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The kinetics of binding of U-U-C-A to a dodecanucleotide anticodon fragment from yeast $tRNA^{Phe}$

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ABSTRACT

The kinetics of U-U-C-A binding to the dodecanucleotide (A-C_m-U-G_m-A-A-Y-A-ψ-^{m5}C-U-Gp) isolated from the anticodon region of yeast $tRNA^{Phe}$ are similar to the kinetics of binding of U-U-C-A to intact $tRNA^{Phe}$. A large enhancement in binding constant over that predicted for U-U-C-A·U-G-A-A is observed for both the complexes of dodecanucleotide and $tRNA^{Phe}$ with U-U-C-A. This strongly suggests that both the anticodon loop in $tRNA^{Phe}$ and the dodecanucleotide can form four base pairs with U-U-C-A. Furthermore, the enhanced stability cannot be attributed to a special conformation of the anticodon loop, but instead the anticodon loop is probably flexible. A likely explanation for the increased binding is the effect of non-base-paired ends. This increased thermodynamic stability comes from a larger entropy gain rather than a larger enthalpy decrease.

INTRODUCTION

The codon-anticodon interaction has been studied extensively due to its biological significance in protein synthesis.[1-7] This complex is more stable than found[8] or predicted[9] for two complimentary oligonucleotides of the same length. This stability has been attributed to the conformation of the anticodon loop.[1-5]

X-ray crystallography has provided detailed atomic coordinates of yeast $tRNA^{Phe}$[10-12] in a crystal. The anticodon loop contains two distinct quasihelical parts; two pyrimidines stacked at the 5' side and five bases (including the anticodon and two purines) stacked at the 3' side. It is important to know the conformation of the anticodon loop in solution and how it differs from a single stranded oligonucleotide of the same sequence. We have tried to answer this question by studying the kinetics of U-U-C-A binding to the dodecanucleotide (A-C_m-U-G_m-A-A-Y-A-ψ-^{m5}C-U-G_p) excised from the anticodon

region of $tRNA^{Phe}$. This dodecanucleotide can form no base pairs to hold it in a loop and presumably has the conformation of a single strand. Its kinetics can be directly compared with the results of U-U-C-A binding to the intact anticodon loop in $tRNA^{Phe}$.[6]

MATERIALS AND METHOD

The dodecanucleotide from the anticodon region of Baker's yeast $tRNA^{Phe}$ was prepared as described previously.[13] A molar extinction coefficient was determined by phosphate analysis to be $\varepsilon(260) = 1.0 \times 10^5$ molar^{-1} cm^{-1}. U-U-C-A was synthesized by the blocked diphosphate method.[6] The $\varepsilon(260)$ for U-U-C-A was estimated to be 3.9×10^4 molar^{-1} cm^{-1}.

A conventional absorbance temperature jump instrument[14] (Messanlagen Studien Gesellshaft, 34 Göttingen, W. Germany) was used for the experiment. The cell volume was 1 ml, with an optical path length of 0.7 cm. A temperature jump of 3.4°C was produced by the discharge of 25 KV with a 0.05 μF capacitor. Details of the procedure were described previously.[6]

RESULTS

The kinetics of U-U-C-A binding to the dodecanucleotide ($A\text{-}C_m\text{-}U\text{-}G_m\text{-}A\text{-}A\text{-}Y\text{-}A\text{-}\psi\text{-}^{m^5}C\text{-}U\text{-}G_p$) were measured at U-U-C-A concentrations ranging from 20 to 120 μM; dodecanucleotide concentrations ranged from 4 to 7 μM. The solvent was 0.1 M NaCl, 0.01 M $MgCl_2$, 0.01 M cacodylate buffer (pH 7). Three distinct relaxation times were observed. One was the instrumental heating time (τ = 5 μs) and its amplitude comprised 1% of the total intensity. The dodecanucleotide showed this relaxation both in the presence and the absence of U-U-C-A. This relaxation time is not dependent on the concentration of either species. Therefore, this fast relaxation can be attributed to single strand stacking changes which occur in times of the order of 100 ns.[15,16] The second ranged from 30 to 100 ms with about 0.2% of the total intensity. This relaxation was observed with the solution of dodecanucleotide in the absence of U-U-C-A; the relaxation time depended on the concentration of dodecanucleotide. Thus, this relaxation can be attributed to the formation of an intermolecular complex of the dodecanucleotide. The third relaxation occurs on a millisecond time

scale and corresponds to a change of 0.2-0.5% of the total intensity, as shown in Figure 1. This relaxation occurs only in the presence of both dodecanucleotide and U-U-C-A. It has a concentration dependence on both dodecanucleotide and U-U-C-A. The amplitude of the relaxation peaks when half of the dodecanucleotide is bound. This is characteristic of biomolecular reactions. Thus, this relaxation must be attributed to the dodecanucleotide and U-U-C-A complex formation;

$$\text{Dodecanucleotide} + \text{U-U-C-A} \underset{k_D}{\overset{k_R}{\rightleftharpoons}} \text{Dodecanucleotide}\cdot\text{U-U-C-A}. \quad (1)$$

