Thermodynamic and Activation Parameters for Binding of a Pyrene-Labeled Substrate by the *Tetrahymena* Ribozyme: Docking Is Not Diffusion-Controlled and Is Driven by a Favorable Entropy Change†

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ABSTRACT: Association and dissociation rates for the pyrene-(pyr)-labeled oligoribonucleotide substrate pyrCUCU binding to the L-21 *Sca*I group I ribozyme are reported as a function of temperature. Combined with thermodynamic parameters for binding of pyrCUCU to rGGAGAA, the results allow calculation of the activation and thermodynamic parameters for docking of pyrCUCU into the catalytic core of the ribozyme. The activation enthalpy for docking is 22 kcal/mol, much larger than the ~4 kcal/mol expected for a diffusion-controlled process. Thus, docking is not diffusion-controlled. The activation and equilibrium entropies for docking are favorable at 21 and 37 eu, respectively. The results suggest the rate-limiting step and the driving force for docking may involve desolvation of RNA functional groups or of Mg^{2+} ions.

The L-21 *Sca*I ribozyme derived from the group I self-splicing intron of *Tetrahymena thermophila* catalyzes a transesterification reaction between small oligonucleotide substrates (Inoue & Kay, 1987; Zaug et al., 1988). The rate for this reaction is enhanced by about 10^{11} relative to the uncatalyzed reaction (Herschlag & Cech, 1990). Since binding of substrates is a major factor in catalysis (Jencks, 1975; Fersht, 1985; Menger, 1992), the mechanism for assembly of substrates with the catalytic site is of interest. This assembly probably involves packing of helixes (Kim & Cech, 1987; Michel & Westhof, 1990), an important step in the tertiary folding of large RNAs. Thus, the thermodynamic and activation parameters for this process are also of fundamental interest. Studies of a miscleavage reaction with a mutant L-21 *Sca*I at 50 °C (Herschlag, 1992) and of binding of fluorescent substrates to L-21 *Sca*I at 15 °C (Bevilacqua et al., 1992, 1994) indicate that substrate binding occurs in at least two steps: formation of a base-paired double helix termed P1 followed by the docking of this helix into the catalytic site. The rates for docking deduced from these studies are dramatically different, however: $>1333\ s^{-1}$ at 50 °C, 10 mM Mg^{2+}, and 50 mM Na^+ (Herschlag, 1992) and between 0.6 and 5 s^{-1} at 15 °C, 5 mM Mg^{2+}, and 160 mM Na^+, depending on fluorescent substrate (Bevilacqua et al., 1992, 1994). This suggests docking has a large activation energy and/or sensitivity to ionic conditions or that the miscleavage reaction and fluorescent substrates are sensitive to different steps in the mechanism. In this paper, we report the temperature dependence for the kinetics of binding a fluorescent substrate to L-21 *Sca*I. The results indicate docking is associated with a large activation energy. This implies docking is not diffusion-controlled. Both the activation and equilibrium entropy changes for docking are positive. This suggests new possibilities for interactions that allow docking of the polyanionic substrate into the catalytic site formed by the polyanionic ribozyme.

MATERIALS AND METHODS

Materials. Unless otherwise stated, L-21 *Sca*I was prepared as described previously (Zaug et al., 1988; Bevilacqua & Turner, 1991). For most experiments, it was renatured in 5 mM $MgCl_2$, 135 mM NaCl, and 50 mM HEPES (25 mM Na^+) (pH 7.5) (reaction buffer) by heating to 50 °C for 10 min and then incubating at room temperature for at least 30 min before use. In order to test how different renaturation procedures affect the reaction, L-21 *Sca*I also was renatured in H_2O by incubating at 95 °C for 3 min and then adding reaction buffer immediately (Walstrum & Uhlenbeck, 1990) and incubating at room temperature for at least 30 min before use.

L-21 *Sca*I transcripts were also purified without denaturation following the procedure of Emerich and Woodson (1993). A chroma spin-100 column (Clontech) was pre-equilibrated three times with 1 mL of reaction buffer by centrifuging for 3 min at 700*g*. After RNA synthesis, the reaction mixture was loaded onto the column, and the column was spun for 5 min at 700*g*. The RNA contained in the flow through was then used immediately for stopped flow experiments.

Oligonucleotides modified with pyrene were chemically synthesized as previously described (Kierzek et al., 1993; Bevilacqua et al., 1994).

