The Association of Complementary Ribonucleic Acids Can Be Strongly Increased without Lowering Arrhenius Activation Energies or Significantly Altering Structures†

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ABSTRACT: The association rates of complementary nucleic acids can be increased by 2−3 orders of magnitude *in vitro* by cellular proteins and low molecular weight compounds including cetyltrimethylammonium bromide (CTAB). In this work, we provide experimental evidence that the CTAB-mediated enhancement of RNA−RNA annealing by approximately 3 orders of magnitude is due to a favorable activation entropy ($\Delta S^{\ddagger}$) and not due to a decrease of the Arrhenius activation energy (E_a) nor to major structural changes of the RNA. Two alternative models for the CTAB-facilitated RNA−RNA annealing will be discussed. First, CTAB could form a positively charged liquid matrix which could steer complementary RNA molecules and thereby increase their collision frequency and annealing rate. Second, increased annealing rates could be explained by stabilization of a non-base-specific precomplex of both complementary RNA molecules in solution.

The association of complementary ribonucleic acids (RNAs) plays a crucial role in the regulation of biological processes such as gene expression (Green et al., 1986; Inouye, 1988), antisense regulation (Wagner & Simons, 1994), and splicing (Madhani & Guthrie, 1994). In living cells, however, RNA is assumed to be complexed with low molecular weight compounds or nucleic acid-binding proteins. A number of mammalian nuclear hnRNP[1] proteins (Portman & Dreyfus, 1994) such as the hnRNP protein A1 (Pontius & Berg, 1990; Kumar & Wilson, 1990; Munroe & Dong, 1992) as well as the tumor suppressor protein p53 (Oberosler et al., 1993; Wu et al., 1995; Nedbal et al., 1997) and the nuclear capsid protein NC of the human immunodeficiency virus type 1 (Dib-Haij et al., 1993; Tsuchihashi et al., 1993) have been shown to enhance the association rates of complementary RNA *in vitro*. The renaturation of DNA double strands can be enhanced by cetyltrimethylammonium bromide (CTAB), a positively charged quarternary ammonium salt with a hydrophobic side chain of 16 carbons, to a similar extent as the hnRNP protein A1 does (Pontius & Berg, 1991). This suggests use of CTAB as a model compound for investigating the facilitator-promoted association of complementary RNA.

The mechanism underlying facilitated annealing of complementary nucleic acids was the subject of various speculations. It was discussed that the half-life of an 'encounter complex' formed between both strands was increased via additional non-base-specific interactions (Pontius, 1993). Others speculated on a possible decrease of the energetic barrier of duplex formation (Tsuchihashi & Brown, 1994; Herschlag et al., 1994) or on protein-induced structural changes of RNA that favor RNA−RNA annealing (Herschlag, 1995; Portman & Dreyfus, 1994; Oberosler et al., 1993). In this work, we performed a mechanistic study on the CTAB-promoted annealing of long-chain complementary RNA and discuss two models that could help to understand the role of CTAB in enhanced RNA−RNA annealing.

MATERIALS AND METHODS

Synthesis of RNA. The RNAs used here have been described: SR6 and αY69 (Homann et al., 1993); αY150 (Sczakiel et al., 1992); αYRz60, SR4 (Homann, 1995). RNA strands were prepared by run-off *in vitro* transcription with T7 RNA polymerase. The linearized plasmids were purified by phenol/chloroform (v/v 1:1) extraction and subsequent precipitation in 80% ethanol. *In vitro* transcription of unlabeled SR6 RNA was run for 90 min at 37 °C and stopped by adding 10 units of RNase-free DNase I and a further incubation for 20 min at 37 °C. Uniformly ^{32}P-labeled antisense RNA αY69 was synthesized as described (Homann et al., 1993). All *in vitro* transcribed RNAs were extracted with chloroform/phenol (v/v 1:1), precipitated with 80% ethanol, and purified by gel-filtration on Sephadex G-50 columns with 10 mM Tris/HCl, pH 7.6, 1 mM EDTA.

Association Reactions in the Presence of Tetraalkylammonium Salts or SDS. Annealing reactions were performed in 30 μL of a buffer containing 20 mM Tris/HCl pH 7.6, 100 mM NaCl and either no or 10 mM $MgCl_2$. The concentration of ^{32}P-labeled αY69 was below 0.5 nM, and unlabeled SR6 was used at a molar excess over αY69 of at least 5-fold. The RNA−RNA annealing reactions were measured at various target RNA concentrations ranging between 0.5 and 200 nM depending on the pair of complementary RNA and the reaction conditions. At 0.5−2 nM

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[1] Abbreviations: CTAB, cetyltrimethylammonium bromide; DTAB, dodecyltrimethylammonium bromide; hnRNP, heterogeneous nuclear ribonucleoprotein; OTAB, octadecyltrimethylammonium bromide; TMAB, tetramethylammonium bromide; TTAB, tetradecyltrimethylammonium bromide.

