

## Density functional study of the phosphodiester hydrolysis of RNA in RNA/DNA hybrid by RNase H

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The phosphate diester hydrolysis is an important reaction in biology. The ribonuclease H (RNase H) catalyzes the nonspecific hydrolysis of RNA in RNA/DNA hybrids, yielding a 3'-hydroxyl and a 5'-phosphate at the hydrolysis site. This enzyme is found that the  $Mg^{2+}$  ion is essential for the enzymatic reaction and that aspartate and glutamate are coordinated the  $Mg^{2+}$  ion in the active site. This catalytic mechanism is ubiquitous among nucleic acid processing enzymes and ribozymes. In particular, RNases H has emerged as important therapeutic targets because RNase H activity is absolutely required for proliferation of HIV and other retroviruses. Although the X-ray crystallographic structures of RNase H are determined [1, 2], there are controversies about the catalytic mechanism of RNase H, the number of  $Mg^{2+}$  ions [3], the proton transfer pathway, and the protonation states of the active site residues.

In the present study, we have explored the hydrolysis of the phosphate diester group of RNA by RNase H, using density functional theory to elucidate how many  $Mg^{2+}$  ions are required for the catalysis of RNase H. Our computation demonstrates that both one- and two-metal models show the step-wise hydrolysis pathway and lower the activation barriers, compared to the non-enzymatic hydrolysis of phosphate diester. However, the activation barrier of the two-metal model is smaller than that of the one-metal model by about 10 kcal mol<sup>-1</sup>.

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[2] Nowotny, M.; Gaidamakov, S. A.; Crouch, R. J. Yang, W. *Cell* **2005**, *121*, 1005-1016.

[3] Nakamura, H.; Oda, Y.; Iwai, S.; Inoue, H.; Ohtsuka, E.; Kanaya, S.; Kimura, S.; Katsuda, C.; Katayanagi, K.; Morikawa, K.; Miyashiro, H.; Ikehara, M. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 11535-11539.