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The N···H hydrogen bond strength in the transition state at the limiting step determines the reactivity of cephalosporins in the active site of L1 metallo-β-lactamase

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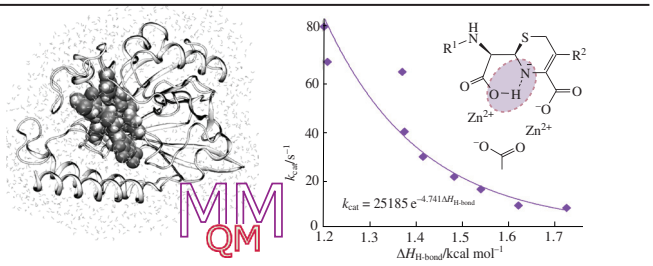
The combined quantum mechanics/molecular mechanics investigation followed by the detailed electron density analysis for 9 cephalosporin–L1 metallo-β-lactamase complexes revealed correlation between the N···H hydrogen bond strength in the transition state at the limiting step and the reactivity of cephalosporin compounds. The stronger interactions were typical of the less reactive species.

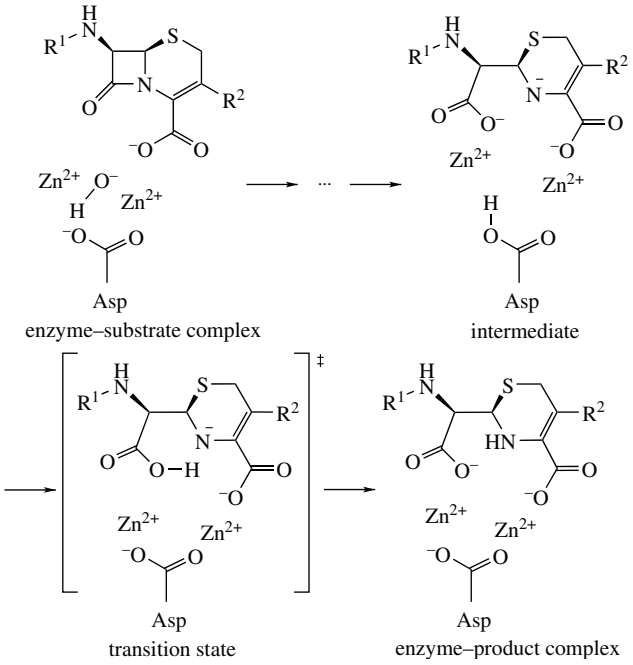


Ongoing developments of the both supercomputers and combined quantum mechanics/molecular mechanics (QM/MM) techniques provide new opportunities for studies of the enzymatic reaction mechanisms.[1] The main attention in this area is usually paid to atomic rearrangements during the elementary steps and energy profiles of the entire processes,[2] while an analysis of the reaction driving forces remains usually beyond the scope of a work. In particular, local properties of the active sites of enzymes, which determine features of the reaction mechanism, are not commonly discussed. However, this is of particular importance for a comparison between reactivities of different substrates inside an enzymatic active site or reactivities of the same substrate with a set of similar proteins.

Thus, this work was aimed at bridging this gap *via* focusing on the practically important reaction of hydrolysis of cephalosporin antibiotics inside the active site of L1 metallo-β-lactamase (MβL), which is one of the mechanisms of bacterial drug resistance.[3] It proceeds in three elementary steps and results in the hydrolysis of C–N bond of the β-lactam ring of cephalosporin compound.[4] The last elementary step is a limiting one and presumes a hydrogen bond transfer from the catalytic aspartic acid to the nitrogen atom of cephalosporin accompanied with the cleavage of N···Zn^{2+} coordination bond (Scheme 1).[4]

We selected a set of nine cephalosporin compounds with available experimental data on their Michaelis–Menten kinetics, *viz.* catalytic constants k_{cat}.[5] Their observed catalytic properties are similar, and the k_{cat} values fall in the range of 7.5–80 s^{-1}. This corresponds to differences in the activation energy of 1.5 kcal mol^{-1} between the slowest and fastest reactions. According to a recent benchmark work[6] on various DFT protocols, the most reliable functional possesses the RMSD of 1.68 kcal mol^{-1} that is larger than the considered energy variation. Thus, the straightforward calculations of energy barriers are not enough accurate for the range of cephalosporin compounds according to their reactivity in the MβL active site, *i.e.* some computational alternatives should be found. Therefore, we had to analyze a possibility of finding the local properties or atomic interactions in the model systems, which mainly determine the observed macroscopic parameter k_{cat} and can be applied for ranging various cephalosporins according to their reactivity.