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target RNA concentration, it was ensured that the radiolabeled RNA strand was below 0.1 nM. The concentration dependence of the oberserved annealing rates showed that the annealing reactions were of second order under the conditions used here. When the annealing reactions were performed in the presence of the detergents, they were added last to the reaction mixture to give the indicated final concentrations. The association reactions were stopped by adding aliquots from the reaction mixture to an 8-fold excess of precooled stop buffer containing 7 M urea, 50 mM Tris/HCl, pH 7.6, 25 mM EDTA, and 0.5% SDS. Reaction products were analyzed by 5% (w/v) polyacrylamide gel electrophoresis as described (Homann et al., 1993). To determine rate constants, the bands containing single-stranded RNA or the duplex RNA were quantified on vacuum-dried polyacrylamide gels by a commercial phosphorimager (Molecular Dynamics).

Association Reactions in the Presence of the hnRNP A1 Protein. The hnRNP A1 protein was isolated and purified as described (Pontius & Berg, 1990). Annealing reactions with the complementary RNAs αY69 and SR6 and the A1 protein were performed in a buffer containing 20 mM Tris/HCl, pH 7.6, 100 mM NaCl, 1 mM DTT, 1 mg/mL BSA, and 0.05 unit/μL RNasin. All components were premixed and preincubated for 30 s at 37 °C before A1 protein was added at the indicated concentrations. Reactions were stopped by adding an equal volume of a solution containing proteinase K (1 mg/mL) and SDS (1%). Complete removal of the A1 protein was controlled by comparison with phenol-extracted samples. After incubation for 2 min at 37 °C and subsequent addition of 30 μL of precooled stop buffer, samples were analyzed on 5% polyacrylamide gels, and rate constants were determined as described above.

Structure Probing of RNA. The 5'-end of *in vitro* transcribed αY150 RNA was dephosphorylated with calf intestine phosphatase and, subsequently, rephosphorylated with [γ-^{32}P]ATP and polynucleotide kinase. Cleavage reactions with RNase T1 (40 units/mL) were performed in the absence or presence of CTAB in a total volume of 10 μL with 5′-^{32}P-labeled RNA (<3 nM) in a buffer containing 100 mM NaCl, 10 mM $MgCl_2$, and 20 mM Tris/HCl, pH 7.6. Aliquots were withdrawn at certain time points, and the cleavage reactions were stopped by adding the 5-fold volume of stop buffer (7 M urea, 50 mM Tris/HCl, pH 7.6, 25 mM EDTA, and 0.5% SDS) and chilling the samples on ice. Subsequently, samples were heated at 95 °C for 5 min and analyzed on 10% polyacrylamide gels containing 7 M urea. Cleavage reactions with RNase A (0.2 unit/mL) were performed in a total volume of 10 μL in 10 mM potassium phosphate (pH 7.0), stopped, and analyzed by gel electrophoresis as described above.

Determination of Thermodynamic Parameters. The Arrhenius activation energy (E_a) was calculated from an Arrhenius plot (ln k versus $1/T$) derived from the temperature dependence of the annealing reaction. The Gibbs's free activation energy ($\Delta G^{\ddagger}$) was calculated from the relationship $\Delta G^{\ddagger} = -RT \ln (kh/k_B T)$ where R is the gas constant, k is the rate constant at the given temperature T, h is Planck's constant, and k_B is Boltzmann's constant. The activation enthalpy ($\Delta H^{\ddagger}$) is given by $\Delta H^{\ddagger} = E_a - RT$, and the activation entropy ($\Delta S^{\ddagger}$) was calculated from the relationship: $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$. Entropy values are given in eu (cal mol^{-1} K^{-1}); 1 eu is equivalent to 4.184×10^{-3} kJ mol^{-1} K^{-1}.

