Adenosine is Inherently Favored as the Branch-Site RNA Nucleotide

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Ligation activity data for all new 10DM and 7DM deoxyribozymes

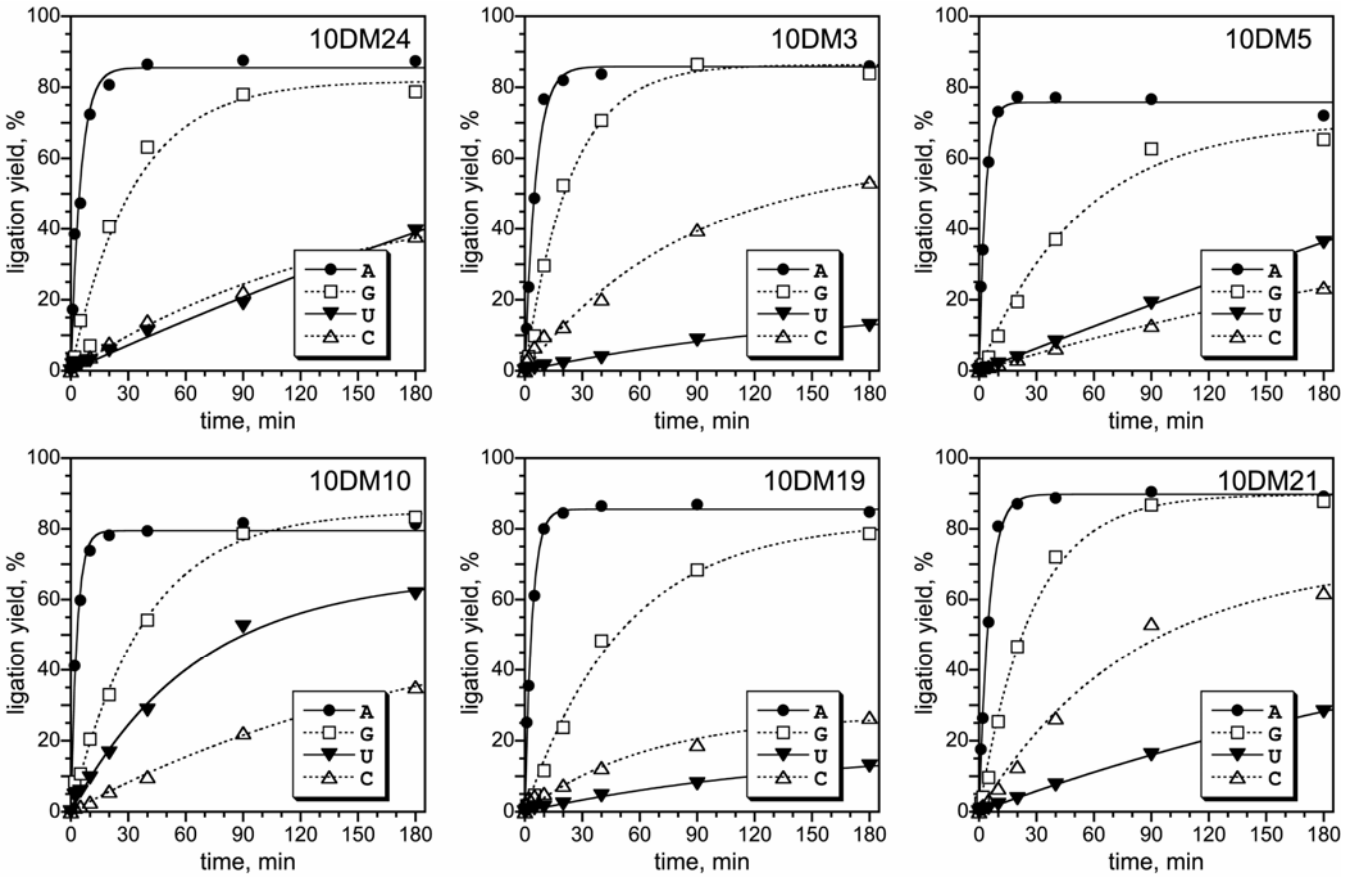

Figure S1. Branched RNA formation by the 10DM deoxyribozymes using the four possible branch-site nucleotides. In all cases, a clear adenosine preference is evident. The k_{obs} values are summarized in Table 1.

