Systematic Evaluation of the Dependence of Deoxyribozyme Catalysis on Random Region Length

Tania E. Velez, Jaydeep Singh, Ying Xiao, Emily C. Allen, On Yi Wong, Madhavaiah Chandra, Sarah C. Kwon, and Scott K. Silverman*

Department of Chemistry, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, IL 61801, USA

Deoxyribozyme sequences

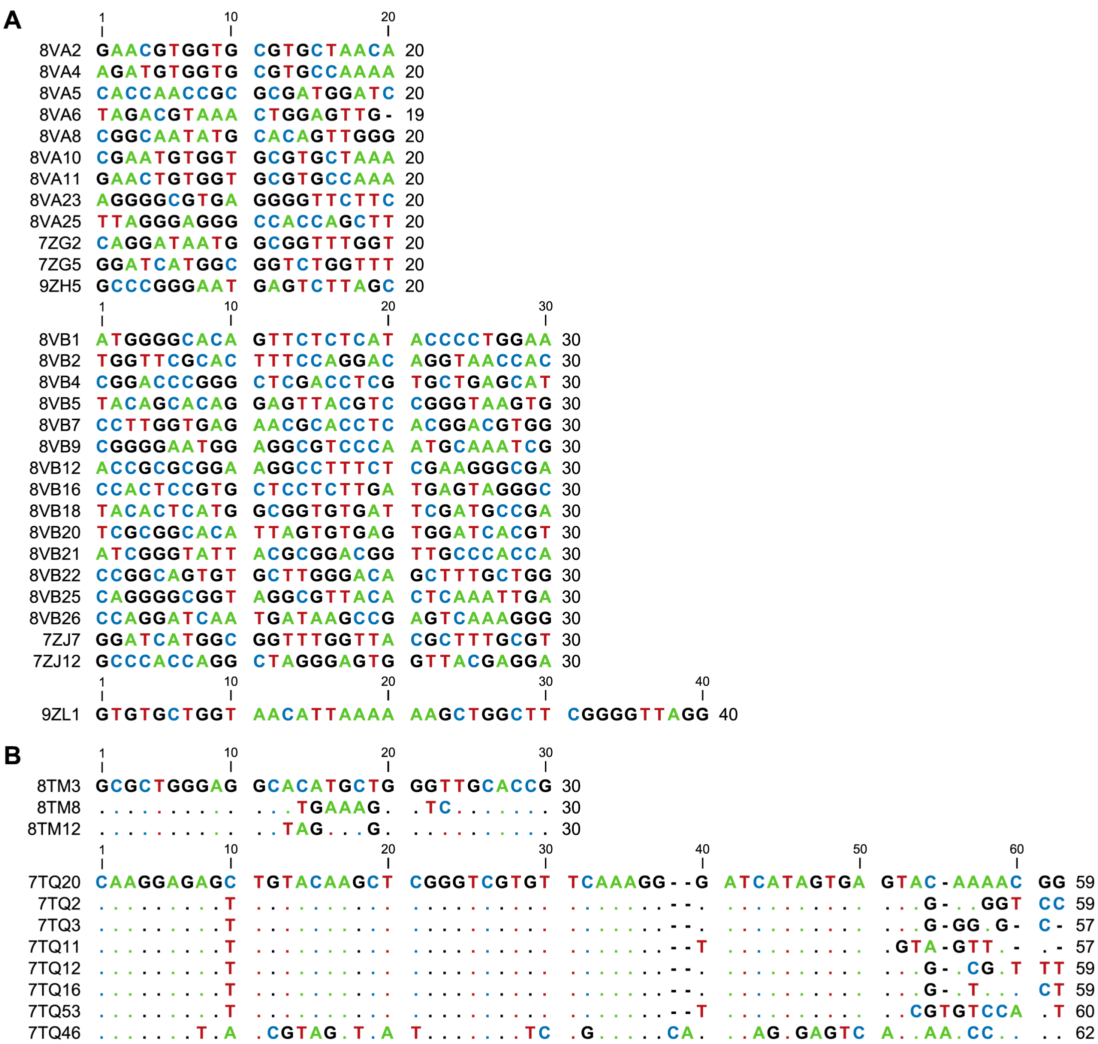

Figure S1. Sequences of the initially random (N_{20}–N_{60}) catalytic regions of the various new deoxyribozymes. The systematic alphabetic nomenclature for each selection experiment (and therefore the resulting deoxyribozymes) is described in the figure captions. (A) Deoxyribozymes for DNA cleavage. (B) Deoxyribozymes for tyrosine-RNA nucleopeptide linkage formation. In all cases, the binding arm sequence to the 5′-side of the catalytic region was 5′-CCGTCGCCATCTCTTC-3′, and the binding arm sequence to the 3′-side of the catalytic region was 5′-ATAGTGAGTCGTATTA-3′. For 8VA23, a G→A binding arm mutation (5′-ATAA...) was identified by sequencing of the clone and found to be required for activity; only a trace amount of DNA cleavage was observed when the original G was included. For 9ZH5, a T→C binding arm mutation (5′-ATAGTGAGC...) was identified by sequencing of the clone and found to be required for activity; no DNA cleavage was observed when the original T was included.

MALDI mass spectrometry data

deoxyribozyme [a]	mass L calcd.	mass L found	L error, % (found – calcd.)	mass R calcd.	mass R found	R error, % (found – calcd.)	hydrolysis site [b]