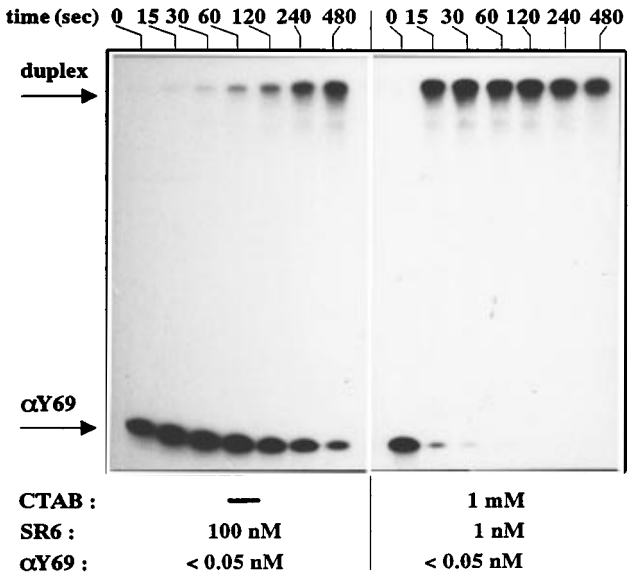

FIGURE 1: Polyacrylamide gel showing the association kinetics of the two HIV-1-derived complementary RNAs SR6 and αY69 (Homann et al., 1993) in the absence (left panel) or presence (right panel) of CTAB.

RESULTS

Enhancement of RNA−RNA Annealing by the hnRNP A1 Protein and CTAB. For measuring the influence of the model compound CTAB on the association of complementary RNA, we chose the HIV-1-derived pair of complementary RNAs αY69 (69 nt) and SR6 (645 nt; Figure 1). Both RNAs had been characterized in terms of their structure and association kinetics in the absence of facilitators (Homann et al., 1993). The annealing reactions were followed by polyacrylamide gel electrophoresis (Figure 1) and led to an RNase T1-resistant duplex (data not shown). Duplex formation was measured at 37 °C in the presence of 10 mM magnesium. The annealing reaction follows second-order kinetics and proceeds at an association rate of $k_{obs} = 2.9 \times 10^4$ M^{-1} s^{-1} (Homann et al., 1993), whereas in the absence of magnesium the rate was found to be approximately 8-fold lower. These values are subsequently used for comparison with association rates in the presence of various facilitators as summerized in Table 1. First, we studied whether protein A1, which had been shown to enhance the reannealing of complementary DNA (Pontius & Berg, 1990), is also able to promote the association of complementary RNA under the same conditions, i.e., without magnesium. At a 100-fold molar excess of the A1 protein (500 nM) over RNA (SR6, 5 nM), we observed an enhancement of the association rate by a factor of approximately 450 (Table 1). However, at 10 mM Mg^{2+}, no significant A1 protein-mediated enhancement of the annealing reaction could be measured.

In the presence of 1 mM CTAB, the observed second-order rate constant (k_{obs}), which characterizes the association step of αY69 and SR6, was increased from 2.9×10^4 M^{-1} s^{-1} to values of approximately 2.7×10^7 M^{-1} s^{-1} (Figure 1; Table 1). The influence of the CTAB concentration on the extent of increased annealing was not linear, and half-maximal increase occurred at a CTAB concentration of approximately 40 μM (Figure 2). Considering the total RNA

Table 1: Facilitator-Mediated Enhancement of the Association of the Complementary RNAs αY69 and SR6

		0 mM magnesium		10 mM magnesium	
facilitator	concentration	k_{obs} (M^{-1} s^{-1})	factor of increased annealing	k_{obs} (M^{-1} s^{-1})	factor of increased annealing
none	−	$(3.4 \times 10^3) \pm (0.27 \times 10^3)$	1	$(2.9 \times 10^4) \pm (0.16 \times 10^4)$	1
spermine	0.5 μM	nd	−	$(1.7 \times 10^5) \pm (0.2 \times 10^5)$	6
A1 protein	1 μM	$(1.4 \times 10^6) \pm (0.35 \times 10^6)$	412	$(3.6 \times 10^4) \pm (0.4 \times 10^3)$	1.2
TMAB	1 mM	$(3.7 \times 10^3) \pm (0.2 \times 10^3)$	1.1	$(3.6 \times 10^4) \pm (0.37 \times 10^4)$	1.3
DTAB	1 mM	$(1.7 \times 10^4) \pm (0.4 \times 10^4)$	5	$(1.0 \times 10^5) \pm (0.33 \times 10^5)$	3.4
TTAB	1 mM	$(1.4 \times 10^7) \pm (0.5 \times 10^7)$	4117	$(3.5 \times 10^7) \pm (1.5 \times 10^7)$	1206
CTAB	1 mM	$(1.0 \times 10^7) \pm (0.25 \times 10^7)$	2941	$(2.7 \times 10^7) \pm (0.40 \times 10^7)$	931
OTAB	1 mM	$(9.0 \times 10^6) \pm (2.0 \times 10^6)$	2647	$(2.0 \times 10^7) \pm (0.53 \times 10^7)$	683