The concentration dependence of the lifetime for such a reaction has the following form

$$\tau^{-1} = k_R \left([\text{U-U-C-A}]_{eq} + [\text{Dodecanucleotide}]_{eq}\right) + k_D \quad (2)$$

where $[\text{U-U-C-A}]_{eq}$ and $[\text{Dodecanucleotide}]_{eq}$ are the equilibrium concentrations of uncomplexed U-U-C-A and dodecanucleotide at the final temperature.

The concentration of dodecanucleotide was kept much lower than the concentration of U-U-C-A to supress the interference of the relaxation signal of the dodecanucleotide itself. The concentration dependent relaxation time was analyzed independent of other relaxations since the three discrete relaxations were sufficiently far apart. Figure 2 shows a plot of τ^{-1} vs. $([\text{U-U-C-A}]_{eq} + [\text{Dodecanucleotide}]_{eq})$; a good linear relation was obtained at each temperature. Equilibrium concentrations were estimated and used to make a trial plot to obtain k_R and k_D. From the equilibrium constants $K = k_R/k_D$, new concentrations were obtained and the process was repeated until k_R and k_D did not change. The recombination (k_R) and dissociation (k_D) rate constants obtained from the plots are given in Table I. The rate constants ($k_R = 1.2 \times 10^7$ ℓ mole^{-1}sec^{-1}, $k_D = 150$ sec^{-1} at 0°C) are very similar to those of U-U-C-A binding to the intact $tRNA^{Phe}$ ($k_R = 1.3 \times 10^7$ ℓ mole^{-1}sec^{-1}, $k_D = 100$ sec^{-1} at 0°C).[6] Arrhenius plots for k_R and k_D yielded 3 ± 1 and 22 ± 3 Kcal mole^{-1} for the activation energies of recombination and dissociation, respectively. The same activation energies have been obtained for the binding of U-U-C-A to $tRNA^{Phe}$.[6]

