

Direct measurement of a pK_a near neutrality for the catalytic cytosine in the genomic HDV ribozyme using Raman crystallography

Bo Gong¹, Jui-Hui Chen², Elaine Chase², Durga M. Chadalavada³, Rieko Yajima³,

Barbara L. Golden^{2}, Philip C. Bevilacqua^{3*}, Paul R. Carey^{1*}*

Contribution from ¹Case Western Reserve University, Department of Biochemistry,
10900 Euclid Avenue, Cleveland, OH 44106, and ²Purdue University, Department of
Biochemistry, 175 South University Street, West Lafayette, IN 47907, and ³The
Pennsylvania State University, Department of Chemistry, 104 Chemistry Building,
University Park, PA 16802

paul.carey@case.edu, pcb@chem.psu.edu, barbgolden@purdue.edu

RUNNING HEAD The pK_a of the catalytic cytosine of the HDV ribozyme

* Corresponding authors

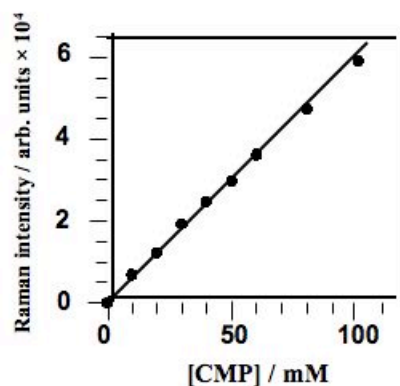


Figure S1. CMP standard concentration curve. A semi-quantitative measure of the concentration of neutral cytosine in HDV ribozyme crystal was obtained by comparing the Raman spectrum of the HDV ribozyme crystal to the CMP standard concentration curve. The experiments were done at ~ 1 pH unit above the pK_a of C75 (pH 7.0 and 20 mM Mg^{2+}). The Raman intensity at 1528 cm^{-1} in HDV ribozyme is approximately 1.5×10^4 arbitrary units. According to Figure S1, this value provides a cytosine concentration of 24 mM, which corresponds to 27 mM when corrected for the pK_a of C75 (see text). The HDV ribozyme concentration is approximately 26 mM in crystal form. Agreement between these two values suggest that a single cytosine in HDV ribozyme crystal is being protonated within the pH range 4.2-8.8.

Full bibliographic information for reference 28:

28.) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A. Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; and Pople, J. A. *Gaussian 98* (Revision A.6), *Gaussian, Inc.* **1998**, Pittsburgh, PA.