Lawrence Berkeley National Laboratory
Recent Work

Title

COMPARISON OF THE KINETICS OF RIBO-, DEOXYRIBO- AND HYBRID OLIGONUCLEOTIDE DOUBLE-STRAND FORMATION BY TEMPERATURE-JUMP KINETICS

Permalink

https://escholarship.org/uc/item/2v13f9vh

Author

Tinoco, I.

Publication Date

1982-05-01

eScholarship.org Powered by the California Digital Library
University of California

LBL-14473
Preprint c.2

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

CHEMICAL BIODYNAMICS DIVISION

RECEIVED
LAWRENCE
BERKELEY LABORATORY
JUN 18 1982
LIBRARY AND
DOCUMENTS SECTION

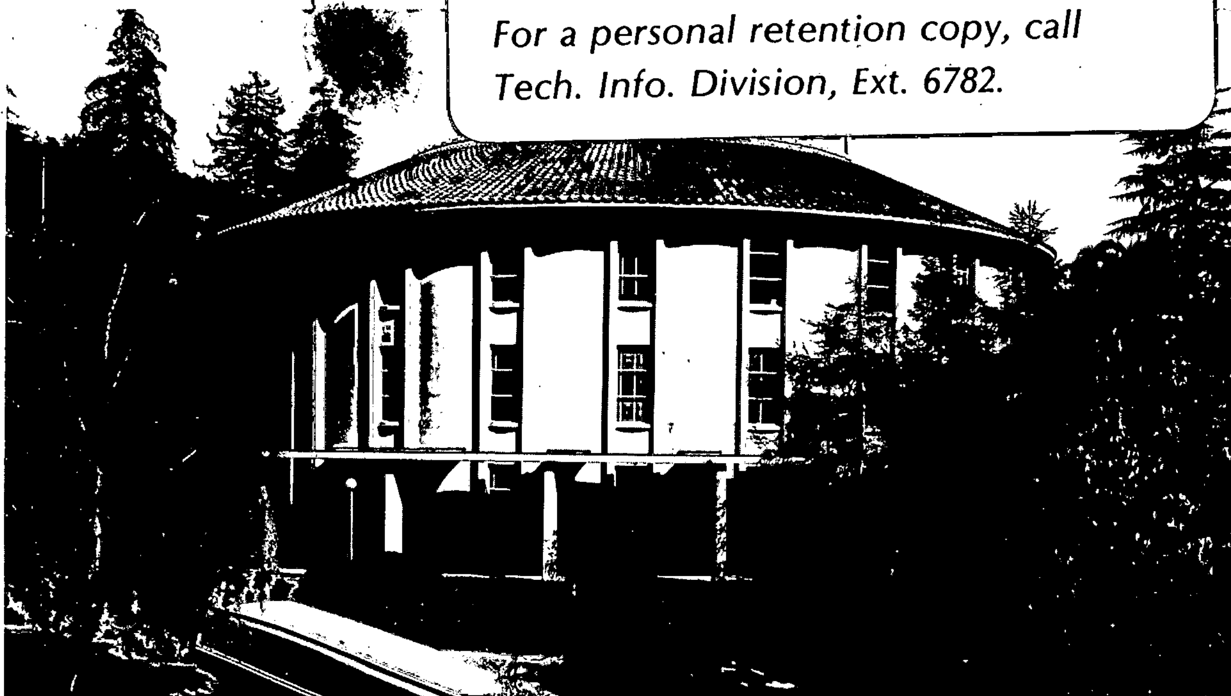
Submitted to Biochemistry

COMPARISON OF THE KINETICS OF RIBO-, DEOXYRIBO- AND HYBRID OLIGONUCLEOTIDE DOUBLE-STRAND FORMATION BY TEMPERATURE-JUMP KINETICS

Jeffrey W. Nelson and Ignacio Tinoco, Jr.

May 1982

TWO-WEEK LOAN COPY
This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 6782.



LBL-14473 c.2

Prepared for the U.S. Department of Energy under Contract DE-AC03-76SF00098

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

LBL-14473

Comparison of the Kinetics of Ribo-, Deoxyribo- and Hybrid Oligonucleotide Double-Strand Formation by Temperature-Jump Kinetics†

Jeffrey W. Nelson and Ignacio Tinoco, Jr.