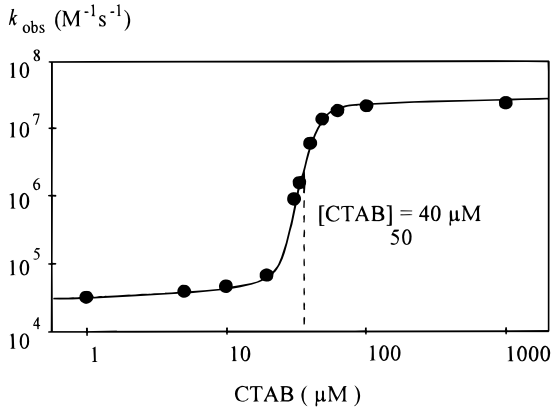

FIGURE 2: Dependence of increased annealing between SR6 (8 nM) and αY69 (<0.5 nM) on the concentration of CTAB.

concentration in the experiment (Figure 2) of 8 nM and 40 μM CTAB, a stoichiometry in solution of approximately eight CTAB molecules per phosphate group can be derived. To further substantiate this observation, we investigated the effect of CTAB on different pairs of complementary RNA at different concentrations. Increasing effects of CTAB on the annealing reaction were observed with all other pairs of complementary RNA used in this work (Table 2). Further, a similar stoichiometry was found when the RNA strands and concentrations were changed (Table 3), indicating that CTAB-facilitated RNA−RNA annealing is not sequence-dependent.

To investigate whether the length of the long alkyl chain of the tetraalkylammonium salt influences the annealing activity, we tested chain lengths of up to 18 carbons. Shortening of the length of the aliphatic side chain to 12 or less carbons (DTAB, TMAB) dramatically decreased or abolished the enhancement of RNA−RNA annealing whereas full activity was measured at a chain length of equal to or greater than 14 carbons (TTAB, CTAB, OTAB) independently of the presence of magnesium (Table 1). The lack of annealing activity with the homolog TMAB further indicates that the CTAB-promoted RNA−RNA annealing is not due to a reduction of electrostatic repulsion.

To test whether the positive charge of the tetraalkylammonium salts is involved in the annealing activity, we compared the effects of DTAB with the negatively charged detergent SDS. Under conditions of approximately 27-fold increased annealing by DTAB (10 mM), SDS showed no significant effects (Figure 3), indicating that the positive charge is necessary for the activity of tetraalkylammonium salts.

Activation Energy of RNA−RNA Annealing. The association of complementary RNA is characterized by a second-order rate constant which is also the case for the facilitator-mediated enhancement of reannealing including CTAB and the A1 protein (Pontius & Berg, 1990, 1991). A decrease of the energy barrier of this reaction represented by the Gibb's free activation energy ($\Delta G^{\ddagger}$) was discussed to be responsible for faster annealing (Herschlag et al., 1994; Tsuchihashi & Brown, 1994). The Gibb's free activation energy can be split into a heat term ($\Delta H^{\ddagger}$) and an entropy term ($\Delta S^{\ddagger}$). Alternatively, the Arrhenius equation $k = Ae^{-E_a/RT}$; $A = PZ_{enc}Z_{col}$ (A, preexponential factor; P, steric factor; Z_{enc}, encounter frequency; Z_{col}, number of collisions per encounter) gives a correlation between the association rate constant and the energy barrier of the annealing reaction which is almost equivalent to $\Delta H^{\ddagger}$ ($\Delta H^{\ddagger} = E_a - RT$). The activation entropy ($\Delta S^{\ddagger}$) is related to the preexponential term of the Arrhenius equation. All parameters can be calculated from the temperature dependence of the annealing reaction and the rate constants (see Materials and Methods; Table 4).

The plot log k versus T^{-1} shows that E_a of the association between the complementary RNAs αY69 and SR6 is increased from 43 to 73 kJ/mol in the presence of CTAB (Figure 4), which is equivalent to an unfavorable activation enthalpy $\Delta H^{\ddagger}$. Remarkably, this occurs under conditions in which the annealing reaction is enhanced by almost 3 orders of magnitude. Thus, the CTAB-mediated increase of RNA−RNA annealing is not due to a decrease of E_a or $\Delta H^{\ddagger}$, respectively. This result strongly suggests consideration of nonenthalpy terms such as the preexponential term ($A = PZ_{enc}Z_{col}$) of the Arrhenius equation or the activation entropy $\Delta S^{\ddagger}$. The calculation of the value for $\Delta S^{\ddagger}$ (+123 eu) shows that this parameter is strongly in favor of RNA−RNA annealing in the presence of CTAB (Table 4).

