Supporting information for:

"An Altered Transition State for the Reaction of an RNA Model Catalyzed by a Dinuclear Zinc(II) Catalyst"

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Raw isotope ratio data (delta values) and fractions of reaction in KIE determinations:

Uncatalyzed reactions of HpPNP

^{15}N KIE: delta of reactant (average of 3 determinations) = -2.687			
reaction #	product delta	residual substrate delta	fraction of reaction
1	-2.5	-1.8	0.57
2	-2.43	-2.34	0.67
3	-2.66	-2.05	0.62

18O leaving group KIE: delta of reactant (average of 3 determinations) = 0.553			
reaction #	product delta	residual substrate delta	fraction of reaction
1	-1.46	5.67	0.610
2	-1.43	4.03	0.610
3	-3.77	6.65	0.520

18O nucleophile KIE: delta of reactant (average of 4 determinations) = -1.588			
reaction #	product delta	residual substrate delta	fraction of reaction
Nuc 1	-19.87	20.64	0.540
Nuc 2	-18.03	26.75	0.640
Nuc 3	-20.21	13.81	0.440
Nuc 4	-19.15	22.4	0.630

Catalyzed reactions of HpPNP

^{15}N KIE: delta of reactant (average of 3 determinations) = -2.687			
reaction #	product delta	residual substrate delta	fraction of reaction
1	-3.26	-1.908	0.56
2	-3.262	-1.812	0.54
3	-3.239	0.460	0.54

18O leaving group KIE: delta of reactant (average of 3 determinations) = 0.553			
reaction #	product delta	residual substrate delta	fraction of reaction
Bridge 1	-6.975	6.241	0.38
Bridge 2	-5.89	7.13	0.54
Bridge 3	-6.811	7.39	0.50

18O nucleophile KIE: delta of reactant (average of 4 determinations) = -1.588			
reaction #	product delta	residual substrate delta	fraction of reaction
Nuc 1	-9.11	3.65	0.495
Nuc 2	-9.37	4.92	0.455
Nuc 3	-9.2	11.39	0.596

Kinetic Isotope Effect Data Analysis. For each isotope effect at least three reactions were run. The $^{15}N/^{14}N$ ratios were measured for the product (R_p) and of the remaining starting material (R_s) at partial reaction, as well as in the original mixture (R_o). The isotope effects were calculated using equations 1 and 2.[1]

$$\text{isotope effect} = \log (1 - f) / \log [(1 - f) (R_S / R_O)] \quad (1)$$

$$\text{isotope effect} = \log (1 - f) / \log (1 - f\ (R_p / R_O)) \quad (2)$$

For each isotope effect the value calculated from R_o and R_p (equation 1) and from R_o and R_s (equation 2) agreed within experimental error and these were averaged to give the results reported. The ^{15}N KIE is given directly from these equations. In the ^{18}O isotope effect experiments the observed KIEs given by the above equations were corrected for the ^{15}N isotope effect and for incomplete levels of isotopic incorporation.

Calculation of corrected ^{18}O kinetic isotope effects

In the ^{18}O isotope effect experiments the observed KIEs were corrected for the ^{15}N isotope effect and for incomplete levels of isotopic incorporation. The derivations of the equations used for these corrections have been described.[2] For remote label isotope effects using one ^{18}O label the following equation is used:

$$^{18}k = \frac{^{15,18}ky}{^{15}k - [^{15,18}k - {}^{15}k][(1-b)z/(bx)] - {}^{15,18}k(1-y)}$$

where,

$^{15,18}k$ = the observed KIE due to both labels

^{15}k = the ^{15}N KIE

^{18}k = the corrected ^{18}O KIE

b = the fraction of doubly labeled compound in the remote labeled mixture

x = the fraction of ^{15}N in the remote label position of the doubly labeled (^{15}N, ^{18}O) compound used for mixing.

y = the fraction of ^{18}O in the doubly - labeled compound used for mixing

z = the fraction of ^{15}N in the remote label position of the ^{14}N - labeled compound used for mixing.

For the labeled substrates and the mixtures used for the $^{18}k_{lg}$ experiments, the fractions of isotopic isomers present as measured by mass spectrometry, used for the correction, were as follows: b=0.003665, x=0.99, y=0.81, z=0.0002.

For the labeled substrates and the mixtures used for the $^{18}k_{nuc}$ experiments, these values were as follows: b=0.003657, x=0.99, y=0.895, z=0.0002.

References

(1) Bigeleisen, J.; Wolfsberg, M. *Adv. Chem. Phys.* 1958, *1*, 15-76.

(2) Cleland, W. W. In *Isotope effects in chemistry and biology*; Kohen, A., Limbach, H.-H., Eds.; CRC Press: Boca Raton, FL, 2006, p 915-930; Hermes, J. D.; Morrical, S. W.; O'Leary, M. H.; Cleland, W. W. *Biochemistry* 1984, *23*, 5479-88.