8VA5 (N_{20})	4823.2	4823.9	+0.01	5701.7	5702.3	+0.01	TAT^CGAA, 5′-p
8VA6 (N_{20})	5192.4	5195.6	+0.06	5332.5	5335.5	+0.06	TATC^GAA, 3′-p
8VB4 (N_{30})	4285.8	4288.2	+0.06	6239.1	6242.3	+0.05	T^ATCGAA, 3′-p
8VB20 (N_{30})	4519.0	4516.4	–0.06	6005.9	6002.0	–0.06	TA^TCGAA, 5′-p
8VB21 (N_{30})	4903.2	4910.3	+0.14	5621.7	5629.8	+0.14	TAT^CGAA, 3′-p
7ZG2 (N_{20})	6084.0	6083.3	–0.01	5996.9	5995.9	–0.02	TATC^GGAA, 5′-p
7ZG5 (N_{20})	6084.0	6082.1	–0.03	5996.9	5995.0	–0.03	TATC^GGAA, 5′-p
9ZH5 (N_{20})	6084.0	6087.4	+0.06	5996.9	6000.1	+0.05	TATC^GGAA, 5′-p
7ZJ7 (N_{30})	6084.0	6086.1	+0.03	5996.9	5999.1	+0.04	TATC^GGAA, 5′-p
7ZJ12 (N_{30})	6084.0	6085.5	+0.02	5996.9	5998.0	+0.02	TATC^GGAA, 5′-p
9ZL1 (N_{40})	6084.0	6085.3	+0.02	5996.9	5997.6	+0.01	TATC^GGAA, 5′-p

Table S1. MALDI mass spectrometry data for products of the new deoxyribozymes that catalyze DNA hydrolysis. L = left-hand cleavage product; R = right-hand cleavage product.

[a] The number of originally random nucleotides is shown in parentheses by the deoxyribozyme name.

[b] The DNA hydrolysis site is marked with ^ within the illustrated portion of the DNA substrate sequence, where the underlined C or CG is unpaired (all other substrate nucleotides are base-paired with the deoxyribozyme binding arms; see full substrate sequences in Experimental Procedures). The location of the phosphate that remains after hydrolysis, either 5′ or 3′, is indicated.