Kinetics. A KinTek stopped flow apparatus was used for rapid mixing experiments, as described previously (Johnson, 1986; Bevilacqua et al., 1994). All experiments were done in 5 mM $MgCl_2$, 135 mM NaCl, and 50 mM HEPES (25 mM Na^+) (pH 7.5) reaction buffer. The concentration of pyrene (pyr)-labeled pyrCUCU is in large excess over L-21 *Sca*I to maintain pseudo-first-order conditions. Chase experiments were used to determine k_{-2}, the rate for undocking the P1 helix from the catalytic core. In the chase experi-

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ments, 1 μM pyrCUCU premixed with 0.1 μM L-21 *Sca*I is rapidly mixed with 10 μM CUCU. Since CUCU is 10 times more concentrated than pyrCUCU and binds somewhat more tightly (Bevilacqua et al., 1993; Bevilacqua & Turner, 1991), any pyrCUCU dissociated from L-21 *Sca*I is replaced by CUCU.

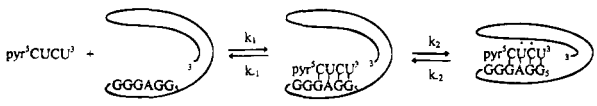
Fluorescence Titrations. Fluorescence titrations were performed on a Perkin-Elmer MPF-44A fluorimeter with excitation at 329 nm and emission at 397 nm. The total concentration of L-21 *Sca*I was held constant at 50 nM for 10 and 15 °C and at 200 nM for 20 and 25 °C with the concentration of pyrCUCU always at least slightly in excess.

Melting Experiments. Optical melting curves of oligoribonucleotides in reaction buffer were measured at 280 nm with a Gilford 250 spectrophotometer. Thermodynamic parameters were obtained by averaging enthalpy and entropy changes obtained from fits of each curve and from plots of inverse melting temperature vs $\log(C_T/4)$, where C_T is total oligonucleotide concentration (Petersheim & Turner, 1983):

$$T_M^{-1} = (2.3R/\Delta H^\circ)\log(C_T/4) + \Delta S^\circ/\Delta H^\circ \quad (1)$$

RESULTS

At 15 °C, it has previously been shown (Bevilacqua et al., 1992) that pyrCUCU binds to L-21 *Sca*I ribozyme in at least two steps, base pairing followed by docking:



(2)

The intermediate never builds up because the base-pairing step has a large dissociation rate (i.e. $k_{-1} \gg k_2$ and k_{-2}). Hence, when [pyrCUCU] $\ll k_{-1}/k_1$ (~40 μM), rapid mixing of ribozyme with fluorescent substrate is expected to result in a single exponential change of fluorescence with a rate, τ^{-1}, given by (Bevilacqua et al., 1992)

$$\tau^{-1} = (k_1k_2/k_{-1})[\text{pyrCUCU}] + k_{-2} \quad (3)$$

Thus, this system allows particularly easy determination of k_2 and k_{-2}, the rate constants for docking and undocking. The rate constant k_{-2} is obtained from the intercept of a plot of τ^{-1} vs [pyrCUCU] and from a chase experiment (see below). The rate constant k_2 is obtained from the slope of τ^{-1} vs [pyrCUCU] and knowledge of k_1/k_{-1}, the equilibrium constant for the base-pairing step. The substrate pyrCUCU was chosen for this study because of these useful kinetic limits.

Figure 1 shows a typical trace of fluorescence vs time after mixing pyrCUCU with L-21 *Sca*I ribozyme. Single exponential transients are observed in all cases. Figure 2 shows plots of the average rates for these transients as a function of pyrCUCU concentration at 10, 15, 20, and 25 °C. Included in Figure 2 are rates at [pyrCUCU] = 0 μM. These were obtained from chase experiments where excess CUCU is mixed with the L-21 *Sca*I·pyrCUCU complex (Bevilacqua et al., 1992), providing the intercepts, k_{-2}. As expected, Figure 2 shows a linear dependence of observed rate on [pyrCUCU], implying similar behavior from 10–25 °C. The data in Figure 2 were fit to eq 3 to provide the apparent association rates, $k_{on,app} = k_1k_2/k_{-1}$, from the slopes, and k_{-2} from the intercepts (Table 1). Rates at 15 °C are similar to

