Two-Metal Ion Catalysis by Ribonuclease H

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The formation and cleavage of phosphate bonds is essential in most biological processes including nucleic acid processing. Many enzymes that catalyze phosphate hydrolysis require two bound divalent metal ions. To elucidate the poorly understood mechanism of this ubiquitous reaction we carry out hybrid quantum-classical QM/MM free energy simulations. In our calculations, we focus on the catalytic cleavage of the RNA backbone in an RNA/DNA hybrid by Bacillus halodurans Ribonuclease H (RNase H). RNase H is a prototypical member of a large family of enzymes that use two-metal ion catalysis to process nucleic acids. The active site of RNase H is almost identical across species with respect to sequence and structure, including the human enzyme and the HIV Reverse Transcriptase (HIV-RT) RNase H domain. HIV-RT is essential to viral replication, which makes it an important target in HIV drug research. In our simulations, we combine [1] Hamiltonian replica exchange with a finite-temperature string method to calculate the QM/MM free energy surface underlying the catalytic reaction. We use a histogram-free reweighting method to obtain this surface from combined multidimensional string simulations. Our method allows us to search for the optimal pathway in multiple dimensions and, therefore, to identify the detailed sequence of steps in the RNA cleavage reaction. From our calculations, coupled proton transfer reactions emerge as central factors in the catalytic RNA cleavage reaction. We also find that both Mg^{2+} ions are required for catalysis. Replacing either one of them with a Ca^{2+} ion abolishes the catalytic activity. Double Mn^{2+} or Ca^{2+} ion replacements have been characterized experimentally and our calculations agree well with measured catalytic activities. Moreover, single ion replacements, which can be performed straightforwardly in simulations, also point to the specific functional role of the metal ions in the catalytic reaction. Our new proton transfer mechanism is consistent with the kinetic effects of protein mutations and RNA backbone modifications. Moreover, the accurate transition state structure provides an ideal target for future structure-based drug design studies of new HIV-specific inhibitors.

[1] E. Rosta, M. Nowotny, W. Yang and G. Hummer, "Catalytic Mechanism of RNA Backbone Cleavage by Ribonuclease H from QM/MM Simulations", *J. Am. Chem. Soc.*, 133:8934, 2011.