deoxyribozyme [a]	mass L calcd.	mass L found	L error, % (found – calcd.)	mass R calcd.	mass R found	R error, % (found – calcd.)	deglycosylation site [b]
8VA2 (N_{20})	4599.0	4603.8	+0.10	5701.7	5707.7	+0.11	TA***T***CGAA
8VA4 (N_{20})	4599.0	4602.0	+0.07	5701.7	not obs.	–	TA***T***CGAA
	4903.2	4903.7	+0.01	5412.5	5412.5	0	TAT***C***GAA
8VA8 (N_{20})	5192.4	5192.8	+0.01	5083.3	5083.9	+0.01	TATC***G***AA
	5521.6	5521.8	+0.004	4770.1	4770.5	+0.01	TATCG***A***A
8VA10 (N_{20})	4599.0	4601.6	+0.06	5701.7	5704.6	+0.05	TA***T***CGAA
8VA11 (N_{20})	4599.0	4599.0	0	5701.7	5701.5	–0.004	TA***T***CGAA
8VA23 (N_{20})	4903.2	4907.3	+0.08	5412.5	5416.5	+0.07	TAT***C***GAA
8VA25 (N_{20})	5521.6	5522.0	+0.01	4770.1	4770.7	+0.01	TATCG***A***A
8VB1 (N_{30})	4285.8	4288.2	+0.06	6005.9	6009.0	+0.05	T***A***TCGAA
8VB2 (N_{30})	5192.4	5190.6	–0.03	5083.3	5081.4	–0.04	TATC***G***AA
	5521.6	5519.7	–0.03	4770.1	4768.2	–0.04	TATCG***A***A
8VB5 (N_{30})	5192.4	5193.6	+0.02	5083.3	5084.2	+0.02	TATC***G***AA
8VB7 (N_{30})	4903.2	4906.0	+0.06	5412.5	not obs.	–	TAT***C***GAA
	5192.4	5196.0	+0.07	5083.3	5084.8	+0.03	TATC***G***AA
8VB9 (N_{30})	5521.6	5528.8	+0.13	4770.1	4776.5	+0.13	TATCG***A***A
8VB12 (N_{30})	5192.4	5191.8	–0.01	5083.3	5082.5	–0.02	TATC***G***AA
8VB16 (N_{30})	5192.4	5192.5	+0.002	5083.3	5083.7	+0.01	TATC***G***AA
	5521.6	5521.5	–0.002	4770.1	4770.7	+0.01	TATCG***A***A
8VB18 (N_{30})	5192.4	5196.0	+0.07	5083.3	5086.7	+0.07	TATC***G***AA
8VB22 (N_{30})	5521.6	5532.7	+0.20	4770.1	4780.3	+0.21	TATCG***A***A
8VB25 (N_{30})	5521.6	5528.6	+0.13	4770.1	4776.0	+0.12	TATCG***A***A
8VB26 (N_{30})	5192.4	5197.3	+0.09	5083.3	5087.7	+0.09	TATC***G***AA

Table S2. MALDI mass spectrometry data for products of the new deoxyribozymes that catalyze DNA deglycosylation and strand scission by two β-elimination reactions. L = left-hand cleavage product; R = right-hand cleavage product.

[a] The number of originally random nucleotides is shown in parentheses by the deoxyribozyme name.

[b] The deglycosylation site is marked in bold italics within the illustrated portion of the DNA substrate sequence, where the underlined C is unpaired (all other substrate nucleotides are base-paired with the deoxyribozyme binding arms; see full substrate sequence in the Experimental Procedures). For deoxyribozymes that deglycosylate at either of two adjacent nucleotides, data for each of the two sites is given on consecutive lines.

deoxyribozyme [a]	mass calcd.	mass found	error, % (found – calcd.)
8TM3 (N_{30})	12357.2	12366.5	+0.08
7TQ20 (N_{60})	12357.2	12364.1	+0.06
7TQ46 (N_{60})	12357.2	12349.8	–0.06

Table S3. MALDI mass spectrometry data for products of the new deoxyribozymes that catalyze tyrosine-RNA nucleopeptide linkage formation.

[a] The number of originally random nucleotides is shown in parentheses by the deoxyribozyme name.