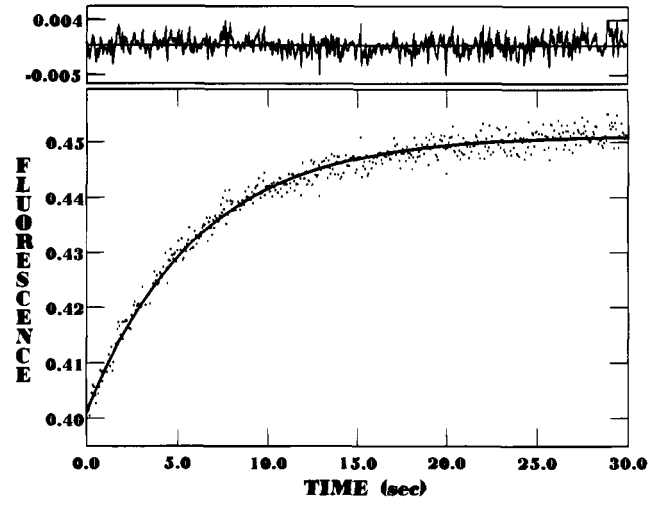


FIGURE 1: Representative trace for dependence of fluorescence intensity, F, on time after mixing equal volumes of pyrCUCU and L-21 *Sca*I at 25 °C. Final concentrations after mixing are 0.3 μM pyrCUCU and 50 nM L-21 *Sca*I. Data represent the average of three separate mixings. Data are fit to $F = F_\infty + Fe^{-t/\tau}$, resulting in $1/\tau = 0.12$ s^{-1}. Residuals for the fit are shown above the trace. Solutions are 5 mM $MgCl_2$, 135 mM NaCl, and 50 mM HEPES (25 mM Na^+) (pH 7.5).

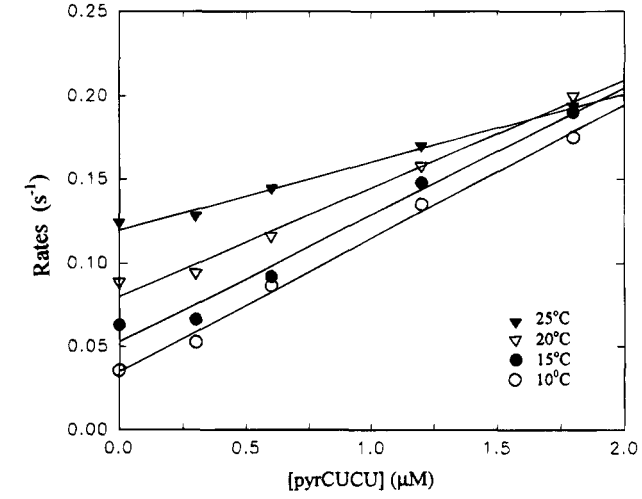


FIGURE 2: Plots of rate, $1/\tau$, as a function of pyrCUCU concentration at 10 (○), 15 (●), 20 (▽), and 25 (▼) °C. Values of rates at zero pyrCUCU concentration come from chase experiments. Solutions are 5 mM $MgCl_2$, 135 mM NaCl, and 50 mM HEPES (25 mM Na^+) (pH 7.5).

those reported previously (Bevilacqua et al., 1992). Activation parameters for the dissociation and apparent association rates were calculated from the Eyring equation

$$k = (eRT/Nh)\exp(\Delta S^\ddagger/R)\exp(-E_a/RT) \quad (4)$$

These values are also listed in Table 1.

Equilibrium constants for binding of pyrCUCU to L-21 *Sca*I were calculated from the rate constants listed in Table 1 and also measured by equilibrium titration. Titration curves are shown in Figure 3, and the results are listed in Table 1. The equilibrium constants from the two methods are in reasonable agreement. The van't Hoff ΔH° and ΔS° calculated from the average of the equilibrium and kinetic results for the overall binding are −22 kcal/mol and −50 eu, respectively.

Previous kinetic studies with a circular form of this group I intron showed that rates for a reverse cyclization reaction

Table 1: Kinetic and Thermodynamic Parameters for Binding of pyrCUCU to L-21 *Sca*I Ribozyme[a]

temperature (°C)	$k_{on,app}$ ($\times 10^{-4}$ M^{-1} s^{-1})	k_{-2} (s^{-1})	kinetic K_d (μM)	titration K_d (μM)
10	8.0 ± 0.4	0.035 ± 0.004	0.4 ± 0.1	0.3 ± 0.2
15	7.6 ± 0.6	0.053 ± 0.006	0.7 ± 0.1	0.8 ± 0.2
	6.2 ± 0.6[b]	0.051 ± 0.007[b]	0.8 ± 0.1[b]	
	8.6 ± 0.5[c]	0.049 ± 0.006[c]	0.6 ± 0.1[c]	
	5.8 ± 0.2[d]	0.076 ± 0.005[d]	1.3 ± 0.1[d]	
20	6.5 ± 0.4	0.081 ± 0.004	1.3 ± 0.1	1.6 ± 0.3
25	4.1 ± 0.2	0.12 ± 0.002	2.9 ± 0.2	2.4 ± 0.2
	3.0 ± 0.5[b]	0.13 ± 0.006[b]	4.3 ± 0.7[b]	
	4.4 ± 0.1[c]	0.14 ± 0.005[c]	3.2 ± 0.1[c]	
E_a (kcal/mol)	−7.6 ± 2.8	14.3 ± 0.1		
$\Delta S^{\ddagger}$ (eu)	−64.5 ± 8.0	−16.4 ± 0.6		
$\Delta H°$ (kcal/mol)			−21.9 ± 2.0	−22.9 ± 2.4
$\Delta S°$ (eu)			−48.1 ± 7.0	−51.3 ± 8.3

[a] Solutions are 5 mM $MgCl_2$, 135 mM NaCl, and 50 mM HEPES (25 mM Na^+) (pH 7.4). Unless noted by superscripts, values are for ribozyme renatured by heating at 50 °C for 10 min and then incubating at room temperature for at least 30 min. [b] Ribozyme renatured in H_2O by incubating at 95 °C for 3 min. [c] Ribozyme purified from transcription mixture without denaturation. [d] Bevilacqua et al., 1992.