We employed a molecular model of transition state at the limiting step, the nitrocefin–MβL complex[4] as the template and substituted the substrate with 8 cephalosporin compounds: cefsulodin, CGP-17520A, cefepime, cephaloridine, CGP-31523A, cephalosporin C, cefotaxime and cefuroxime. Thus, the nine model



Scheme 1

† See Online Supplementary Materials for the computational details.

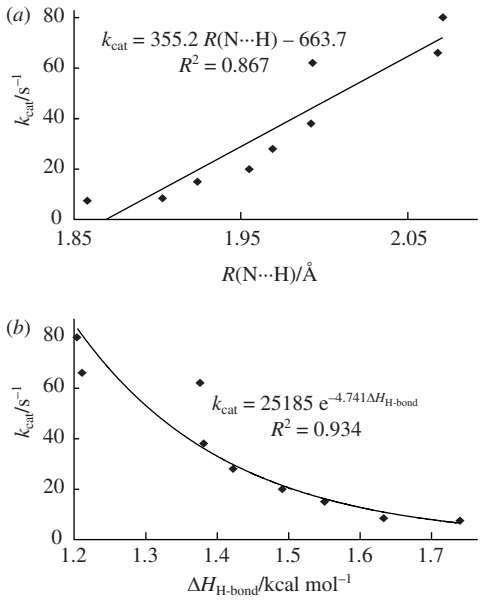


Figure 1 Dependencies of the experimental k_{cat} values on (*a*) the calculated equilibrium N···H distances and (*b*) estimated enthalpy of the hydrogen bond.

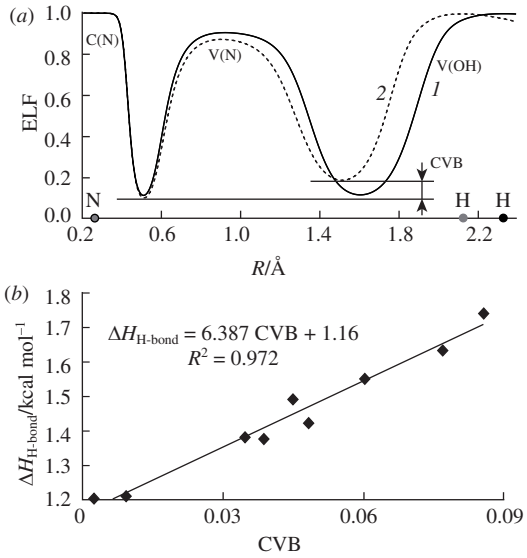


Figure 2 (*a*) ELF distribution along the N···H bond for (*1*) the fastest substrate, cefuroxime, and (*2*) the slowest one, cefsulodin. Points on the horizontal axis show positions of N and H atoms. (*b*) Dependency of the calculated hydrogen bond enthalpies on CVB indexes.

systems were considered† according to the known computational protocol.[6]

We selected the transition state at the limiting step and preceding intermediate that most likely characterize the reactivity of the system (see Scheme 1).[6] The concept of key interaction or property was suggested and tested as the local atomic interaction or property correlating with the observed quantity (k_{cat} in our particular case). The only correlations between the macroscopic parameters k_{cat} for the compounds and equilibrium distances at the stationary points on the potential energy surfaces were found for the N···H hydrogen bond distances in the transition states at the limiting step (Figure 1). The model systems corresponding to the less reactive species (*i.e.*, with the lower k_{cat} values) possess shorter N···H distances.

The most of QM/MM studies on the reaction mechanisms analyze only equilibrium geometry parameters, whereas shorter hydrogen bond distances are usually associated with the stronger hydrogen bonds.[7] However, the modern quantum chemistry provides various advanced methods to estimate bond energies or some other descriptors related to the bond strength.[8] These methods are successfully employed to characterize molecular clusters and crystals, although their applications to biological systems are scanty (*e.g.*, see ref. 9). Thus, we have consistently analyzed the N···H hydrogen bond for the set of considered systems using the known (*e.g.*, ref. 10) descriptors based on the electron density.

Various correlations suggested to estimate the hydrogen bond energy (as well as their other parameters) from the electron density at the bond critical point, $\rho(r_{BCP})$, were tested.[11,12] First of all, we used the relationship obtained for the great diversity of dimers, which reflects the correlation between hydrogen bond enthalpy $\Delta H_{H\text{-}bond}$ and IR intensity of the hydrogen bond stretching vibration in a wide range of bond strengths.[13] Combining it with dependency of $\rho(r_{BCP})$ on the above IR intensity,[12] we obtained the relationship between the $\Delta H_{H\text{-}bond}$ and $\rho(r_{BCP})$:

$$\Delta H_{H\text{-}bond} = 286.8\,\rho(r_{BCP}) \quad (1),$$

where $\Delta H_{H\text{-}bond}$ and $\rho(r_{BCP})$ are expressed in kcal mol^{-1} and arbitrary units (a.u.), respectively. The Arrhenius type dependency between the experimental k_{cat} values and estimated $\Delta H_{H\text{-}bond}$ values was found. Figure 1 shows that complexes possessing the weaker hydrogen bonds, *i.e.* smaller $\Delta H_{H\text{-}bond}$ values, are more reactive. Importantly, the $\Delta H_{H\text{-}bond}$ varies in the range of 1.2–1.8 kcal mol^{-1} that is comparable to the activation energy range of 1.5 kcal mol^{-1}, which indicates that it can be responsible for the observed reactivity variations.