CTAB Does Not Significantly Influence RNA Structure. The influence of CTAB on RNA structures was investigated by limited RNase cleavage experiments with the same conditions which were used to determine the association rate constants. Structural probing was performed with any of the antisense RNA, i.e., αY69 (69 nt), αYRz60 (100 nt), and αY150 (150 nt), that were used in the annealing experiments (see Table 2). In all cases, no differences in the accessibility of single-stranded regions of the probed RNA for RNase A, RNase T1, and RNase CL3 were observed in the presence or absence of CTAB, suggesting that structures are not changed significantly by CTAB (data not shown). Since the number of accessible cleavage sites for single-strand-specific RNases in αY69 and αYRz60 is limited, the cleavage pattern of RNase T1 and RNase A of 5'-radiolabeled αY150 is shown in Figure 5. In sum, the probing data strongly suggest that CTAB does not have a

Table 2: CTAB-Mediated Increase of RNA−RNA Annealing

complementary RNA	k_{ass} (M^{-1} s^{-1}) 0 mM CTAB	k_{ass} (M^{-1} s^{-1}) 1 mM CTAB	factor of increased RNA−RNA annealing
αY150, SR6	$(1.3 \times 10^4) \pm (0.1 \times 10^4)$	$(2.4 \times 10^7) \pm (0.1 \times 10^7)$	1846
αY69, SR6	$(2.9 \times 10^4) \pm (0.2 \times 10^4)$	$(2.7 \times 10^7) \pm (0.4 \times 10^7)$	931
αYRz60, SR6	$(1.4 \times 10^4) \pm (0.2 \times 10^4)$	$\approx 1.0 \times 10^7$	≈714
αYRz60, SR4	$(2.8 \times 10^4) \pm (0.8 \times 10^4)$	$\approx 1.1 \times 10^7$	≈392

Table 3: Stoichiometry of CTAB and RNA at Half-Maximal Annealing of Different Pairs of Complementary RNA

complementary RNA1) (length in nucleotides)	phosphate (μM)	$CTAB_{50}$[b] (μM)	CTAB per phosphate[c]
4 nM SR4 (420 nt), ^{32}P-αYRz60 (100 nt)	1.68	32	15.0 ± 5
4 nM SR6 (640 nt), ^{32}P-αY69 (69 nt)	2.56	15	9 ± 4
8 nM SR6 (640 nt), ^{32}P-αY69 (69 nt)	5.12	40	7.8 ± 1
16 nM SR4 (420 nt), ^{32}P-αYRz60 (100 nt)	6.72	50	7.4[d]
16 nM SR6 (640 nt), ^{32}P-αY69 (69 nt)	10.24	100	8.8 ± 1.4

[a] Unlabeled RNA (SR6 or SR4) was used at high molar excess as indicated over ^{32}P-labeled RNA (αY69 or αYRz60, <0.5 nM). [b] $CTAB_{50}$, concentration of CTAB at half-maximal increase of RNA−RNA annealing. [c] The standard deviation was calculated from at least three measurements. [d] This value has been determined once.

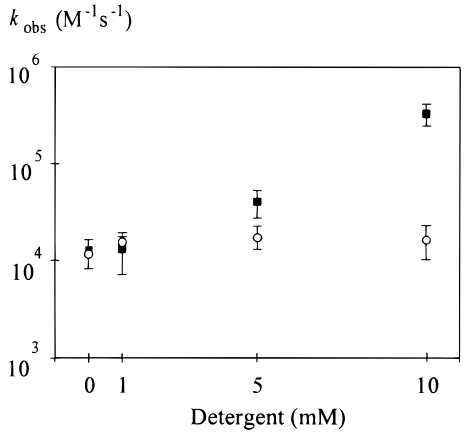

FIGURE 3: Association rate constants of αY150 and SR6 in the presence of different concentrations of DTAB (■) or SDS (○). Annealing reactions were performed at 37 °C in the presence of 10 mM Mg^{2+}. The standard deviation was calculated from three experiments.