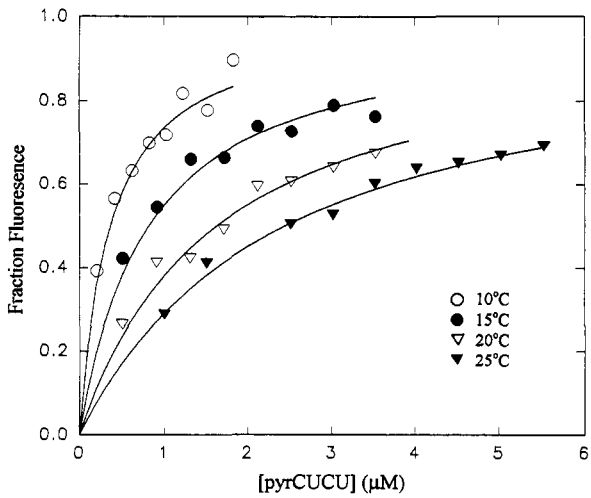


FIGURE 3: Equilibrium fluorescence titrations for pyrCUCU binding to L-21 *Sca*I at 10 (○), 15 (●), 20 (▽), and 25 (▼) °C. Solutions are 5 mM $MgCl_2$, 135 mM NaCl, and 50 mM HEPES (25 mM Na^+) (pH 7.5). To obtain K_d for pyrCUCU binding to L-21 *Sca*I, the fraction of maximal fluorescence increase (f) with [pyrCUCU] was directly fit by nonlinear least-squares to the quadratic equation $f = 0.5([\text{L-21 } \textit{Sca}\text{I}]_{ini} + [\text{pyrCUCU}]_{ini} + K_d) - [0.25([\text{L-21 } \textit{Sca}\text{I}]_{ini} + [\text{pyrCUCU}]_{ini} + K_d)^2 - [\text{L-21 } \textit{Sca}\text{I}]_{ini}[\text{pyrCUCU}]_{ini}]^{1/2}/[\text{L-21 } \textit{Sca}\text{I}]_{ini}$, where $[\ ]_{ini}$ represents initial concentration. The solid lines represent the best fits to the data.

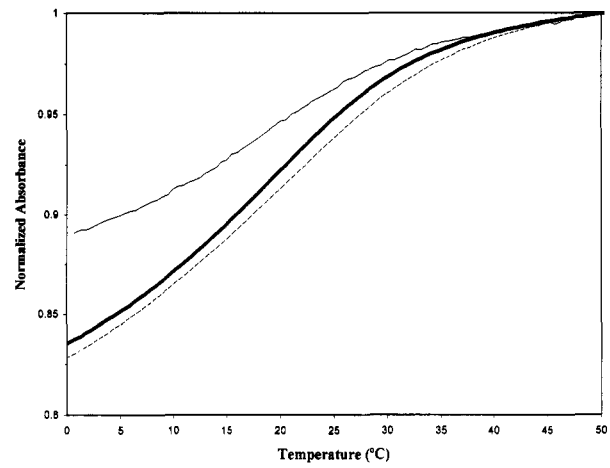


FIGURE 4: Plots of normalized absorbance vs temperature for rGGAGAA with rCrUrCrU (heavy line) at C_T = 0.49 mM, pyrCrUrCrU (dashed line) at 0.47 mM, and dCrUrCrU (light line) at 0.48 mM. Solutions are 5 mM $MgCl_2$, 135 mM NaCl, and 50 mM HEPES (25 mM Na^+) (pH 7.5).

could differ by orders of magnitude, depending on the method used for renaturation (Walstrum & Uhlenbeck, 1990). Moreover, the activation energy for reverse cyclization changed from 50 kcal/mol when no renaturation step was used (Sugimoto et al., 1988) to 6 kcal/mol when the RNA was renatured by heating to 95 °C (Walstrum & Uhlenbeck, 1990). To see if the rates for pyrCUCU binding to L-21 *Sca*I ribozyme were strongly dependent on renaturation protocol, kinetics experiments were also conducted at 15 and 25 °C on ribozyme renatured by heating to 95 °C (Walstrum & Uhlenbeck, 1990) and isolated directly from a transcription mixture without denaturation (Emerich & Woodson, 1993). These results are also listed in Table 1. The rates are similar for all three methods of ribozyme preparation. Either all three methods give the same ribozyme folding, or the rates for binding are not strongly dependent on any differences in folding.