Chemical Biodynamics Division
Lawrence Berkeley Laboratory
University of California
Berkeley, CA 94720

†From the Department of Chemistry and Laboratory of Chemistry Biodynamics, University of California, Berkeley, CA 94720. This work was supported by NIH Grant GM 10840 and by the Office of Health and Environmental Research of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

[1]Abbreviations used:

NMR	nuclear magnetic resonance
EDTA	ethylenediaminetetraacetate

Abstract

The kinetics of double-strand formation were measured using temperature-jump kinetics techniques for the DNA oligonucleotides $dCA_5G + dCT_5G$, the analogous RNA oligonucleotides $rCA_5G + rCU_5G$, and the hybrid $rCA_5G + dCT_5G$. The DNA oligonucleotides have a faster rate of recombination and a slower rate of dissociation at 12.0°C than the RNA oligonucleotides; the hybrid has about the same recombination rate and a slightly faster dissociation rate than the RNA oligonucleotides. The activation energy for recombination for the DNA and RNA oligonucleotides are both near zero kcal/mol. The difference in dissociation and recombination activation energies are consistent with the thermodynamic results obtained earlier.

The relaxation process is composed of two exponential components for the RNA and hybrid oligonucleotides at temperatures of 12.0°C and lower. One exponential component is observed for these oligonucleotides above 12.0°C and for the DNA oligonucleotides at all temperatures.

The kinetics of double-strand formation for a number of ribo-oligonucleotides have been studied using the temperature-jump technique (Craig et al., 1971; Podder, 1971; Pörschke & Eigen, 1971; Pörschke et al., 1973; Ravetch et al., 1974; Breslauer & Bina-Stein, 1977). Studies on deoxyribo-oligonucleotides are more limited (Drobnies, 1979; Freier et al., 1982). One RNA-DNA hybrid oligonucleotide double helix has been studied (Hoggett & Maass, 1971). However, no thorough study has been made comparing the kinetics of the same sequence for RNA, DNA and hybrid oligonucleotides. Such knowledge is important in determining how chemical differences between DNA and RNA manifest themselves in the dynamic properties of the double strands.

In this report we present results of temperature-jump kinetics studies of double-strand formation of the RNA oligonucleotides rCA_5G + rCU_5G, the analogous DNA oligonucleotides dCA_5G + dCT_5G, and a hybrid double helix composed of rCA_5G + dCT_5G. The other hybrid dCA_5G + rCU_5G does not form stable double strands (Martin & Tinoco, Jr., 1980).

Methods and Materials

The ribo-oligonucleotides rCA_5G and rCU_5G were synthesized enzymatically using polynucleotide phosphorylase as described earlier (Uhlenbeck et al., 1971). The deoxyribo-oligonucleotides dCA_5G and dCT_5G were chemically synthesized using the diester method (Khorana, 1968). Extinction coefficients for the ribo-oligonucleotides were determined by base hydrolysis and the extinction coefficients of the mononucleotides. Extinction coefficients for the deoxyribo-oligonucleotides were estimated using dinucleotide and mononucleotide extinction coefficients. The extinction coefficients in terms of $1\ cm^{-1}$ (mole strands)$^{-1}$ at 25°C were: rCA_5G, 7.1×10^4; rCU_5G, 6.3×10^4; dCA_5G, 7.9×10^4; dCT_5G, 5.8×10^4.

Concentrations of all mixtures capable of forming double strands were determined by measuring absorbances at 50°C, where the strands exist as single strands. The oligonucleotides are subject to degradation due to the high intensity of the UV lamp used in temperature-jump experiments. Therefore, concentrations were determined before and after the temperature-jump measurements, and were always within 3%. Additionally, absorbances were recorded at 0°C before and after, to check the hypochromicity. The hypochromicity, $(A_{50°}-A_{0°})/A_{50°}$, generally decreased 0.5 to 2% after the measurements. Samples with larger discrepancies were not used in the analysis.

The buffer used throughout these studies, 1M NaCl, 0.01M sodium phosphate buffer, pH=7, 0.1mM EDTA[1], was filtered using Uni-Pore Polycarbonate Membranes (Bio-Rad) with a pore size of 3 μm. Samples were degassed prior to temperature-jump measurements by purging the buffer with helium for 3 to 5 minutes before mixing with a small volume of a stock solution of oligonucleotides.

