HAMMERHEAD RIBOZYME LIGATES RNA IN FROZEN SOLUTION.

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Abstract & Introduction

Although considerable evidence supports a key role for RNA at the origin of life, its inherent fragility in solution poses a difficulty for the emergence of catalytic and self-replicating RNA. Previous work showed that the interstitial liquid within frozen ice provides an environment that minimizes RNA degradation yet supports condensation of activated nucleotides into RNA [1], RNA ligation by the hairpin ribozyme ([2], and RNA polymerization by a ribozyme [3].

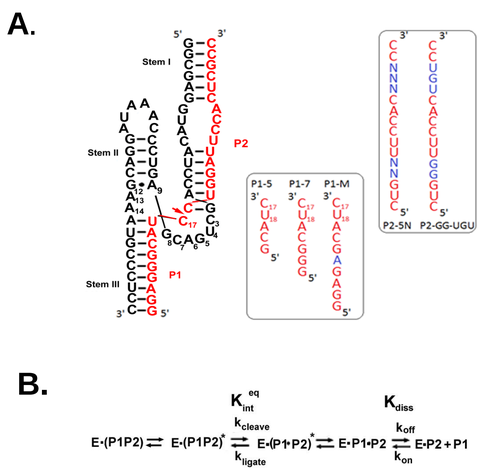
The current study examined the catalytic activity of the *Schistosoma mansoni* hammerhead ribozyme *(*HHR) in frozen solution. This ribozyme (**Fig. 1)** predominantly cleaves RNA at 25 °C, but can ligate its cleaved products (P1,P2) in single turnover experiments with yields up to 23%. $Mg^{2+}$is required**.**

Our results show that the HHR ligates RNA oligomers in frozen solution lacking divalent metal ions. Anions with carboxylate groups and properties associated with strong ion-water interaction enhance ligation.

Yields up to 43% are observed in one freeze-thaw cycle and a maximum of 60% is obtained after several freeze-thaw cycles. The HHR can ligate P2 substrates with mismatched base pairs at 5 of its 11 WC base paired positions, as well as truncated and mutated P1 substrates . Freeze-thaw induced ligation can generate an ensemble of diverse RNA sequences from short RNA oligomers.

1. *S. mansoni* Hammerhead Ribozyme

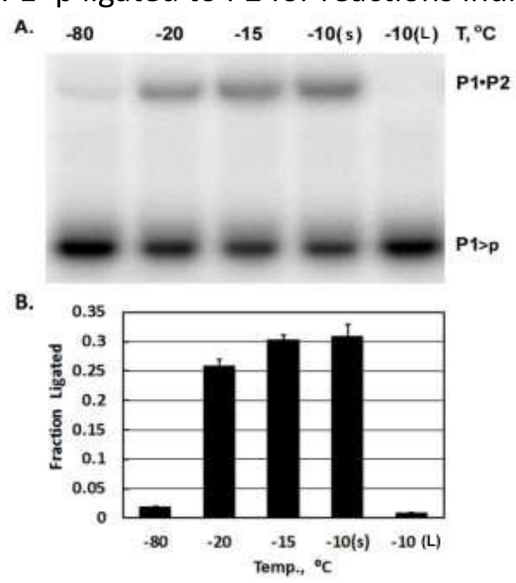
Fig. 1 A. Enzyme or E strand (black) and substrate strand, P1P2,(red). Arrow indicates cleavage/ligation site between P1 and P2 RNAs. P1-5, P1-7, and P1-M are truncated or mutant forms of P1. P2 with a random mixture of A,U,G,C at five positions is represented by P2-5N. The P2 sequence most frequently observed when P2-5N is ligated to P1 is P2-GG-UGU.
B. Reaction scheme; * denotes catalytically active forms



2. HHR ligates RNA oligomers in frozen solutions with no divalent cations

Fig. 2 A. 12 % denaturing PAGE. Ligation of P1>p to P2 by HHR in ice using 0.5uM E strand, 2 uM P2, 5 nM P1>p. P1>p is labeled P1 with 2'3' cyclic phosphate at 3' end. First 4 lanes show samples quick-frozen in NTE (0.1 M NaCl +50 mM Tris(8.2)+1 mM EDTA) and then incubated for 24 hr at -80°C, -20°C, -15°C, and -10°C. Lane 5 is a supercooled sample to -10°C that remained liquid for 24 hr.