Table 4: Thermodynamic Parameters of CTAB Increased RNA−RNA Annealing at 37 °C

CTAB (1 mM)	k_{ass} (M^{-1} s^{-1})	E_a (kJ/mol)	$\Delta G^{\ddagger}$ (kJ/mol)	$\Delta H^{\ddagger}$ (kJ/mol)	$\Delta S^{\ddagger}$ (eu)
+	2.7×10^7	73 ± 6	32	70 ± 5	$+123 \pm 18$
−	2.9×10^4	43 ± 3	49	40 ± 3	-30 ± 9

major influence on RNA structures at conditions of CTAB-promoted RNA−RNA annealing.

To further investigate whether CTAB influences RNA secondary structure, we measured CD spectra of αY150 in the presence or absence of CTAB (Figure 6). The concentrations of CTAB were chosen such that the stoichiometry with phosphates of RNA was 8:1 (1.8 mM CTAB and 1.5 μM αY150; see Figure 6), which corresponds to the stoichiometry at half-maximal CTAB-promoted RNA−RNA annealing. In addition, CD spectra were measured at 1 mM CTAB which was used in most of the experiments of this work. Minor differences in the CD spectra were observed in the presence of CTAB which, however, already occurred at a ratio of CTAB to RNA (4.4:1) which does not lead to strongly increased annealing. The CD spectrum of αY150 resembles a typical A form spectrum with a maximum at 265−270 nm and negative ellipticities at 240 and 210 nm.

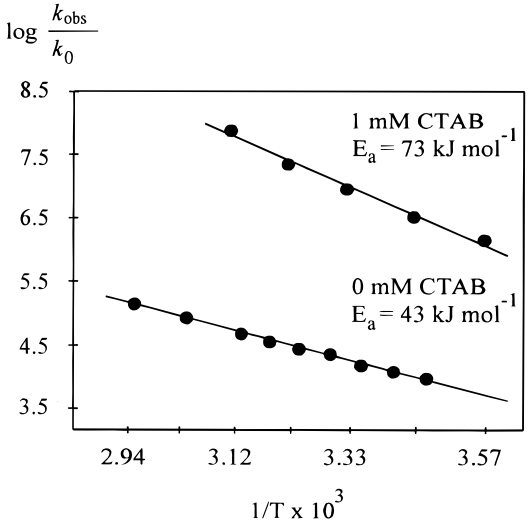

FIGURE 4: Arrhenius plot of the annealing reaction between αY69 and SR6 in the presence or absence of 1 mM CTAB. The standard deviation of the rate constants of reactions without CTAB was less than 10%, and between 15% and 30% in the presence of CTAB.

The A form spectrum of DNA had been shown to be similar to that of a G,C ribopolymer and RNA from rice dwarf virus, respectively (Samejima et al., 1968; Ivanov et al., 1974), allowing one to derive some interpretations of the CD spectrum of αY150 RNA from the CD spectra of A form DNA (Ivanov et al., 1974). For example, there is a notable decrease of the maximum at 270 nm analogous to the A to B form transition of DNA, which is consistent with the observation that cations can stabilize the B form of DNA. The decreased signal at 270 nm could indicate an influence of CTAB on stacking involving stacked unpaired bases as well as intramolecular double-helical portions. The helix geometry of double-helical portions of αY150 could be influenced by CTAB analogous to the A to B form transition of DNA (Ivanov et al., 1974). The slight shift of the maximum at 270 nm toward longer wavelength could be due to the increased ionic strength in the presence of CTAB, but this shift has also been observed as a direct consequence of the A to B form transition of DNA (Ivanov et al., 1974).

Moreover, the relationship of the association rate constants of a set of 40 different HIV-1-directed antisense species