Measurements were taken at the mercury line near 267 nm for the ribo- and deoxyribo-oligonucleotides. For instrumental problems related to the sample cell, the hybrid oligonucleotides were measured at 250 nm. The temperature was measured to an accuracy of 0.2°C using a coppper-constantan digital thermocouple (Fluke model 2100A) in contact with the upper electrode.

The Temperature-Jump Instrument.

The temperature-jump instrument was manufactured by DiaLog (West Germany) and has been previously described (Rigler et al., 1974; Drobnies, 1979). The data were collected digitally by a Biomation model 805 transient recorder, using 2048 data points. A PET micro-computer was used to transfer the data from the Biomation to a VAX 11/780 computer, where the data were analyzed.

The program DISCRETE written by S. W. Provencher (Provencher, 1976a,b) was used to analyze the data. The program determines, from the data, what number of exponential components results in the best fit. The program was allowed to search for three exponential components. Two exponentials were reproducibly found for the ribo- and hybrid oligonucleotides at 6.7°C and 12.0°C. One exponential was found above this temperature and for the deoxyribo-oligonucleotides.

Analysis of the Temperature-Jump Kinetic Data.

For the reaction scheme given by equation 1, k_r corresponds to the

$$A + B \underset{k_d}{\overset{k_r}{\rightleftharpoons}} AB \qquad (1)$$

recombination rate constant for the formation of double strands AB from the non-selfcomplementary strands A and B, whereas k_d corresponds to the dissociation rate constant. When the system at equilibrium is perturbed by producing a fast jump in temperature, the system relaxes exponentially to its equilibrium at the final temperature according to equation 2.

$$1/\tau = 2k_r C_s + k_d \qquad (2)$$

C_s is the equilibrium concentration of A at the final temperature, and the total concentrations of A and B are assumed to be equal. τ is the relaxation time characterizing the process.

We have considered three methods to determine k_r and k_d by measuring $1/\tau$ at several concentrations of strands. The first is to calculate C_s at each total concentration using the equilibrium constant determined thermodynamically, and plotting $1/\tau$ vs. C_s. The slope gives k_r; the intercept gives k_d.

The second method is to use the kinetic equilibrium constant determined

by the first method, $K_{kinetic} = k_r/k_d$, and calculate new values for C_s, k_r and k_d. Iteration will result in the determination of a kinetically determined equilibrium constant consistent with the data.

The third method is to square equation 2 and eliminate C_s from the equation to get equation 3:

$$1/\tau^2 = 4k_r k_d C_{tot} + k_d^2 \quad (3)$$

A plot of $1/\tau^2$ vs. C_{tot}, the total concentration, gives an intercept = k_d^2 and slope = $4k_r k_d$.

The second and third analysis methods have the advantage that previous thermodynamic knowledge is not used in the final determination of the kinetics. A comparison of the results of the kinetics and thermodynamics allows a check of the consistency of the reaction scheme. We used all three methods of analysis.

Results

Figure 1 shows a trace of voltage _vs._ time for the ribo-oligonucleotides $rCA_5G + rCU_5G$ at 14.2 μM concentration and a final temperature of 6.7°C. The size of the temperature jump was 1.8°C; the initial signal was 5 volts. Figure 2 shows a semi-log plot of the trace in figure 1. The small amount of curvature indicates that there are two exponential components. The two lines indicate the best fits assuming one exponential (-----) and two exponentials (———). Due to the small amplitude of the second component, the relaxation time for the predominant relaxation for both fits are within 3%. Since the second component is seen only at low temperature, the one-component fit is used to characterize the bimolecular reaction. Using the two exponentials, when observed, does not change the results. The second component is seen only for the ribo- and hybrid oligonucleotides at temperatures at or below 12°C.

It is not observed at higher temperatures or for the deoxyribo-oligonucleotides at all temperatures.

The relaxation times for the predominant relaxation were averages of 5 to 10 relaxations for each sample. The standard deviation of the relaxation times was generally 2-6%. These standard deviations were used in linear regression to give the errors for the recombination and dissociation rate constants reported. Using these errors in the linear regression to determine the activation energies yielded the errors reported for the activation energies.