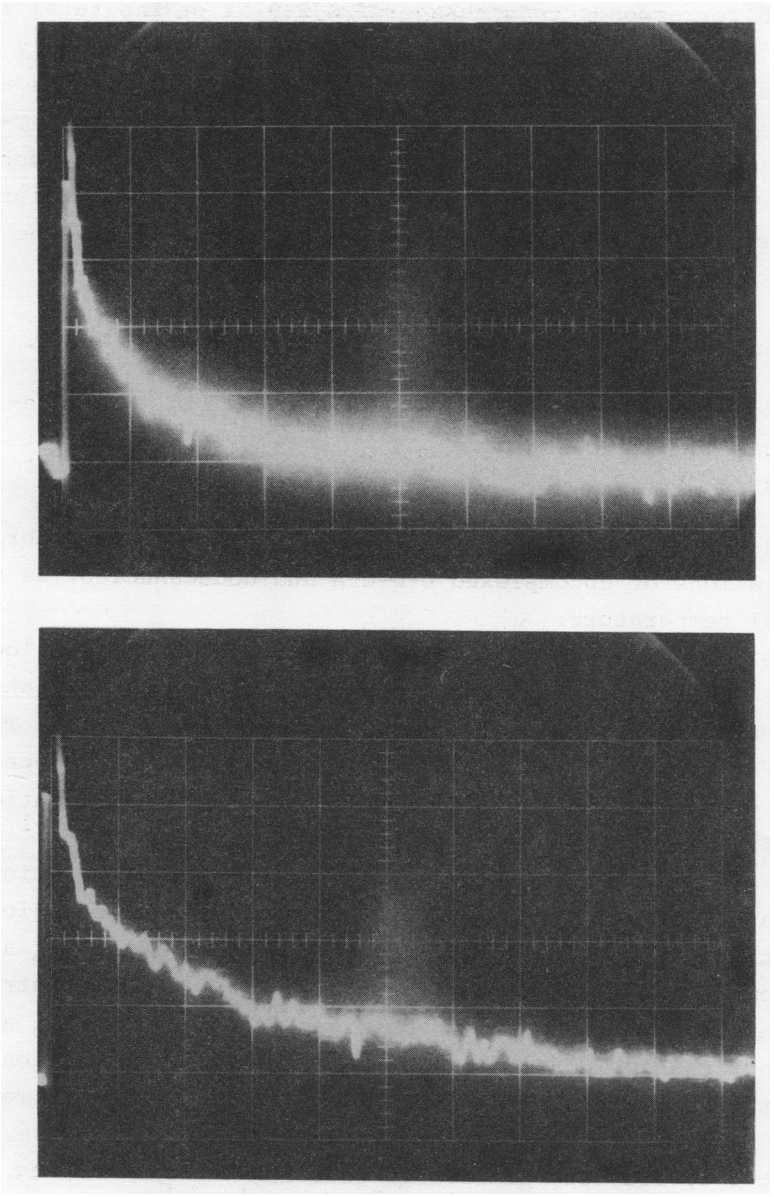

Figure 1. Oscillograms of temperature-jump relaxation curves of U-U-C-A binding to dodecanucleotide in 0.1 M NaCl, 0.01 M $MgCl_2$ and 0.01 M cacodylate buffer pH7. Concentrations were 7.4 μM dodecanucleotide and 49.2 μM U-U-C-A. Final temperature 10.5°C; oscilloscope setting: upper trace 1ms/division, 2mV/division lower trace 400μs/division, 2mV/division. Total intensity corresponds to 2V and wavelength was at 265nm.

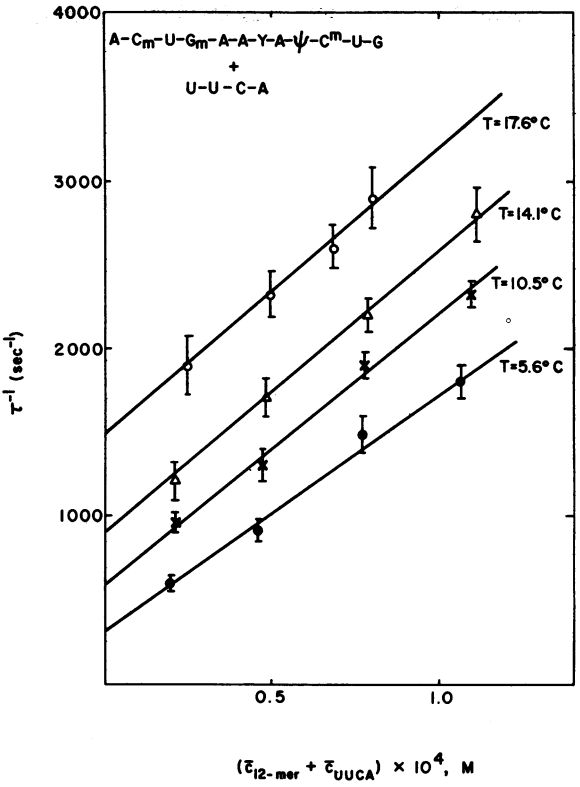

Figure 2. Reciprocal of relaxation time vs. concentration of unbound ([U-U-C-A]eq) and ([Dodecanucleotide]eq) at final temperature.

Table I: Kinetic and Thermodynamic Parameters for the Interaction Between Dodecanucleotide and U-U-C-A.

Temperature	k_R (M^{-1} sec^{-1})	k_D (sec^{-1})	K (M^{-1})
5.7°C	(1.4 ± 0.2) x 10^7	300 ± 30	(4.7 ± 0.9) x 10^4
10.5°C	(1.6 ± 0.2) x 10^7	590 ± 60	(2.7 ± 0.5) x 10^4
14.0°C	(1.7 ± 0.2) x 10^7	900 ± 100	(1.9 ± 0.4) x 10^4
17.0°C	(1.8 ± 0.2) x 10^7	1500 ± 200	(1.2 ± 0.3) x 10^4
5 ∿ 17°C	Ea = 3 ± 1 Kcal $mole^{-1}$	Ea = 22 ± 3 Kcal $mole^{-1}$	ΔH = - 19 ± 4 Kcal $mole^{-1}$
	$\Delta H_R^{\neq}$ = 2 ± 1 Kcal $mole^{-1}$	$\Delta H_D^{\neq}$ = 21 ± 3 Kcal $mole^{-1}$	
	$\Delta S_R^{\neq}$ = - 16 ± 2 cal deg $mole^{-1}$	$\Delta S_D^{\neq}$ = 32 ± 6 cal deg $mole^{-1}$	ΔS = - 48 ± 8 cal deg $mole^{-1}$

DISCUSSION

The kinetics of U-U-C-A binding to the dodecanucleotide show a remarkable similarity to the kinetics of U-U-C-A binding to the intact anticodon loop.