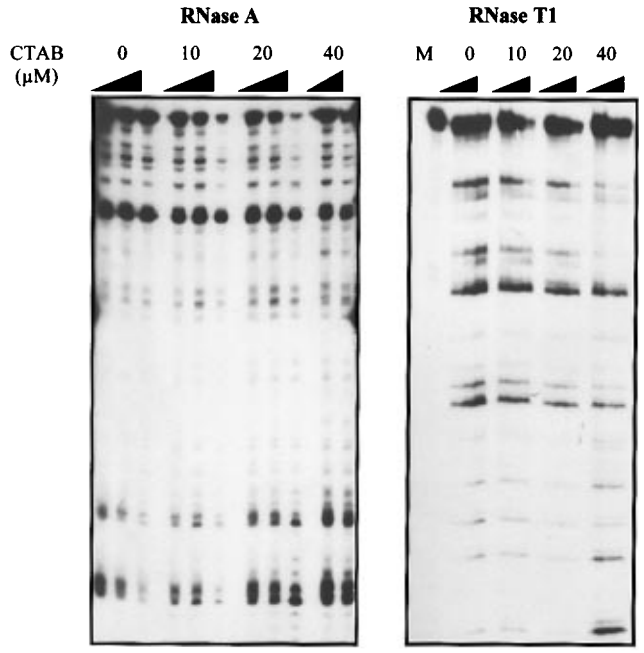

FIGURE 5: Structural probing of αY150 (5 nM) with RNase A (1×10^{-4} units/μL) and RNase T1 (5×10^{-2} units/μL) at increasing concentrations of CTAB. The reaction volume was 10 μL.

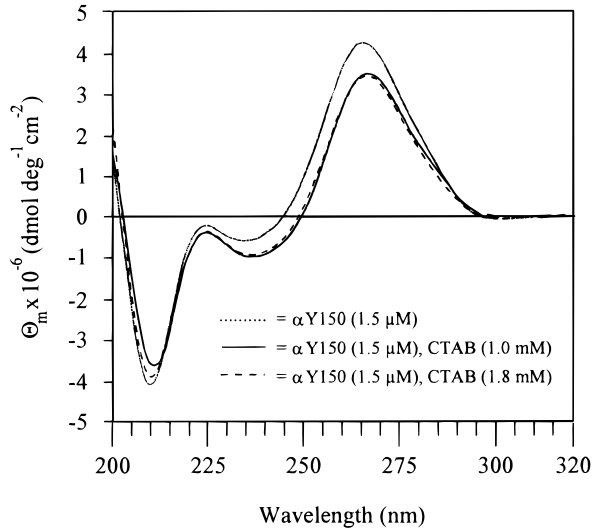

FIGURE 6: Circular dichroism spectra of 1.5 μM αY150 in the absence or presence of CTAB (1.0 or 1.8 mM) at 25 °C in 10 mM Tris/HCl, pH 7.6, and 10 mM $MgCl_2$. Spectra were measured on a Tasco t-710 spectropolarimeter in a 1 mm quartz cuvette. Each curve is signal-averaged from two scans with a similarly signal-averaged base line subtracted and then subjected to Fourier transformation to remove high-frequency noise.

remained qualitatively different among each other (Rittner et al., 1993) in the presence of 1 mM CTAB; i.e., all rate constants were increased by a similar factor (Nedbal, unpublished). Assuming that structural characteristics of these antisense RNAs are the cause for their different kinetic behavior, this result provides additional evidence that CTAB has no major influence on RNA structures.

DISCUSSION

In this work, we show that RNA−RNA annealing is increased up to 3000-fold by tetraalkylammonium salts with one alkyl chain containing 14 methyl groups or more (Table 1). This effect is not dependent on the RNA sequence and does not seem to involve major global structural changes of the RNA though probing data are also consistent with a minor structural influence of CTAB. The CD spectra of αY150 indicate a slight influence of CTAB on stacking in a way that has been described in qualitative terms for the A to B form transition of DNA (Ivanov et al., 1974), and unstacking could occur to some extent in the presence of CTAB. Both effects could contribute to increased RNA−RNA annealing.

There is a clear influence of magnesium on the extent of the observed protein A1-mediated enhancement of RNA−RNA annealing (Table 1). At least under some conditions, the hnRNP A1 protein does not work as well if one of the strands is much less than 100 nucleotides long (Pontius, unpublished). Since the A1 protein is known to bind in a cooperative manner (Cobianchi et al., 1988; Swanson & Dreyfuss, 1988; Nadler et al., 1991), one could speculate that Mg^{2+} may block the A1 protein-facilitated reactions simply by displacing A1 protein from such short strands as αY69 (69 nt). However, since neither DTAB nor TMAB had major effects on the reannealing of complementary RNA in the absence of magnesium (Table 1), it is unlikely that this lack of annealing activity of DTAB and TMAB in the presence of magnesium could be due to competitive effects of the cations. Consistently, the positively charged spermine increases RNA−RNA annealing only moderately by a factor of 6 (Table 1). The stoichiometry of CTAB to phosphates of RNA was found to be approximately 8:1 (Figure 2, Table 3). This is remarkable in view of a similar stoichiometry in the case of the CTAB-mediated enhancement of DNA reannealing that was measured under different experimental conditions (Pontius & Berg, 1991). These findings suggest that there may be a well-defined stoichiometry of CTAB molecules on nucleic acid strands under a broad range of conditions. If this was true, then the ratio of 8:1 could give a clue to the structure of CTAB-coated nucleic acid strands and, hence, one could shed some light on possible entropic influences as well as the role of hydrophobic interactions on the association mechanism.