The temperature range of the instrument is limited by the sample cell to initial temperatures no lower than 5°C. The upper end of the temperature range is reached when the concentration of double strands becomes too low and the signal becomes too small to measure. Thus, the temperature ranges studied were 6.7 to 21.1°C for the ribo-oligonucleotides and 8.0 to 24.8°C for the deoxyribo-oligonucleotides.

Table I lists the rate constants and activation energies determined for the ribo-oligonucleotides $rCA_5G + rCU_5G$ at four temperatures, using the three methods of analysis discussed above. The first uses thermodynamic equilibrium constants to calculate the equilibrium single strand concentrations, C_s, in equation 2; the recombination rate constant, k_r, and the dissociation rate constant, k_d, are determined from the slope and intercept of a plot of $1/\tau$ _vs._ C_s. This procedure has the disadvantage of requiring previous thermodynamic knowledge, which might bias the kinetic results. The second method avoids this problem by calculating the equilibrium constant determined from kinetics, $K_{kinetic} = k_r/k_d$. This equilibrium constant is then used to calculate the next set of rate constants. Iteration produces rate constants which give a consistent equilibrium constant determined kinetically; previous thermodynamic

parameters do not bias the results. The third procedure uses equation 3, from which a plot of $1/\tau^2$ vs. C_{tot}, the total concentration, gives directly k_d^2 from the intercept, and $4k_rk_d$ from the slope. For our data the intercept of such a plot is small, and hence k_d has a large uncertainty. This also contributes to the error of the recombination rate constant, k_r. Thus, although this procedure requires no thermodynamic knowledge, it is less accurate than the second procedure.

Of course, if the mechanism is correct, and the kinetics and thermodynamics are consistent, the three methods should give consistent results. The data in Table I show the three analytical methods give consistent results within experimental error. The second method is preferred, since it requires no previous thermodynamics results, and is more accurate than the third method. It will be used for the remainder of the kinetic results reported in this paper. Figure 3 shows plots of $1/\tau$ vs. C_s for the ribo-oligonucleotides, using the second method of analysis.

Comparison of $rCA_5G + rCU_5G$, $dCA_5G + dCT_5G$ and $rCA_5G + dCT_5G$.

Table II shows the kinetic results for the ribo-, deoxyribo- and hybrid oligonucleotides. Activation energies were determined for the first two; rate constants at 12.0°C were determined for the third. The rate constants are all compared at 12.0°C. The recombination rate for the deoxyribo-oligonucleotides (8.3×10^6 ℓ mol^{-1} sec^{-1}) is 34% faster than for the ribo-oligonucleotides (6.2×10^6); the hybrid is essentially the same as the ribo-oligonucleotides, (6.6×10^6 ℓ mol^{-1} sec^{-1}).

The activation energy, E_a, and the activation entropy, $\Delta S^\ddagger$, are obtained from the temperature dependence of the rate constants. Figure 4 shows an Arrhenius plot for the recombination and dissociation rate constants for the ribo- and deoxyribo-oligonucleotides. The recombination activation energies

for the ribo- and deoxyribo-oligonucleotides are small and the same within experimental error: 0 ± 2 and -0.5 ± 2, kcal/mol, respectively. The activation entropies are negative and nearly equal, -29 and -30 e.u., respectively. The activation parameters were not measured for the hybrid oligonucleotides; they are assumed to be consistent with the ribo- and deoxyribo-oligonucleotide data since the recombination activation energies are the same in the ribo- and deoxyribo-oligonucleotides.

The dissociation rate constant at 12.0°C for the deoxyribo-oligonucleotides (9.6 sec^{-1}) is several times smaller than the ribo-oligonucleotides (38 sec^{-1}). The hybrid dissociation rate constant (50 sec^{-1}) is slightly faster than that of the ribo-oligonucleotides. The dissociation activation energies are large. The deoxyribo-oligonucleotide activation energy (43 kcal/mol) is somewhat larger than that for the ribo-oligonucleotide (39 kcal/mol). The activation entropies for the deoxyribo- and ribo-oligonucleotides (95 e.u. and 84 e.u., respectively) are both large and positive.