From the fast rate ($k_R \approx 10^7$ ℓ $mole^{-1}sec^{-1}$) and small activation energy (E_a = 3 kcal), the rate determining step can be assigned to the formation of the first base pair (G·C) as in the case of U-U-C-A·$tRNA^{Phe}$. The enthalpy of formation (ΔH = -19 ± 4 kcal) is the same as found for U-U-C-A and $tRNA^{Phe}$ and is in agreement with the enthalpy estimated[9] for the formation of four base pairs (ΔH = -20 kcal). This strongly suggests that both the anticodon loop in $tRNA^{Phe}$ and the dodecanucleotide can form four base pairs with U-U-C-A.

The binding constant of U-U-C-A to $tRNA^{Phe}$ is 1.3 x 10^5 at 0°C.[16] This is about 3000 times that calculated from the model compound U-U-C-A·U-G-A-A.[9] It has been suggested that this greatly enhanced binding is due to the conformation of the anticodon loop. The experiments reported here indicate that the binding constant, $K = k_R/k_D$, for U-U-C-A to a single stranded dodecanucleotide excised from the anticodon loop 8 x 10^4 at 0°C. This is within a factor of two of the binding to the intact tRNA. Moreover, the kinetic parameters in both cases are very similar. Thus it seems that the large binding cannot be attributed to a special conformation of the anticodon loop.

The reason for the enhanced binding of U-U-C-A to the anticodon remains a mystery. Several possible causes are: (1) modified bases, (2) non-base-paired ends, and (3) Mg^{++} effects.

There are a number of modified bases in the sequence, and these may alter the stability. However, the effect of the hypermodified base Y has been found to be at most a factor of seven in the binding constant of two tRNAs with complementary anticodons.[7] The modified ribose in G_m is probably not important since this does not strongly affect the base stacking in $G_{mp}A$ or $G_{mp}U$.[17,18] Thus base modifications do not seem to be the explanation.

A more likely explanation for the increased binding is the effect of non-base-paired ends. The first observation that

these ends can affect stability was made by Martin, et al.[19] Grosjean, et al.[7] have pointed out that this might be important in the codon-anticodon interaction. They studied the binding of the decamer, C-C-C-U-^{s}U-U-C-$^{m^2}$A-C-G,[19] with the same dodecamer used in the experiments reported here. This is a situation in which both oligomers have non-base-paired ends. Their equilibrium constant for this complex formation is 1×10^6 at 0°C. In the work reported here, only one of the oligomers has unpaired ends. The association constant is $\sim 10^5$ at 0°C. Thus it is clear that the effect of unpaired ends can be very significant. We find that the stability comes from a smaller decrease in entropy than expected for two oligonucleotides, just as in the case of U-U-C-A·tRNA^{Phe}.[6,21] Model systems of A_mU_n ($m > n$)[20] have also shown a large increase in binding constant relative to A_nU_n due to a gain in entropy. For example, the melting temperature of A_7U_4 is 9°C higher than A_4U_4; A_7U_4 shows an unfavorable increase in enthalpy (2 kcal mole^{-1}) and a favorable increase in entropy (12 cal deg^{-1} mole^{-1}) compared to A_4U_4. Previous codon-anticodon interactions have been compared to two complementary oligomers of the same length. This experiment has shown that oligomers of the same size are not a good model to use. Rather, an oligomer with non-base-paired dangling ends should be used.

A third possibility for the large codon-anticodon association constant is the effect of Mg^{2+} ion. Both the anticodon loop and the dodecanucleotide have one strong binding site for Mg^{2+}.[13,22] Preliminary experiments show Mg^{2+} concentration affects the kinetics and binding even in the presence of 0.1 M Na^+ ion.

X-ray crystallography[10-12] has shown the anticodon loop to have two discrete helices with a sharp kink between U and G_m. According to this model, the formation of an A·U base pair with U-U-C-A at the 5' side of the anticodon needs a conformational change of the loop to bring these together. Comparison of the kinetic studies, however, leads to the same results for the case of the intact loop and the dodecanucleotide in the temperature range of $0 \sim 20$°C. It is suggested that the anticodon loop is flexible and that a minor conformational change of the

anticodon loop can accommodate an A-U base pair at the 5' side of the anticodon.

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