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Binding of a Fluorescent Oligonucleotide to a Circularized Intervening Sequence from Tetrahymena thermophila

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Binding behavior of a fluorescent oligonucleotide εACUCU to a circularized intervening sequence from Tetrahymena thermophila has been investigated by fluorescence titration. The binding constant was 9.5×10^6 mol^{-1} dm^3 at 10 °C, roughly 3 orders of magnitude larger than expected for simple Watson-Crick base pairing.

The discovery of RNA catalysis (ribozyme) has led to great interest in the role of RNA in the origin of life and in the mechanisms for such reactions.[1-7] The first RNA known to facilitate cleavage and ligation reactions in RNA is the intervening sequence (IVS) from the rRNA precursor of Tetrahymena thermophila.[1] The IVS produces a covalently closed, circularized form (CIVS). The CIVS can be linearized by addition of oligonucleotides[8] or by hydrolysis.[9] Our previous studies[10-12] for the reaction of CIVS with oligonucleotides have indicated that 1) binding of oligonucleotide substrate is unusually strong, that is, the binding constant is 3-4 orders of magnitude larger than expected for simple Watson-Crick base pairing, 2) a weakly bound Mg^{2+} ion may be required for reaction, 3) the 2'OH of the 5' sugar of a oligonucleotide is involved in substrate binding, and 4) the 2'OH of the 3' sugar of a dinucleotide phosphate is involved in Mg^{2+} binding. These results on the binding, however, were not directly measured but were obtained from the kinetic analysis.

In this work, we have measured the binding constant of a fluorescent oligonucleotide εACUCU (εA; 1,N^6-ethenoadenosine which is a fluorescent derivative of adenosine) with CIVS from the rRNA precursor of Tetrahymena thermophila. This is the first report of the binding behavior between an oligonucleotide and a ribozyme using a fluorescent probe which is being developed rapidly due to current interest.[13] The results suggest the binding constant is unusually large and consistent with that from the kinetic analysis.

CIVS was obtained by the method described previously,[10,11] and purified by running the reaction mixture on a 4% polyacrylamide/8 M urea gel. Final purification was by chromatography on Sephadex G-50. 1,N^6-Ethenoadenosine (εA) which was one of the starting materials for synthesizing εACUCU was obtained by the method of Tolman et al.[14] The oligonucleotide εACUCU was synthesized on solid support with a phosphoramidite method,[15] and purified by high-performance liquid chromatography.[16] Concentrations of CIVS and εACUCU were determined optically with extinction coefficients at 260 nm.[11,17]

All fluorescence measurements were made at 10 °C because the intermediate with εACUCU bound is easily trapped at low temperature. Indeed, the kinetic results[10] predicted the half-life for the circle opening reaction from the intermediate is longer than a week at 10 °C . The buffer was 7 mmol dm^{-3} NaCl, 3 mmol dm^{-3} Na_2HPO_4, 10.5 mmol dm^{-3} $MgCl_2$, and 0.5 mmol dm^{-3} Na_2EDTA (pH 7.0). Figure 1 shows the fluorescence spectra of εACUCU in the absence and presence of CIVS when excitation is at 305 nm. The peak at around 405 nm did not shift, but the fluorescence intensity appreciably decreased upon addition of CIVS. The

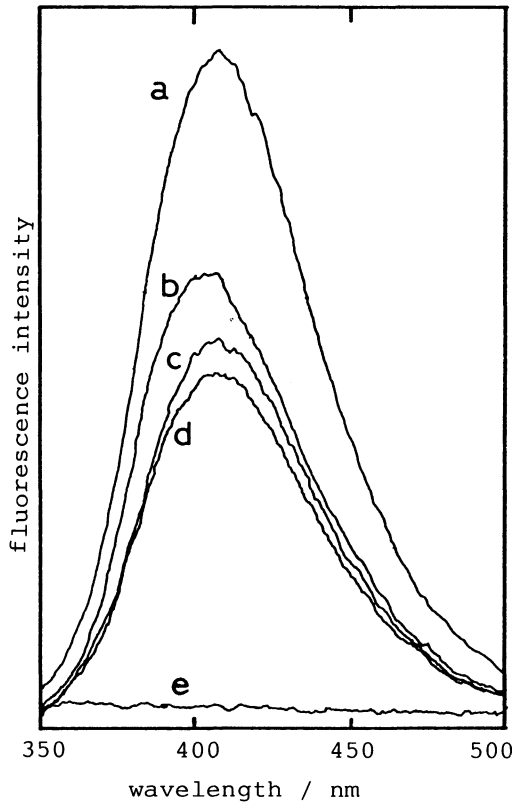


Fig. 1. Fluorescence spectra of εACUCU (a) in the absence and in the presence of (b) 0.24, (c) 1.1, and (d) 4.5 μmol dm^{-3} CIVS, and (e) fluorescence spectrum of buffer only.