The activation energies determined from kinetics can be used to calculate the enthalpy and entropy for the reaction scheme. Comparison of these values with those determined thermodynamically provides a useful check for consistency of the reaction scheme. The thermodynamic parameters for these oligonucleotides have been previously determined from melting curve analysis (Nelson et al., 1981) and are included in Table II. The kinetically determined enthalpy is given by $\Delta H_{kinetic} = E_{a,r} - E_{a,d}$; the entropy is given by $\Delta S_{kinetic} = \Delta S_r^{\ddagger} - \Delta S_d^{\ddagger}$. For the ribo-oligonucleotides, the values of the enthalpy determined from thermodynamics and kinetics are -41 and -39 kcal/mol, respectively, in excellent agreement. The corresponding values for the entropy are -120 and -113 e.u., respectively. For the deoxyribo-oligonucleotides, the comparisons for the enthalpy are -49 and -44 kcal/mol; for the

entropy, -145 and -125 e.u. The consistency of these values derived from independent techniques lends confidence to the procedures used to characterize the double-strand formation of these oligonucleotides.

The Second Exponential Component.

Table III summarizes the relaxation times found for the faster second component for the ribo- and hybrid oligonucleotides at 6.7°C and 12.0°C. For these molecules the second component was not observed above 12°C; no second component was observed for the deoxyribo-oligonucleotides at any temperature. There was no trend of the relaxation time with concentration. The data shown are averages of 16 to 23 relaxations at several concentrations for each temperature. Due to the small amplitude of the effect (5-10% of the total amplitude), the errors in determining the relaxation times are large. This relaxation was 7-12 times faster at 6.7°C and 15-25 times faster at 12.0°C than the predominant bimolecular component. No trend in the amplitude with concentration or temperature was determinable.

The relaxation times for the ribo- and hybrid oligonucleotides are the same within experimental error at both temperatures: 440 and 470 sec^{-1}, respectively, at 6.7°C; and 2000 and 1900 sec^{-1} at 12.0°C. The apparent activation energies determined were large and nearly equal for the ribo- and hybrid oligonucleotides: 45 and 42 kcal/mol, respectively. Since we do not know what the process is, we cannot resolve the apparent rate into forward and reverse components.

The predominant relaxation is due to the bimolecular single-strand-to-double-strand transition. The fast minor component may be a rearrangement between two double helical species (Freier et al., 1982) (see Discussion).

Discussion

Comparison of the Ribo-, Deoxyribo- and Hybrid Oligonucleotides.

These studies allow us to make direct comparisons of the kinetics of ribo-, deoxyribo- and hybrid oligonucleotides of equivalent sequences under identical conditions. Thermodynamic results on these same oligonucleotides showed the order of stability was $dCA_5G + dCT_5G > rCA_5G + dCT_5G > rCA_5G + rCU_5G$ (Martin & Tinoco, Jr., 1980). The hybrid $dCA_5G + rCU_5G$ was very unstable, and formed triple strands at low temperatures (Martin & Tinoco, Jr., 1980; Pardi et al., 1981); it was not studied here.

The recombination rate constant for the deoxyribo-oligonucleotides at 12.0°C is faster than that for the ribo-oligonucleotides (Table II). The hybrid recombination rate constant is essentially the same as that for the ribo-oligonucleotides. The dissociation rate constant for the deoxyribo-oligonucleotides at 12.0°C is much smaller than for the ribo-oligonucleotides; the hybrid dissociation rate constant was slightly larger than the ribo-oligonucleotides. From this, we can conclude that the deoxyribo-oligonucleotide double helix is more stable than the ribo-oligonucleotide double helix because of both a greater recombination rate and a smaller dissociation rate. However, the difference in dissociation rates is the more important factor. The data for the hybrid double helix is only at one temperature, and thus comparisons with it are not as meaningful.

The activation energy for recombination of the deoxyribo- and ribo-oligonucleotides were both small; 0 and -0.5 kcal/mol, respectively. This suggests that although the rates are different, the mechanism is nearly the same for both oligonucleotides. The dissociation activation energies were both large, with the deoxyribo-oligonucleotide activation energy being greater than that of the ribo-oligonucleotides. The values of the activation energies are

consistent with the greater enthalpy of double strand formation for the deoxyribo-oligonucleotides (Nelson et al., 1981). The activation energies for the hybrid oligonucleotides were not determined, but are presumably consistent with the deoxyribo- and ribo-oligonucleotides.