Thermodynamic parameters for duplex formation between pyrCUCU and GGAGAA were measured by optical melting. GGAGAA is a mimic of the "internal guide sequence" substrate binding site on the ribozyme, GGAGGG (Davies et al., 1982; Michel et al., 1982; Waring et al., 1986; Been & Cech, 1986). GGAGAA was used because it forces pyrCUCU to bind in a single register and does not aggregate at the high concentrations required for melting. Parameters were also measured for duplex formation of GGAGAA with CUCU, dCUCU, CdUCU, CUdCU, and CUCdU. Typical melting curves and a T_M^{-1} plot are shown in Figures 4 and 5, and the results are listed in Table 2.

DISCUSSION

It has been shown previously that substrate binding by the *Tetrahymena* ribozyme involves at least two steps (Herschlag, 1992; Bevilacqua et al., 1992). The temperature dependence of the rates reported in Table 1 provides confirmatory evidence for this since the rate constant $k_{on,app}$ is slower at higher temperatures. Thus, the activation energy measured for $k_{on,app}$ is negative, whereas any elementary process must have a positive activation energy. Qualitatively similar results were obtained for three different methods of preparing ribozyme, including one that does not involve denaturation. Thus, the negative activation energy is probably not due to a misfolding. While negative activation energies have been observed for simple base pairing of

Table 2: Thermodynamic Parameters for Binding to GGAGAA[a]

	$1/T_M$ vs $\log(C_T/4)$ parameters				curve fit parameters			
oligomer	$-\Delta G°_{15}$ (kcal/mol)	$-\Delta H°$ (kcal/mol)	$-\Delta S°$ (eu)	T_M[b] (°C)	$-\Delta G°_{15}$ (kcal/mol)	$-\Delta H°$ (kcal/mol)	$-\Delta S°$ (eu)	T_M[b] (°C)
pyrCrUrCrU	5.77 ± 0.07	30.4 ± 2	85.4 ± 9	25	5.72 ± 0.2	28.7 ± 3	79.7 ± 9	25
rCrUrCrU	5.62 ± 0.06	35.7 ± 2	104 ± 7	23	5.68 ± 0.2	37.4 ± 3	110 ± 11	23
dCrUrCrU	4.92 ± 0.04	29.3 ± 2	84.7 ± 7	16	4.95 ± 0.2	34.4 ± 5	102 ± 16	17
rCdUrCrU	4.73 ± 0.05	26.7 ± 2	76.2 ± 9	15	4.71 ± 0.1	28.8 ± 2	83.7 ± 6	14
rCrUdCrU	4.96 ± 0.04	29.6 ± 2	85.6 ± 8	17	4.99 ± 0.1	31.5 ± 2	92.1 ± 7	17
rCrUrCdU	5.12 ± 0.01	35.8 ± 0.7	106 ± 2	19	5.10 ± 0.04	33.0 ± 1	96.9 ± 5	18

[a] Solutions are 5 mM $MgCl_2$, 135 mM NaCl, and 50 mM HEPES (25 mM Na^+) (pH 7.4). Significant figures beyond error estimates are given to allow accurate calculation of T_M and other parameters. [b] Calculated for a 10^{-3} M oligomer concentration.

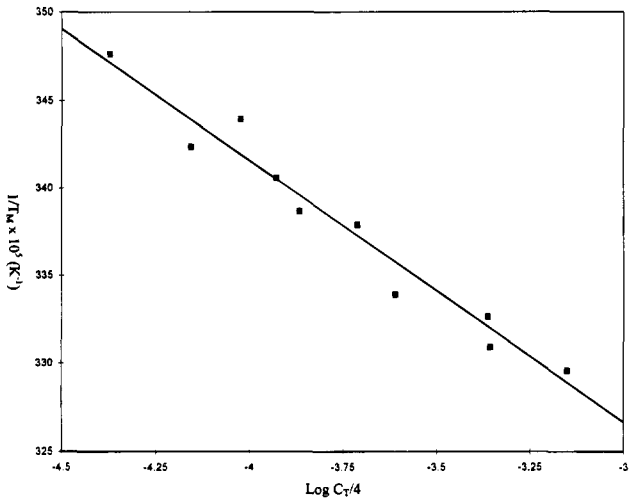


FIGURE 5: Reciprocal melting temperature vs $\log(C_T/4)$ plot for pyrCUCU and rGGAGAA.

