Metal ions activate cleavage and lower catalytic pK_a in RNase P

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RNase P is a ribonucleoprotein complex that is responsible for 5′ maturation of pre-tRNAs. The metal ion dependence of RNase P is well documented but less well understood, with Hill numbers ranging from 1 to 4 under different conditions [1, 2]. To better understand metal ion interactions in RNase P we are using cobalt (III) hexaammine as a magnesium hexahydrate mimic to substitute for outer sphere metal ions. RNase P RNA (PRNA) can fold in cobalt hexaammine, and bind the RNase P protein and pre-tRNA^{Asp} with high affinity, but is catalytically inactive. The addition of Mg(II), Zn(II), Mn(II), or Cd(II) to pre-formed RNase P•pre-tRNA (ES) complexes restores activity. At high pH (>7.5) pre-formed complexes display a hyperbolic dependence on the metal ion concentration ($K_{1/2,app}$ = 6 mM), however, at lower pH the apparent metal ion affinity decreases ($K_{1/2,app}$ = 14 mM) and becomes more cooperative (n_H = 2). These data can be explained by a model where one metal ion enhances catalysis at high pH (>7.4), while at low pH additional metal ions function to lower the pK_a for catalysis . This model provides new insight into the link between metal ion affinity and the pH dependence of catalysis by RNase P. Using this model, we have examined the role of the 2′-OH at the pre-tRNA cleavage site. Previously, we showed that this deoxy substitution increases the pK_a for cleavage under single turnover conditions with saturating enzyme; however, the origin of this effect is unclear, perhaps reflecting a decrease in metal ion affinity or the inability of the enzyme to form the correct ES conformation [3, 4]. Here, we show that in cobalt hexamine, the apparent metal ion affinity at high pH ($K_{1/2,app}$ = 7 mM) is unaffected by the deoxy modification, indicating that the 2′-OH does not interact with the catalytic metal ion. Furthermore, we observe no rescue of cleavage activity by Zn(II), Mn(II), or Cd(II) with a 2′-fluoro or 2’-amino modification at the cleavage site of pre-tRNA. Taken together these data indicate that the 2′-OH does not interact with an inner sphere metal ion but may form a weak hydrogen bond with a metal-water complex.

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