The kinetics of exchange with water for the base-pairing imino protons of the three helices in this work were studied by NMR (Pardi & Tinoco, Jr., 1982). For $dCA_5G + dCT_5G$ in 0.18M NaCl, the rate constant and activation energy for exchange at 12.0°C were 7 sec^{-1} and 47 ± 7 kcal/mol, respectively, for the interior A-T base pairs. The similarity to the values in the present work, 9.6 sec^{-1} and 43 ± 3 kcal/mol, led the authors to conclude that the rate limiting step for exchange of the imino protons was the dissociation of the double strands to single strands. The exchange from the single strands was fast. The agreement of kinetic studies using techniques as diverse as temperature-jump and NMR lends valuable support to the interpretations of the results from both techniques.

The Second Exponential Component.

As reported in the results section, the relaxation data are best fit using a two-exponential fit for the ribo- and hybrid oligonucleotides at temperatures of 12.0°C and below. One-exponential fits are observed above 12.0°C and for the deoxyribo-oligonucleotides at all temperatures.

Freier et al. (1982) obtained two-exponential fits for the deoxyribo-oligonucleotide dG-C-G-C-G-C at temperatures below the melting temperature (T_m). They attribute the second relaxation to a reaction between two different double helical species. Since the oligonucleotides in this study are less stable than dG-C-G-C-G-C, we obtained less data below the T_m. They found that the relaxation time of the second component was independent of concentration. The amplitude of their signal was also independent of concentration and

temperature. Our data also indicate no trend of relaxation time with concentration; the data do not allow a conclusion to be drawn concerning the behavior of the amplitude.

The second component found by Freier et al. had an amplitude of 10-15% of the maximum signal; it comprised a larger fraction of the signal at low temperatures, where the signal from the bimolecular process was small. The second component observed in this study was never more than 5 to 10% of the total amplitude. The second component of Freier et al. was 4 to 10 times faster than the predominant component; in this study, it was 7 to 25 times faster, depending on the temperature (see Results).

The rates for the fast process in dG-C-G-C-G-C are calculated to be 9.6 and 24 sec^{-1} at 6.7°C and 12.0°C, respectively, with an activation energy of 23 kcal/mol (Freier et al., 1982). For the ribo-oligonucleotides in this study, the corresponding rates are 440 and 2000 sec^{-1} at 6.7°C and 12.0°C, respectively, with an activation energy of 45 kcal/mol. The process observed in this study behaves very differently from that observed by Freier et al. They observe the effect in the deoxyribo-oligonucleotide; we observe it for the ribo- and hybrid oligonucleotides, but not for the deoxyribo-oligonucleotide. It is not clear that the process we observe is similar to the one observed by Freier et al. Further studies on this effect will be necessary before the process can be characterized to any extent.

The Hybrid Behaves Similarly to the Ribo-Oligonucleotide.

The rate constants of the hybrid oligonucleotides at 12.0°C are closer to the ribo-oligonucleotides than the deoxyribo-oligonucleotides. This suggests that the properties of the hybrid more closely resemble the RNA than the DNA. More convincing evidence comes from the behavior of the fast second component. The rates and activation energies were essentially the same for

the ribo- and hybrid oligonucleotides, and the effect was not observed in the deoxyribo-oligonucleotides (Table III).

NMR studies of the chemical shifts of the base-pairing imino protons and the non-exchangable base and sugar protons of the helices used in this study (Pardi et al., 1982) indicate that the structure of the hybrid is close to the structure of the ribo-oligonucleotide. The NMR study on exchange rates of base-pairing imino protons mentioned earlier (Pardi & Tinoco, Jr., 1982) showed that at 5°C the dissociation rate of the hybrid and ribo-oligonucleotides are nearly equal, and an order of magnitude faster than for the deoxyribo-oligonucleotide.

The evidence seems to indicate that the properties of the hybrid double helices are governed mainly by the ribo-oligonucleotide strand. RNA generally exhibits less structural diversity than DNA. This lower freedom of flexibility of the RNA might dictate the properties of the more flexible DNA strand in the hybrid double helix.