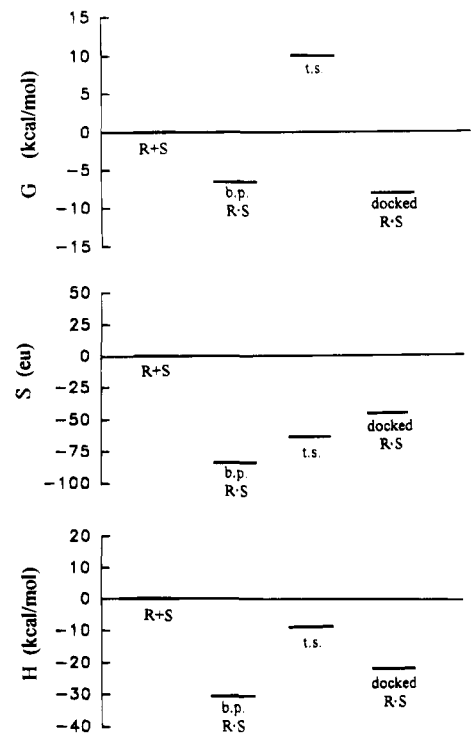


FIGURE 6: Approximate relative free energies, G_{15}, entropies, S, and enthalpies, H, for free ribozyme and substrate (R+S), base-paired intermediate (bp R·S), transition state (t.s.), and docked pyrCUCU·L-21 *ScaI* complex (docked R·S) based on kinetic results in Table 1 and $\log(C_T/4)$ parameters in Table 2.

oligomers containing only AU pairs (Porschke & Eigen, 1971; Craig et al., 1971), pairing involving GC pairs typically is associated with zero or positive activation energies (Podder, 1971; Pörschke et al., 1973; Nelson & Tinoco, 1982; Freier et al., 1983). For example, E_a is 3 kcal/mol for binding of UUCA to the anticodon loop of phenylalanine tRNA (Yoon et al., 1975). Since the pairing or first step in binding to ribozyme involves 2 GC pairs, it is expected to have a positive activation energy. The measured activation energy of −7.6 kcal/mol presumably arises because a subsequent step, docking into the catalytic core, requires the base-paired intermediate. The concentration of this paired intermediate decreases with increasing temperature since increasing temperature disfavors helix formation. This leads to a slower $k_{on,app}$ as temperature increases. That is, $k_{on,app} = k_1k_2/k_{-1} = K_1k_2$, and the association constant for the pairing step, K_1, decreases with increasing temperature.

The activation energy for $k_{on,app}$ provides new insight into the docking step that brings the helix formed by pyrCUCU into the catalytic core of the ribozyme. For the two-step mechanism of eq 2, the apparent activation enthalpy, $\Delta H^‡_{on,app} = E_a - RT = -7.6 - 0.6 = -8.2$ kcal/mol, is the sum of $\Delta H°_1$, the enthalpy change for the base-pairing step, and $\Delta H^‡_2$, the activation enthalpy for the docking step:

$$\Delta H^‡_{on,app} = \Delta H°_1 + \Delta H^‡_2 \quad (5)$$

The value of $\Delta H°_1$ can be approximated by the $\Delta H°$ of −30.4 kcal/mol measured for pyrCUCU binding to GGAGAA (Table 2). Thus, $\Delta H^‡_2 \approx 22.2$ kcal/mol. This value is much larger than the activation energy of about 4 kcal/mol for diffusion-controlled processes (Lohman, 1986). Evidently, docking is not controlled by simple diffusion. In a similar manner, $\Delta S^‡_2 = \Delta S^‡_{on,app} - \Delta S°_1 = -64.5 - (-85.4) = 20.9$ eu. In contrast to $\Delta H^‡_2$ and $\Delta S^‡_2$, $\Delta H^‡_{-2}$ is modest at 13.7 kcal/mol, and $\Delta S^‡_{-2}$ is unfavorable at −16.4 eu. Diagrams that summarize the various activation and thermodynamic parameters are shown in Figure 6.

There are several possible origins for the large, unfavorable $\Delta H^‡_2$ and favorable $\Delta S^‡_2$. One is uptake of Mg^{2+} ions. For comparison, binding of Mg^{2+} to ATP^{4-} is associated with a $\Delta H^‡$ of 11.3 kcal/mol and a $\Delta S^‡$ of 12 eu (Banyasz & Stuehr, 1973). Thus, the activation parameters for docking are consistent with a rate-limiting step involving uptake of Mg^{2+} ions.