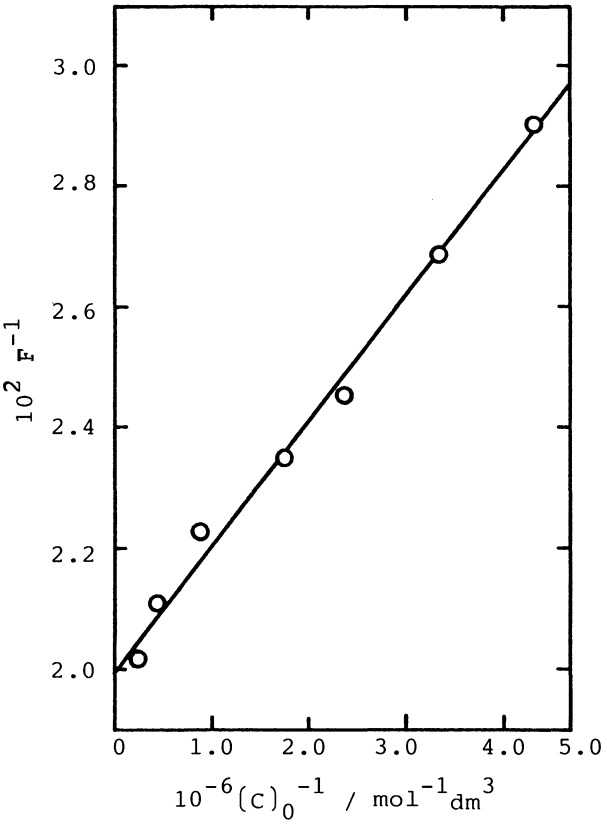
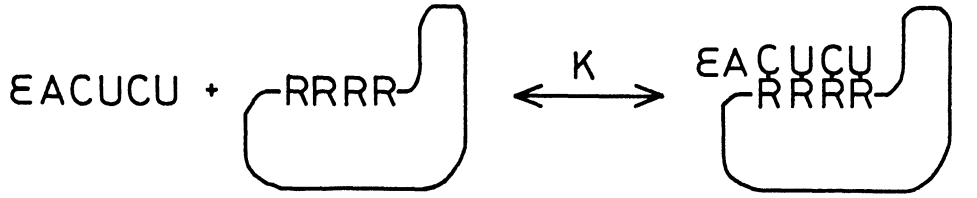


Fig. 2. Dependence of the percentage of fluorescence intensity decrease, F, at 405 nm on the CIVS concentration.

dependence of the fluorescence intensity decrease on the concentration of added CIVS was hyperbolic, and the minimal fluorescent intensity was almost reached by 4×10^{-6} mol dm^{-3} CIVS. The fluorescent probe is sensitive to environment. For example, the fluorescence intensity of poly(εA) is less than one-seventh that of εA,[14,18] indicating that the fluorescence of εA is quenched by its neighbors in the polynucleotide chain. The fluorescence intensity decrease of εACUCU by CIVS in this work indicates the oligonucleotide binds to the intervening sequence consistent with the kinetic analysis for the circle opening reaction.[10]

If the mechanism for the oligomer binding to CIVS is simple as Eq. 1;



(1)

CIVS εACUCU·CIVS complex

(RRRR; internal guide sequence[2])

and the concentration of CIVS is in excess over the oligonucleotide concentration, then plots of $1/F$ against $1/[C]_0$ should be linear according to Eq. 2;

$$1/F = (1/K_1F_m)(1/[C]_0) + (1/F_m) \quad (2)$$

where F is the percentage decrease of fluorescence intensity relative to that of εACUCU only, F_m the maximum value of F, $[C]_0$ the initial concentration of CIVS, and K_1 the binding constant of the complex. As shown in Fig. 2, a linear plot is observed. A linear least-squares fit to Eq. 2 of the data shown in Fig. 2 provides the K_1 value of 9.5×10^6 mol^{-1} dm^3. The binding constant for this association can be predicted from nearest-neighbor parameters determined for RNA duplex formation.[19] The predicted value is 3.0×10^3 mol^{-1} dm^3 in 1 mol dm^{-3} NaCl at 10 °C if only base pairs are considered.[20] If the effect of the dangling εA is added with the assumption that the stacking stability of εA is similar to that of adenosine, the predicted value is at most 10^4 mol^{-1} dm^3. The results of melting of deoxyoligonucleotide[21] and ribooligonucleotide[22] duplexes suggest the predicted value will be similar for the salt condition used here. The difference between the measured K_1 and the predicted binding constant is equivalent to an additional 15-20 kJ mol^{-1} in binding free energy. Evidently, the binding step involves more than a simple association of the oligonucleotide to CIVS by base-pairing such as a potential base triplet involving the GU pair,[11] and a hydrogen bond between the phosphate of the substrate and CIVS. This was indirectly shown by the results of the kinetic analysis using radioactivity.[10,11]

The previous results indicated the mechanism for the circle opening reaction involved separate steps for binding an oligonucleotide and Mg^{2+}.[10] The sequence for those binding steps and additional steps in the mechanism can be revealed by

transient kinetic studies. The fluorescent probe used in this work should be very useful for such kinetic studies because fluorescence detection is widely used with stopped-flow and temperature-jump techniques.

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