The activation enthalpy ($\Delta H^{\ddagger}$) of CTAB-promoted RNA−RNA annealing is clearly unfavorable whereas the activation entropy ($\Delta S^{\ddagger}$) favors the reaction. It is largely a matter of speculation how the positive value of $\Delta S^{\ddagger}$ (+123 eu) has to be interpreted in mechanistic terms. However, according to the Arrhenius theory, an increase of the preexponential factor A could mean that either the steric factor P or the number of encounters in solution Z_{enc} or the number of collisions Z_{col} is increased. The increase of E_a by 30 kJ/mol (and $\Delta H^{\ddagger}$) in the presence of CTAB is significantly above the error range. Formally, this unfavorable increase of the activation enthalpy means that the positive entropy effect of CTAB-promoted RNA−RNA annealing is even greater than indicated by the measured increase of annealing rates.

One way to interpret the data of this work in mechanistic terms is the following model: tetraalkylammonium salts could form a liquid matrix-like structure via sufficiently long alkyl chains (≥14 methyl groups, Table 1). This arrangement could occur below the critical micelle concentration (CMC), which is not reached for CTAB at the experimental conditions used in this work (Pontius & Berg, 1991). Hence, a positively charged matrix could be formed in solution along which RNA strands preferentially diffuse. This kind of restricted diffusion or steering mechanism would be consis-

tent with the thermodynamic and structural data presented here. Alternatively, according to Pontius (1993), factors such as CTAB could increase the half-life of the 'encounter complex' formed between complementary nucleic acids which does not involve base-specific pairing. This precomplex could be stabilized by hydrophobic interactions between the aliphatic side chains of the CTAB-decorated RNA strands and result in its decreased dissociation, thereby favoring duplex formation. This means that the binding of CTAB or proteins to RNA increases non-base-specific interactions and results in a higher frequency of reorienting and recolliding of the RNA molecules in the course of a given encounter. The consequence is an increase of the frequency of productive collisions, i.e., an increase in Z_{col} according to the Arrhenius equation which is reflected in increased association rates.

A slight unstacking of RNA by CTAB was indicated by the CD spectrum of αY150. Since CTAB seems to increase non-base-specific intermolecular interactions, as discussed above, a contribution of unstacking to increased RNA−RNA annealing should not involve Watson−Crick-like interactions. Slightly altered CTAB-induced structural changes of RNA could eventually positively influence non-base-specific RNA−RNA interactions. If CTAB binds to duplex RNA, increased annealing could also be due to the stabilization of the product. This possibility, however, seems to be unlikely because the association of long-chain complementary RNA is kinetically controlled and is not related to the thermodynamic stability of the formed double strands (Persson et al., 1988; Rittner et al., 1993).

This work provides strong support and new insights into a mechanism described previously (Pontius, 1993) that may be a powerful way to increase rates of association between complementary macromolecules. The conclusions seem to be of general meaning for macromolecular assembly rather than being specific for CTAB or the complementary RNA chosen here. A consistent observation is the even stronger CTAB-mediated enhancement by up to 10^4-fold of strand displacement occurring in double-stranded RNA which also occurs at an increased Arrhenius activation energy (Homann et al., 1996). The rate-limiting step of this process is thought to be the association between the RNA duplex and the displacer strand.

Steering mechanisms such as hydrophobic and electrostatic steering (Brune & Kim, 1994), which minimize steric or entropic hindrances by preorienting the interacting molecules as well as stabilization of the transition state by lowering the Arrhenius activation energy E_a, are well-known ways by which enzymatic catalysis occurs for reactions involving at least one small molecule (enzyme−ligand interactions). Conversely, in the case of interactions between macromolecules such as complementary RNA, these mechanisms might not be as effective as increasing the frequency of productive collisions or as steered diffusion possibly occurring according to the above described models.

It is noteworthy that the thermodynamic parameters of the p53 protein-promoted RNA−RNA annealing are different; i.e., there, the activation heat ($\Delta H^{\ddagger}$, E_a) is in favor of the annealing reaction whereas $\Delta S^{\ddagger}$ is not (Nedbal & Sczakiel, 1996; Nedbal et al., 1997), strongly suggesting that there is more than one mechanism underlying facilitator-mediated RNA−RNA annealing.

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