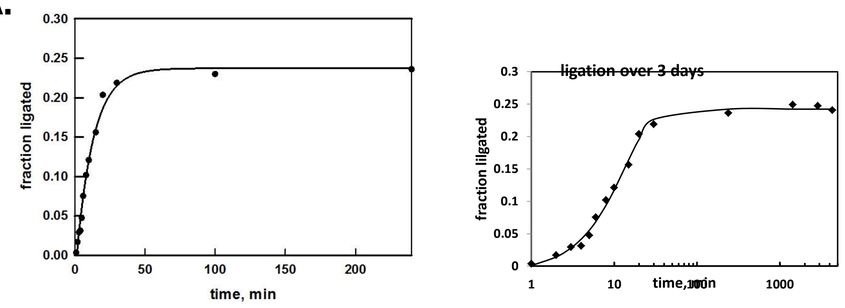
B. Fraction of P1>p ligated to P2 for reactions indicated in **A**.



3. Freeze-induced ligation occurs within ~30 minutes.

Fig. 3 Kinetics of ligation at -20°C using single turnover conditions and NTE solvent; k_{obs} ~ 0.063/min.

A.



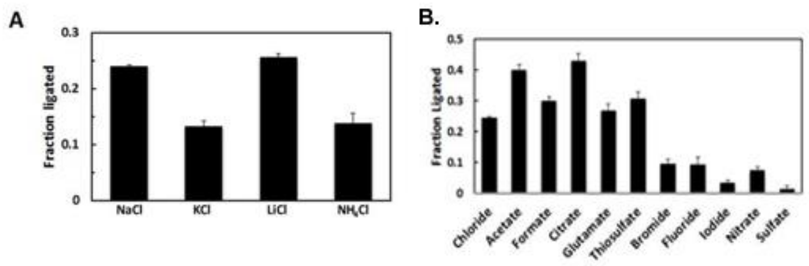
4. Solvent cations and anions affect ligation in ice.

No ligation was observed by freezing in the absence of NaCl.

Fig. 4 A. Impact of cation substitutions for Na^+ on ligation.

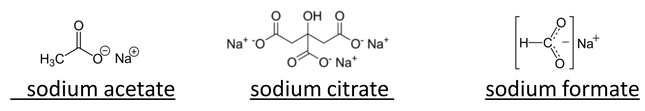
B. Effect of anion substitution for Cl^- on ligation.

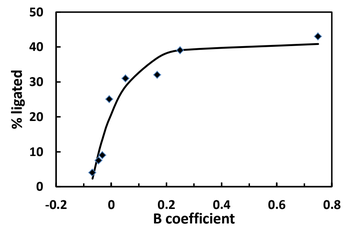
Citrate and other anions with carboxylate groups and thiosulfate enhanced ligation.



5. Anions that strongly interact with water enhance HHR ligation in ice.

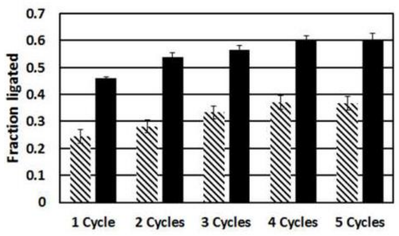
Fig. 5 A correlation was observed between the Jones-Dole B-coefficients of anions and ligation yield. This term measures the strength of ion-water interaction [4]. {Plot excludes Na^+ salt solutions with low solubility; SO_4, Fl}





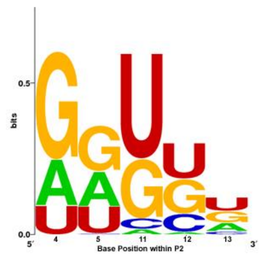
6. Freeze-thaw cycles enhance ligation

Fig. 6 Freeze-thaw re-equilibrates active vs. kinetically trapped inactive HHR-substrate complexes. Cross hatch bars; samples in NTE solution. Black bars; samples with sodium citrate replacing NaCl. One cycle: -20°C for 24 hr, thaw at 70°C for 2 min. Maximum ligation yield 60%.



7. HHR ligates P1 and P2 substrates with mismatched base pairs in ice.

Fig. 1 shows 3 mutant P1 that ligated to P2 at ≥ 14%. Sequencing of P1•P2-5N products showed 220 of 1024 with ≥ 150 reads/million. **Fig. 7** Sequence distribution at 5 'N' positions for 100 most frequently ligated P2-5N oligomers (95% of all reads).



Summary:

I. Freezing concentrates the HHR strands and solutes and induces ligation in the absence of divalent cations.
II. Citrate and other anions enhance ligation in ice.
III. Freezing induced ligation by HHR exceeds ligation at 25°C with Mg^2+. (60% vs 23%)
IV. Freeze-thaw ligation by the HHR can generate an ensemble of RNA sequences from short RNA oligomers.

References

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