Comparison with Previous Results.

The kinetics of double-strand formation have been studied for a number of ribo-oligonucleotide sequences containing only A•U base pairs (Craig et al., 1971; Pörschke & Eigen, 1971; Breslauer & Bina-Stein, 1977), both A•U and G•C base pairs (Pörschke et al., 1973; Ravetch et al., 1974), and one sequence containing only G•C base pairs (Podder, 1971). The data for deoxyribo-oligonucleotides are much more limited (Drobnies, 1979; Freier et al., 1982). One hybrid has been studied (Hoggett & Maass, 1971).

The rate of recombination increases considerably as the ionic strength is increased. The dissociation rate is essentially independent of ionic strength (Pörschke et al., 1973). Both recombination and dissociation activation energies are independent of salt concentrations between 0.05 and 1M NaCl

(Pörschke et al., 1973). Thus we can compare activation energies and dissociation rate constants from experiments done at different ionic strengths; recombination rate constants can be compared only when measured at the same ionic strength. Table IV summarizes the results for the activation energy for recombination for several oligonucleotides.

Ribo-oligonucleotides containing only A•U base pairs recombine with a negative activation energy: -9 kcal/mol for rA_n + rU_n and -4 to -6 kcal/mol for rA_nU_n (Table IV). Rate constants and activation energies were essentially independent of chainlength for both systems. The negative activation energy suggests that the mechanism of recombination requires the formation of a stable nucleus with two or three base pairs; the rate-determining step is the formation of the next base pair (Craig et al., 1971; Pörschke & Eigen, 1971) (see below).

Ribo-oligonucleotides containing both G•C and A•U base pairs have positive activation energies for recombination: +6 to +9 kcal/mol for sequences like rA_nGCU_n, rA_nCG + $rCGU_n$ and rA_nG_2 + rC_2U_n (Table IV). The activation energy was roughly independent of length or sequence. However, the rate constants decreased with increasing chainlength. The rates were faster if the G•C base pairs were at the end rather than the middle of the sequence. It appears recombination is faster when formation of the stable nucleus involves more stable G•C base pairs. Adding more A•U base pairs slows the rate due to increased steric hindrance; G•C bases at the end could be more accessible than in the middle of the sequence (Pörschke et al., 1973). The ribo-oligonucleotides rG-G-G-C + rG-C-C-C, which contain only G•C base pairs, have a recombination activation energy of +4.5 kcal/mol. The positive activation energy of oligonucleotides with G•C base pairs probably means the nucleus involved in the rate-determining step requires one or two G•C base pairs (Pörschke et al., 1973).

Much less work has been done on deoxyribo-oligonucleotide kinetics. The recombination activation energy for dG-C-G-C-G-C is +1 kcal/mol (Freier et al., 1982). For $dA_8 + dT_8$ and dA-T-G-C-A-T, the corresponding values are -2 and -4 kcal/mol, respectively (Drobnies, 1979). The hybrid oligonucleotides $rA_n + dT_n$ have an activation energy of -1 kcal/mol (Hoggett & Maass, 1971).

The trend in activation energies for the ribo-oligonucleotides is from negative values when only A•U base pairs are present (-4 to -9 kcal/mol), zero when isolated G•C base pairs are present, and positive when two or more G•C base pairs are adjacent (+5 to +9 kcal/mol). Due to the greater stability of G•C base pairs, fewer base pairs are required to form a stable nucleus, thus the larger activation energy when G•C base pairs are present. This trend is not as apparent in the deoxyribo-oligonucleotides. The activation energies vary from -2, -0.5, -4, +1 kcal/mol for the series $dA_8 + dT_8$, $dCA_5G + dCT_5G$, dA-T-G-C-A-T, and dG-C-G-C-G-C. This might mean the number of bases required for the stable nucleus does not depend on the presence of G•C base pairs. The difference in stability between A•T and G•C base pairs in DNA is not as great as for RNA; thus, in DNA the stability of the nucleus will not depend as strongly on the presence of G•C base pairs, and the number of bases in the nucleus and thus the activation energies vary less than for RNA.