Another descriptor allowing one to estimate the hydrogen bond strength is the electron localization function (ELF).[14] Figure 2(*a*) shows the ELF profiles along the N···H hydrogen bond for the fastest (cefuroxime) and slowest (cefsulodin) substrates. The core regions of nitrogen atom, C(N), are the same for the both systems, and the values at the ELF minima between the core C(N) and valence V(N) regions are almost equal as well. In the case of cefuroxime, small variations were observed for the V(N) maxima of atom exhibiting the higher electron localization. The minima between the N and OH valence regions differ significantly, and the deeper minimum corresponds to the fastest substrate. This is a clear indication of the weaker hydrogen bond interaction for the cefuroxime as compared to the cefsulodin. The core–valence bifurcation (CVB) index was suggested as a measure of the H-bond strength for small molecular systems.[15] Figure 2(*b*) demonstrates the relationship between the hydrogen bond enthalpy estimated according to equation (1) and CVB index. For all the considered systems, the CVB indexes are positive and linearly growing along with the increase in the hydrogen bond enthalpy.

The electrostatic potential (ESP) is one more characteristic used widely to explain the reactivity or interactive behavior, especially if the interactions between two distant pieces are mainly of the electrostatic nature.[16] The ESP measures the potential energy that is a positive test unit of charge external to a given molecule, acquired or lost upon the transportation from infinity to point *r*, while all the geometric and electronic deformations are ignored. The ESP is useful for the assignment of partial atomic charges in a parametrization of the classical force fields,[17] however its usefulness in bonding analysis inside the existing molecular systems is questionable.[18] The potential acting on an electron in the molecule (PAEM) quantifies the total interaction energy of any one electron with the rest $(n-1)$ electrons of a molecule and with all the nuclei, thus being more informative in this context.[19]

We considered the PAEM picture in the N···H hydrogen bond region starting from an analysis of the 3D maps of PAEM and taking the transition state at the limiting step containing CGP-17520A substrate as an example (Figure 3). The PAEM isosurfaces of –0.51 a.u. covering the N and H atoms cross in the region of N···H hydrogen bond line. Therefore, the PAEM profiles

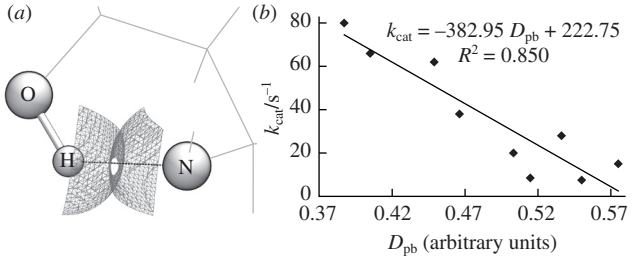


Figure 3 (*a*) 3D map of the PAEM at the isovalue of –0.51 a.u. for the system containing CGP-17520A substrate and (*b*) dependency of the experimental k_{cat} values *vs.* calculated D_{pb} ones.

along the N···H hydrogen bonds should be informative. The PAEM is always negative, whereas the less negative values correspond to the regions that are less preferable for electrons, and the absolute value of PAEM at its maximum, D_{pb}, characterizes the system reactivity or the bond strength.[20] We have found that the plot of D_{pb} values at the N···H hydrogen bond *vs.* experimental k_{cat} shows a linear correlation. It demonstrates that the systems possessing the higher D_{pb} values (*i.e.*, weaker N···H interactions) are less reactive. This conclusion is in agreement with the above mentioned results acquired using other descriptors based on the electron density.

To conclude, we have demonstrated that it is possible to discriminate the local calculated properties or atomic interactions that mainly contribute to the entire process even in the case of complex enzymatic reactions. The descriptors based on the electron density previously applied mainly to small model systems or crystals containing a limited number of interactions have been herein expanded for the broader range of considered systems. In our particular case of hydrolysis reaction of cephalosporin compounds inside the MβL active site, the N···H hydrogen bond at the limiting step of reaction determines the experimental rate constant k_{cat}. We have also revealed that the hydrogen bond strength determines the reactivity of particular cephalosporins, *viz.* the stronger O–H···N hydrogen bonds at the transition state of limiting step correspond to the less reactive species and, *vise versa,* the weaker bonds, to the more reactive ones. We suppose that the approach proposed and illustrated herein on the particular example may be successfully applied to other enzymatic reactions, which should open new horizons in the rational design of novel compounds or mutated enzymes possessing the desired reactivity.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.09.004.

