Application of NIR Raman Spectroscopy and Bio-reactor to probe the RNA' flexibility and self-catalytic activity of the « *Avocado Sunblotch* » viroid in response to changes in extreme conditions of environments

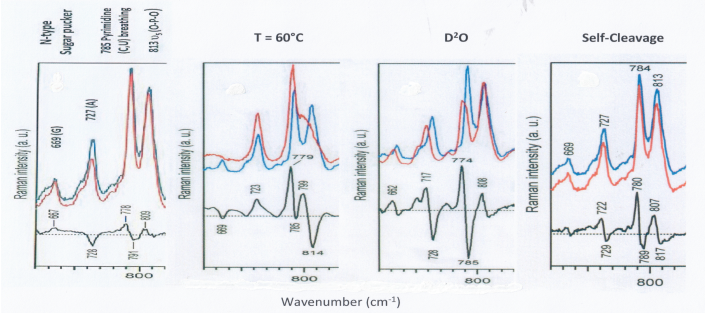
Gaston HUI BON HOA, Ph. D., INSERM France

Abstract

The typical environment for biomolecules in vivo is highly crowded. In vitro, the study of RNA's folding and activity may be radically affected by molecular crowding effects. To mimic the crowding effects, high pressure, D_2O solvent and crowding agents are excellent tools. This perturbation approach is applied to the study of the *Avocado Sunblotch* viroids. They are non encapsidated RNA plant pathogens which possess a catalytic hammerhead ribozyme motif responsible for crucial cleavage step during viroid replication. To date, little is known regarding the conformation of the RNAs, and the ways by which such viroid induces diseases. This prompts us to develop a NIR Raman spectroscopy which is a sensitive technique for monitoring RNA's molecular structure. The results (Figures) show that the Raman structural markers are sensitive to RNA' structure and folding. ASBVd viroid exhibits a typical A-type RNA conformation with ordered double helical content and a C3'-endo/anti sugar pucker configuration. High temperature destabilizes the RNA's secondary structures. Deuteration not only decreases RNA's self-cleavage activity but induces a new secondary structure. Surprisingly, the RNA's Raman vibrational modes after Mg^{2+} - activated self-cleavage, exhibit noticeable frequency downshifts suggesting that phosphodiester and phosphodioxy structures of the cleaved viroids are modified.

The second developed technique is a baro-bio-reactor. The new designed reactor allows to rapid sampling in of activators and sampling out of products, the pressure of which is kept constant by a remote-controlled motor. Bell-shape temperature-profiles of RNA's cleavage activity are obtained which is independent of the pressure. However pressure decreases the rates in a non-linear manner suggesting that the active site is controlled by complex multi-conformers. The initial activation volume $\Delta V^{\neq}$ = + 17 ml/mole. However, study on a model full-length ribozyme shows clearly two distinct pressure-sensitive activities indicating the participation of a fast cleaving and a slow cleaving conformers.

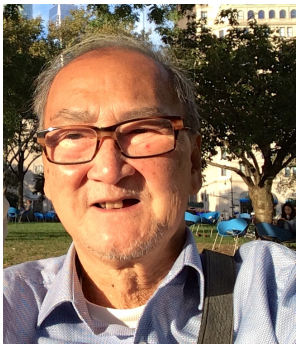
Image



Figures: Effects of polarity (-) and (+), temperature, deuterium solvant and self-cleavage activity on Raman specific marquers of RNA's pyrimidine bases and phosphodiester vibrational modes of *Avocado Sunblotch* viroids. Blue curves are RNA polarity (-) spectra in normal condition.

Recent Publications

1. Rocheleau L, Pelchat M : The subviral RNA database: a toolbox for viroids, the hepatisis delta virus and satellite RNAs research. BMC Microbiol. 2006, 6:24.
2. Thomas GJ, Hartman KA : Raman studies of nucleic acids. 8. Estimation of RNA secondary structure from Raman scattering by phosphate group. Biochem. Biophys. Acta 1978, 312:311-322.
3. Hui-Bon-Hoa G, Kaddour H, Vergne J, Kruglik S.G, Maurel M-C : Raman characterization of *Avocado Sunblotch* viroid and its response to external perturbations and self-cleavage. BMC Biophysics 2014, 7:2.
4. Hui-bon-Hoa G, Hamel G, Else A, Weil G, Hervé G : A reactor permitting injection and sampling out for steady state studies of enzyme reactions at high pressure : Tests with aspartate transcarbamylase. Analytical Biochemistry 1990, 187(2):258-61.
5. Kaddour H, Vergne J, Hervé G, Maurel M-C : High-pressure analysis of a hammerhead ribozyme from *Chrysanthemum Chlorotic* mottle viroid reveals two different populations of self-cleaving molecules. FEBS J. 2011, 278:3739-3747.



Biography

Gaston HUI BON HOA has completed his Ph.D. in 1974 from the University of Paris XI and obtained his positions in I.N.S.E.R.M. since 1975. He is an Emeritus Director of Research, since 2000, in the Hospital Kremlin Bicêtre Center, France. His expertise is focus on the studies of cryo-enzymology and enzyme intermediates (1978-1980), cytochrome P450's structure and function (1981-1992), pressure-induced protein's stability, compressibility and dynamics as well as osmotic stress studies (1992-2015). In 2012, he starts in the study of plant viroid in the University Pierre et Marie Curie, (UPMC), Paris. The aim of such study is to probe the effects of extreme conditions of environments on the structure and function of the RNA from *Avocado Sunblotch* viroids. He has published more than 90 papers in reputed international journals and has been serving as an editorial board member.

Email: gasthui3@yahoo.fr