Kinetic data for deoxyribozymes from the initial selections for DNA cleavage

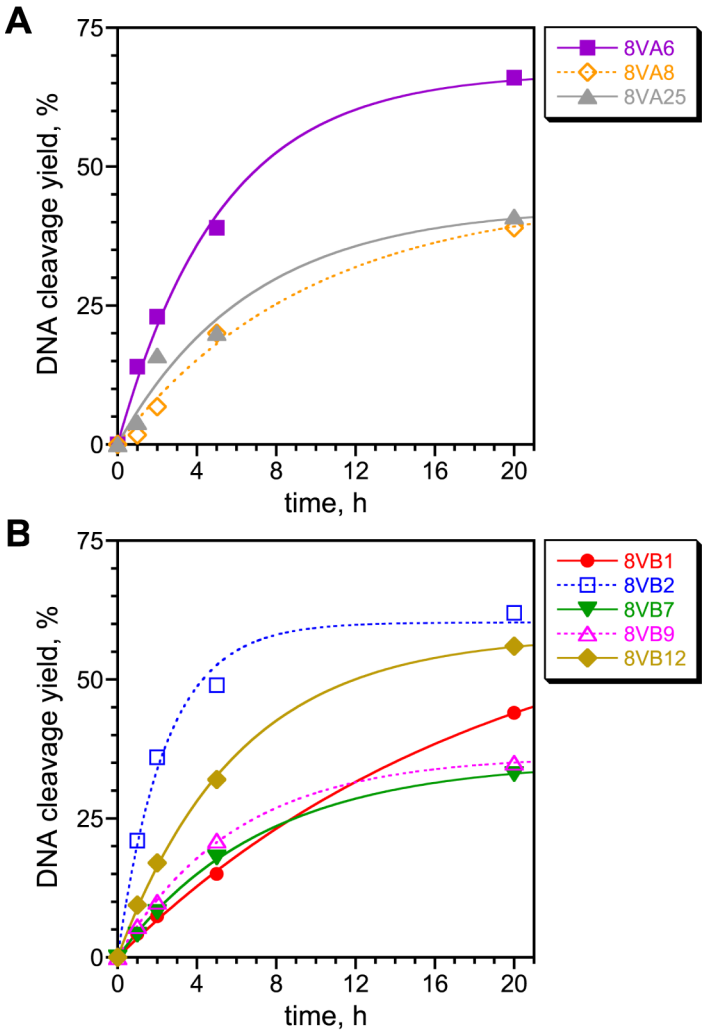

Figure S2. Kinetic plots for additional individual deoxyribozymes from the initial selections for DNA cleavage (see Figure 3 for other deoxyribozymes).

k_{obs} values for kinetic plots

deoxyribozyme [a]	k_{obs}, h^{-1}
8VA2 (N_{20})	0.12
8VA4 (N_{20})	0.16
8VA5 (N_{20})	0.011*
8VA6 (N_{20})	0.19
8VA8 (N_{20})	0.009*
8VA10 (N_{20})	0.22
8VA11 (N_{20})	0.19
8VA23 (N_{20})	0.051
8VA25 (N_{20})	0.15
8VB1 (N_{30})	0.052
8VB2 (N_{30})	0.41
8VB4 (N_{30})	0.18
8VB5 (N_{30})	0.52
8VB7 (N_{30})	0.14
8VB9 (N_{30})	0.17
8VB12 (N_{30})	0.17
8VB16 (N_{30})	0.22
8VB18 (N_{30})	0.23
8VB20 (N_{30})	0.006*
8VB21 (N_{30})	0.094
8VB22 (N_{30})	0.080
8VB25 (N_{30})	0.086
8VB26 (N_{30})	0.041
7ZG2 (N_{20})	0.053
7ZG5 (N_{20})	0.30
9ZH5 (N_{20})	0.063
7ZJ7 (N_{30})	0.19
7ZJ12 (N_{30})	0.29
9ZL1 (N_{40})	0.52
8TM3 (N_{30})	0.15
11MN5 (N_{40})[33]	0.95
7TQ20 (N_{60})	0.073

Table S4. k_{obs} values for the kinetic plots shown in Figures 3, 5 and 6 and Figure S2. Unmarked values were obtained from direct fits to first-order kinetics, $Y = Y_{max} \bullet (1 - e^{-kt})$. Values marked with an asterisk were obtained from linear fits due to the low rate constants.

[a] The number of originally random nucleotides is shown in parentheses by the deoxyribozyme name.