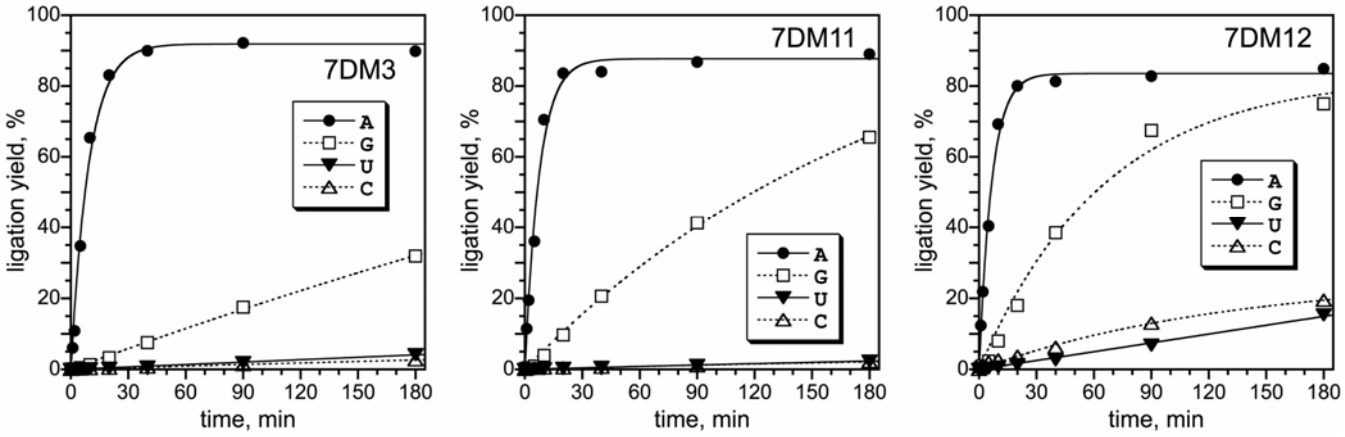

Figure S2. Branched RNA formation by the 7DM deoxyribozymes using the four possible branch-site nucleotides. In all cases, a clear adenosine preference is evident. The k_{obs} values are summarized in Table 1.

Partial alkaline hydrolysis to confirm branch-site nucleotides used by 10DM24

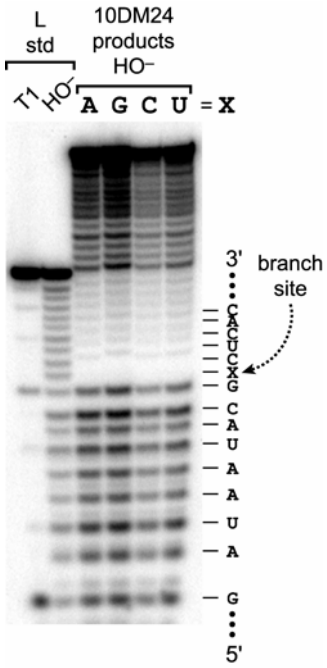

Figure S3. Partial alkaline hydrolysis assays using the branched RNA products synthesized by the 10DM24 deoxyribozyme with branch-site A, G, C, or U. The assays were performed for 10 min at 90 °C in 50 mM $NaHCO_3$ (pH 9.2) and reveal the expected branch site in all cases. For preparing the 10DM24 products, the left-hand (L) RNA substrates were transcripts of sequence 5′-GGAUAAUACGXCUCACUAUA-3′, where X is the branch site. For the L standards (left two lanes), X = A; T1 denotes RNase T1 digestion (G-specific ladder), and HO^- denotes partial alkaline hydrolysis.