References

1 H. M. Senn and W. Thiel, *Angew. Chem., Int. Ed.*, 2009, **48**, 1198.
2 (*a*) M. G. Khrenova, I. D. Solovyev, G. D. Lapshin and A. P. Savitsky, *Mendeleev Commun.*, 2017, **27**, 157; (*b*) M. G. Medvedev, M. V. Panova, G. G. Chilov, I. S. Bushmarinov, F. N. Novikov, O. V. Stroganov, A. A. Zeifman and I. V. Svitanko, *Mendeleev Commun.*, 2017, **27**, 224.
3 (*a*) D. A. Dik, J. F. Fisher and S. Mobashery, *Chem. Rev.*, 2018, **118**, 5952; (*b*) J. F. Fisher, S. O. Meroueh and S. Mobashery, *Chem. Rev.*, 2005, **105**, 395.
4 (*a*) M. G. Khrenova and A. V. Nemukhin, *J. Phys. Chem. B*, 2018, **122**, 1378; (*b*) M. G. Khrenova, A. V. Krivitskaya and V. G. Tsirelson, *New J. Chem.*, 2019, **43**, 7329.
5 (*a*) A. Felici and G. Amicosante, *Antimicrob. Agents Chemother.*, 1995, **39**, 192; (*b*) M. W. Crowder, T. R. Walsh, L. Banovic, M. Pettit and J. Spencer, *Antimicrob. Agents Chemother.*, 1998, **42**, 921.
6 N. Mardirossian and M. Head-Gordon, *Mol. Phys.*, 2017, **115**, 2315.
7 (*a*) E. Kwiatkowska and I. Majerz, *J. Phys. Org. Chem.*, 2008, **21**, 867; (*b*) *Hydrogen Bonding – New Insights*, ed. S. J. Grabowski, Springer, Dordrecht, 2006.
8 C. F. Matta, *J. Comput. Chem.*, 2014, **35**, 1165.
9 (*a*) R. Chaudret, J.-P. Piquemal and G. A. Cisneros, *Phys. Chem. Chem. Phys.*, 2011, **13**, 11239; (*b*) E. G. Vega-Hissi, R. Tosso, R. D. Enriz and L. J. Gutierrez, *Int. J. Quantum Chem.*, 2015, **115**, 389.
10 E. V Bartashevich and V. G. Tsirelson, *Russ. Chem. Rev.*, 2014, **83**, 1181.
11 M. V. Vener, A. N. Egorova, A. V. Churakov and V. G. Tsirelson, *J. Comput. Chem.*, 2012, **33**, 2303.
12 M. Rozenberg, *RSC Adv.*, 2014, **4**, 26928.
13 A. V. Iogansen, *Spectrochim. Acta, Part A*, 1999, **55**, 1585.
14 (*a*) B. Silvi and A. Savin, *Nature*, 1994, **371**, 683; (*b*) Y. Grin, A. Savin and B. Silvi, in *The Chemical Bond: Chemical Bonding Across the Periodic Table*, Wiley-VCH, Weinheim, 2014, vol. 1 p. 345; (*c*) A. D. Becke and K. E. Edgecombe, *J. Chem. Phys.*, 1990, **92**, 5397; (*d*) A. Savin, A. D. Becke, J. Flad, R. Nesper, H. Preuss and H. G. von Schnering, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 409.
15 F. Fuster and B. Silvi, *Theor. Chem. Acc.*, 2000, **104**, 13.
16 V. G. Tsirelson and R. P. Ozerov, *Electron Density and Bonding in Crystals: Principles, Theory and X-ray Diffraction Experiments in Solid State Physics and Chemistry*, CRC Press, 1996.
17 (*a*) U. C. Singh and P. A. Kollman, *J. Comput. Chem.*, 1984, **5**, 129; (*b*) V. M. Anisimov, G. Lamoureux, I. V. Vorobyov, N. Huang, B. Roux and A. D. MacKerell, *J. Chem. Theory Comput.*, 2005, **1**, 153.
18 E. V. Bartashevich, I. D. Yushina, A. I. Stash and V. G. Tsirelson, *Cryst. Growth Des.*, 2014, **14**, 5674.
19 D.-X. Zhao, L.-D. Gong and Z.-Z. Yang, *J. Phys. Chem. A*, 2005, **109**, 10121.
20 (*a*) Z.-Z. Yang, Y.-L. Ding and D.-X. Zhao, *J. Phys. Chem. A*, 2009, **113**, 5432; (*b*) Z.-Z. Yang, Y.-L. Ding and D.-X. Zhao, *ChemPhysChem*, 2008, **9**, 2379.

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