Petrella, B. Roux, Y. Won, G. Archontis, C. Bartels, S. Boresch, A. Caflisch, L. Caves, Q. Cui, A. R. Dinner, M. Feig, S. Fischer, J. Gao, M. Hodoscek, W. Im, K. Kuczera, T. Lazaridis, J. Ma, V. Ovchinnikov, E. Paci, R. W. Pastor, C. B. Post, J. Z. Pu, M. Schaefer, B. Tidor, R. M. Venable, H. L. Woodcock, X. Wu, W. Yang, D. M. York and M. Karplus, *J. Comput. Chem.*, 2009, **30**, 1545.

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