Another possible origin for the activation parameters is that the rate-limiting step for docking involves opening some ribozyme structure before the pyrCUCU helix can dock. At least two types of conformational change can be envisaged. In one type, structure could be broken away from the docking site. In a second type, nucleotides required for the docking

site might rearrange to form the docking site. While a conformational change involving disruption of 2−3 base pairs is consistent with the measured activation enthalpy of 22 kcal/mol, the activation entropy is at least 3-fold less than the $\Delta S°$ associated with breaking such base pairs in oligomers (Freier et al., 1986). This could arise from several possible considerations. First, calculation of activation entropy requires knowledge of the frequency factor, normally considered to be *RT/Nh* (see eq 4). This may not be reasonable for this type of reaction in a macromolecule. Second, the $\Delta S°$ for breaking base pairs in the constricted environment of a ribozyme may be considerably less than that for oligomers. Third, a combination of conformational changes and, separately, metal ion uptake may be involved in the rate-limiting step.

Previous studies have provided very different estimates for the rate of docking in ribozymes derived from the *Tetrahymena* intron. Herschlag (1992) used a miscleavage assay with substrates of the form $pppG_2CCCUCUA_5$, $pppG_2$-CCCUCUAGU and derivatives thereof with versions of the L-21 *Sca*I ribozyme mutated in region J 1/2 to estimate a lower limit for the rate of docking of 1333 s^{-1} at 50 °C in 10 mM Mg^{2+} and 50 mM Na^+. Bevilacqua et al. (1992, 1994) used fluorescence-detected stopped flow with the wild type L-21 *Sca*I ribozyme to measure a rate of docking of 3 s^{-1} for pyrCCUCU and 0.6 s^{-1} for pyrCCUCUA at 15 °C. The rate of docking for pyrCUCU at 50 °C calculated with eq 4 and $\Delta H^{\ddagger}_2$ ($=E_{a,2}$ − RT) and $\Delta S^{\ddagger}_2$ reported here is 200 s^{-1}. Considering the differences in substrate, ribozyme, and salt conditions, the data are consistent with miscleavage and fluorescence reporting on the docking step, but it remains possible that they measure different steps.

Comparison of the thermodynamic parameters for pyrCUCU binding to L-21 *Sca*I ribozyme (Table 1) and to GGAGAA (Table 2) provides interesting insights into the tertiary interactions important for binding to ribozyme. While pyrCUCU binds about 60-fold more tightly to ribozyme than to GGAGAA, the $\Delta H°$ of −22 kcal/mol for binding to ribozyme is less favorable than the $\Delta H°$ of −30 kcal/mol for binding to GGAGAA. Evidently, the tighter binding to ribozyme is associated with a more favorable $\Delta S°$. Assuming the thermodynamic parameters for the pairing step are the same for ribozyme and for GGAGAA, the formation of tertiary interactions with the ribozyme must be associated with an unfavorable $\Delta H°$ of about 8 kcal/mol and a favorable $\Delta S°$ of about 40 eu (see Figure 6). That is, at least some of the tertiary interactions formed must be driven by favorable entropy changes. Several possibilities exist. Binding of metal cations by ion-pair formation is entropically driven, partly because the charge neutralization leads to less ordered water (Kauzmann, 1959; Cantor & Schimmel, 1980). For example, for Mg^{2+} binding to ATP^{4-}, $\Delta S°$ is a favorable 28 eu and $\Delta H°$ is an unfavorable 3 kcal/mol (Banyasz & Stuehr, 1973). Since organization of the catalytic core involves packing of helixes (Kim & Cech, 1987; Michel & Westhof, 1990), the increased density of negative charges would likely result in uptake of Mg^{2+} ions (Record et al., 1978; Manning, 1978). In addition, it has been suggested that specifically held Mg^{2+} ions are involved in the catalysis (Sugimoto et al., 1988; Toh et al., 1987; Freemont et al., 1988; Steitz & Steitz, 1993; Piccirilli et al., 1993). A cross-linking study indicated about 8 Mg^{2+} ions are taken up upon substrate docking (Wang & Cech, 1994), and a thiophosphate substitution study identified several phosphates likely to coordinate Mg^{2+} (Christian & Yarus, 1993). Another favorable contribution to entropy could arise from expansion of the volume occupied by condensed counterions upon packing of helixes, as predicted by counterion condensation theory (Ray & Manning, 1994). A third possibility for the entropy-driven interactions important for docking is classical hydrophobic bonding associated with release of bound water (Kauzmann, 1959). This would be surprising, however, given the hydrophilic nature of the periphery of the substrate helix.

