P039 EPR spectroscopic analysis of U7 hammerhead ribozyme dynamics during metal ion folding

Thomas E. Edwards and Snorri Th. Sigurdsson
University of Iceland, Science Institute, Dunhaga 3, IS-107 Reykjavik, Iceland

Electron paramagnetic resonance (EPR) spectroscopy was used to examine changes in internal structure and dynamics of the hammerhead ribozyme upon metal ion-induced folding, changes in pH and the presence and absence of ribozyme inhibitors. A nitroxide spin-label was attached to U7 of the catalytic core and this modified ribozyme was observed to be active as analyzed by ion exchange HPLC and EPR spectroscopy. A two-step divalent metal ion dependent folding pathway was observed with the first transition at 0.25 mM Mg^{2+} and the second transition around 10 mM Mg^{2+}, in agreement with studies using other biophysical and biochemical techniques. Over a range of temperatures, similar dynamics were observed for U7 in the presence of 4 M Na^{+} or Li^{+}, which were distinctively different than that observed in the presence of 10 mM Mg^{2+}, indicating that U7 of the catalytic core experiences a different microenvironment under these conditions. Interestingly, the dynamics of U7 observed in the presence of 10 mM Mg^{2+} were recovered from a solution containing 1 M Na^{+} upon addition of one equivalent of a divalent metal ion. These results support the theory of an efficient divalent metal ion-dependent and an inefficient monovalent metal ion-dependent catalytic pathway for the hammerhead ribozyme.