Assays to investigate generality of 10DM24 for the RNA substrate sequences

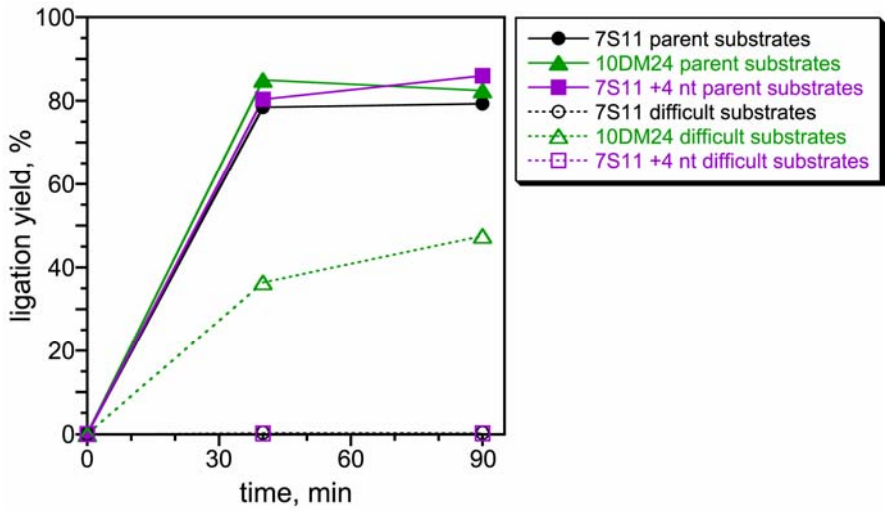

Figure S4. Testing the generality with the difficult RNA substrates of the 10DM24 deoxyribozyme and of the 7S11 deoxyribozyme that has a 4-nucleotide insertion in loop A. Because only 10DM24 leads to appreciable ligation with the difficult substrates, the results show 10DM24 is much more general than either 7S11 itself or 7S11 with the 4-nucleotide insertion. The difficult RNA substrates were those of the "worst-case scenario" as described on page 2903 of ref. 4. The four nucleotides CCGT were inserted into the 5′-end of 7S11 loop A (see deoxyribozyme sequences in Figure 3).