Table 3: Comparison (kcal/mol) of $\Delta\Delta G°_{15}$ for Binding to L-21 *Sca*I and rGGAGAA[a]

oligomer	$\Delta G°_{15}$ binding to L-21 *Sca*I[b]	$\Delta\Delta G°_{15}$ relative to rCrUrCrU[c]		tertiary $\Delta\Delta\Delta G°_{15}$ (L-21 *Sca*I −rGGAGAA)[e]
		L-21 *Sca*I	rGGAGAA[d]	
rCrUrCrU	9.98 ± 0.08	−	−	−
dCrUrCrU	9.36 ± 0.05	−0.62 ± 0.1	−0.70 ± 0.07	0.08 ± 0.1
rCdUrCrU	7.68 ± 0.06	−2.3 ± 0.1	−0.89 ± 0.07	−1.4 ± 0.1
rCrUdCrU	8.48 ± 0.09	−1.5 ± 0.1	−0.66 ± 0.07	−0.8 ± 0.1
rCrUrCdU	9.24 ± 0.06	−0.74 ± 0.1	−0.51 ± 0.06	−0.2 ± 0.1

[a] Solutions are 5 mM $MgCl_2$, 135 mM NaCl, and 50 mM HEPES (pH 7.4). [b] Bevilacqua & Turner, 1991. [c] $\Delta\Delta G°_{15} = \Delta G°_{15}$ (all ribo) − $\Delta G°_{15}$ (deoxyribo). [d] Calculated from $1/T_M$ vs $\log(C_T/4)$ parameters. [e] $\Delta\Delta\Delta G°_{15} = D\Delta G°_{L\text{-}21Scal} - \Delta\Delta G°_{rGGAGAA}$.

A fourth possibility for entropy-driven interactions is desolvation of RNA functional groups involved in tertiary interactions. Previous studies have identified hydrogen bonding to 2′-OH groups on substrates as one of the favorable tertiary interactions holding substrate (Sugimoto et al., 1989; Bevilacqua & Turner, 1991; Pyle et al., 1992; Pyle & Cech, 1991; Herschlag et al., 1993; Strobel & Cech, 1993). In those studies, various methods were used to estimate the free energy increments associated with these interactions. The data in Table 2 coupled with previous results for binding of the same chimeric substrates to L-21 *Sca*I ribozyme (Bevilacqua & Turner, 1991) permit an estimate for the free energy increments for the 2′-OH tertiary interactions at 15 °C, $\Delta G°_{2'\text{-}OH,15}$, at 5 mM Mg^{2+} and 160 mM Na^+. The increments are empirically estimated by taking the difference in the effect of a deoxy substitution on binding to ribozyme and to GGAGAA (see Table 3). The results indicate the 2′-OH groups at positions −2 and −3 contribute −0.8 and −1.4 kcal/mol, respectively, similar to the values of −0.6 and −1.0 kcal/mol previously reported at 50 mM Mg^{2+} and 25 mM Na^+ (Bevilacqua & Turner, 1991). Thus, the 2′-OH interactions are thermodynamically important under the conditions used for the experiments reported here. Hydrogen-bonding tertiary contacts with the exocyclic amino group of the G·U pair are also thought to favor docking (Pyle et al., 1994; Knitt et al., 1994; Strobel & Cech, 1995). It is possible that the desolvation required to allow all these functional groups to form hydrogen bonds might provide the favorable entropy change for docking. On the basis of available data for hydrogen bonds in nucleic acids (Turner et al., 1987; SantaLucia et al., 1991, 1992), however, it would be surprising if hydrogen bonds completely account for the large favorable entropy change observed for docking. Thus, it is likely that additional interactions are also important for helix packing.

As shown in Figure 6, the equilibrium for docking the P1 helix is driven by a favorable entropy change. A recent study of the binding of another substrate required for splicing,

guanosine 5′-monophosphate, showed that its binding is also entropically driven (McConnell & Cech, 1995). The prominant role of favorable entropy changes for those processes may have functional consequences. In particular, binding and alignment of substrates is associated with unfavorable entropy terms due to loss of translational and rotational freedom. If all this unfavorable entropy was compensated by interactions associated with favorable enthalpy changes, then the temperature dependence of the binding constants, K, would be considerable since $K = \exp(-\Delta G°/RT) = \exp(-\Delta H°/RT + \Delta S°/R)$. This is the typical case, for example, when associations are driven by base pairing. Such a large temperature dependence could be beneficial in the early stages of evolution when reactions were slow and associations and dissociations could be driven by temperature (Turner & Bevilacqua, 1993). In later stages, however, this large temperature dependence could be deleterious. Thus, there might be a selection pressure to employ interactions such as salt bridges that are associated with favorable entropy changes, in order to reduce the enthalpic component of binding. This would allow the organism to function efficiently over a wider temperature range. Interestingly, much of the binding of oligopyrimidine substrate to the group I ribozyme is driven by base pair formation driven by a large enthalpy change (Table 2). In the natural self-splicing group I intron, however, this enthalpy change is reduced since formation of the base pairs in the P1 helix requires disruption of previously formed base pairs with an upstream element of the ribosomal RNA precursor (Woodson & Cech, 1991). Thus, switching base pairs may represent another strategy for reducing the temperature dependence of an equilibrium. Such switching of base pairs to provide function is also seen in nuclear splicing (Madhani & Guthrie, 1992) and trans splicing (LeCuyer & Crothers, 1994).

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