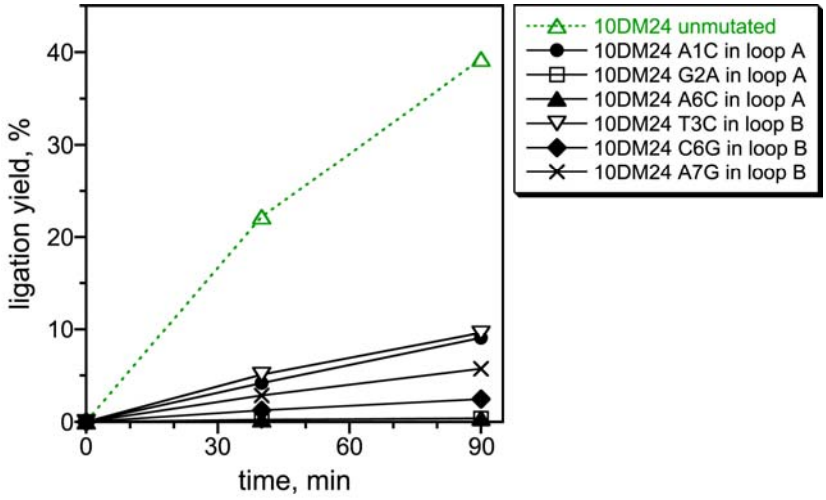

Figure S5. Changing nucleotides of 7S11 to match those of 10DM24 does not increase ligation activity with the difficult RNA substrates. Six specific nucleotides in loops A and B differ between 7S11 and 10DM24 (pink nucleotides in Figure 3). Here, six deoxyribozyme mutants were synthesized; each mutant is derived from the 7S11 sequence with five of six nucleotides changed to those found in 10DM24. An equally valid way to view these six mutants is that each is derived from the 10DM24 sequence with only one of six nucleotides changed to that found in 7S11 (see legend in this figure). For all six mutants, the modified deoxyribozyme has substantially lower ligation activity than 10DM24 itself, indicating that all six nucleotides which differ between 10DM24 and 7S11 are required for high ligation activity (in addition to the 4-nucleotide insertion in loop A; see Figure S4). For comparison, 7S11 has almost no activity with these difficult RNA substrates (~2% yield in 90 min; ref. 4).

Loop B mutants of 10DM24 are inconsistent with base-pairing of branch-site nucleotide

Nucleotide G1 of 10DM24 loop B was mutated to either A, T, or C (Figure S6), and kinetic data were obtained using RNA substrates with the four possible branch-site nucleotides (Table S1). As anticipated on the basis of data for 7S11 loop B G1A (ref. 4), any change to the G1 nucleotide of loop B reduces the k_{obs} values. Importantly, for each of the mutants, the selectivity in favor of branch-site A is retained. This is inconsistent with an inhibitory base pair forming between the G1 nucleotide of loop B and the branch-site RNA nucleotide (i.e., G1 of loop B could form a wobble base pair with branch-site U or Watson-Crick base pair with branch-site C, thus suppressing activity). If such base-pairing were responsible for the branch-site A selectivity of the parent 10DM24 deoxyribozyme, then the 10DM24 G1A mutant should work well with branch-site C, unlike the parent 10DM24. Similarly, the 10DM24 G1T mutant should work well with branch-site U or C but no longer with branch-site A or G, and the 10DM24 G1C mutant should work well with branch-site U, C, or A but not G. None of these reactivity patterns are actually observed, which is strong evidence against the possible G1–X base pairing.

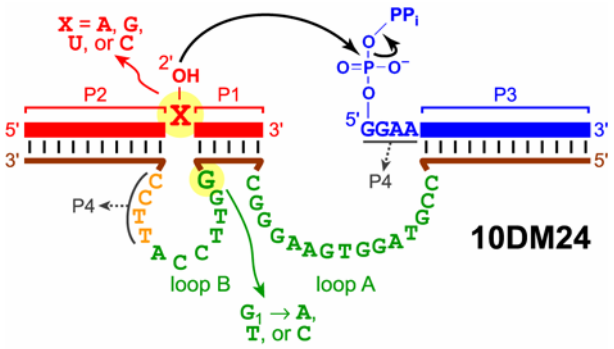

Figure S6. Design of experiments to test a potential base-pairing interaction between the G1 nucleotide of 10DM24 loop B and the branch-site RNA nucleotide (shown as X).

10DM24 deoxyribozyme	k_{obs}(A) min^{-1}	k_{obs}(G) min^{-1}	k_{obs}(U) min^{-1}	k_{obs}(C) min^{-1}	k_{obs}(A)/ k_{obs}(G)	k_{obs}(A)/ k_{obs}(U)	k_{obs}(A)/ k_{obs}(C)
parent [a]	0.26	0.035	0.0035	0.0030	7.4	74	87
loop B G1 → A	0.055	0.00014	<0.00001	0.000015	390	>5000	3700
loop B G1 → T	0.0039	0.00004	<0.00001	<0.00001	100	>400	>400
loop B G1 → C	0.0035	0.00003	<0.00001	<0.00001	120	>400	>400

Table S1. Kinetic data for loop B mutants of 10DM24. When no discrete ligation product band was observed, the upper limit on k_{obs} [and therefore the lower limit on selectivity k_{obs}(A)/k_{obs}(X)]was calculated on the basis of the ~0.2% background signal.

[a] Data for the parent 10DM24 deoxyribozyme is from Table 1.