Because of the restriction that recombination rate constants must be compared at the same salt concentration, there are fewer results to compare. Values determined in 1M NaCl at 12.0°C from earlier work (in terms of $\ell\ mol^{-1}\ sec^{-1}$) are: rA_7U_7, 2×10^6 (Breslauer & Bina-Stein, 1977); rA_2GCU_2, 5×10^6 (Pörschke et al., 1973); dA-T-G-C-A-T, 8×10^6 (Drobnies, 1979); dG-C-G-C-G-C, 11×10^6 (Freier et al., 1982). No systematic comparisons may be made from the data available.

The Mechanism of Double-Strand Formation.

The data presented in Tables II and III allow us to investigate the differences between the kinetics of ribo-, deoxyribo- and hybrid oligonucleotides.

The negative activation energy found for the recombination of ribo-oligonucleotides $rA_n + rU_n$ means there is a pre-equilibrium step involved in double-strand formation. The rate-determining-step of the recombination is hypothesized to be the addition of the next base pair to the nucleus composed of a few base pairs (Craig et al., 1971). The nucleus is the species which adds the next base pair faster than it dissociates. Thereafter the double helix quickly zippers up to the fully base-paired double helix. The nucleus is in a fast equilibrium with the single strands. Thus the forward rate is given by k_f(nucleus), where k_f is the rate of forming the next base pair. Dissociation of the strands occurs by breaking enough base pairs to get to the nucleus, which then quickly dissociates. The forward rate is then characterized by a small activation energy, positive or negative depending on the number of base pairs in the nucleus. The dissociation rate is characterized by a large activation energy required for breaking several base pairs to get to the nucleus.

Differences in the forward rate will depend on two factors: the concentration of the nucleus and the rate of adding base pairs to an existing nucleus.

It was stated earlier that the recombination of $dCA_5G + dCT_5G$ was faster than $rCA_5G + rCU_5G$. The hybrid $rCA_5G + dCT_5G$ recombines at essentially the same rate as $rCA_5G + rCU_5G$. The dissociation rates went as hybrid $\gtrsim$ ribo > deoxyribo. The behavior of the second exponential component also suggests that the behavior of the hybrid is similar to the ribo-oligonucleotide.

The similarity of the recombination activation energies for the deoxyribo- and ribo-oligonucleotides might suggest that the nuclei for double-strand formation are of similar stability. Thus the increased rate for the deoxyribo-oligonucleotides might be manifested in a larger k_f than for the ribo-oligonucleotides. Freier et al. suggest that the zippering rate, k_f, might be limited by the diffusion of the single-stranded bases into a helical conformation (Freier et al., 1982). The faster rate of recombination of the deoxyribo-oligonucleotides might be a result of faster single-strand stacking. From laser temperature-jump studies, it was determined that single-strand stacking in poly(dA) is about 4 times faster than in poly(rA): k(single strand stacking) = 2.7×10^7 and 0.7×10^7 sec^{-1}, respectively, at 25°C in 0.05M sodium cacodylate (Dewey & Turner, 1979). This reasoning suggests that the hybrid recombination rate would be similar to the ribo-oligonucleotides, since the helix zippering would be limited by the stacking of the slower rCA_5G strand.

The data presented in this paper are insufficient to fully justify the explanations for the differences between deoxyribo- and ribo-oligonucleotide kinetics. More studies comparing deoxyribo- and ribo-oligonucleotides would greatly clarify the situation. Ribo-G•C base pairs are more stable than deoxyribo-G•C base pairs. It would be very informative to compare the kinetics of double-strand formation for two analogous G-C-containing deoxyribo- and ribo-oligonucleotides. This would determine if the differences in stability are due to the ribo-oligonucleotide having a greater rate of recombination, a slower rate of dissociation, or both. Also comparing recombination activation energies and single-strand stacking rates would determine whether the differences in recombination rates are due to a more stable nucleus as manifested by a more negative activation energy, or a faster zippering rate.

Acknowledgements

The authors thank Dr. Francis H. Martin, Mr. David Koh and Ms. Barbara Dengler for synthesis of the oligonucleotides.

This work was supported by the Office of Health and Environmental Research of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098, and by National Institute of Health Grant GM 10840.

References

Bevington, P. R. (1969) Data Reduction and Error Analysis for the Physical Sciences, McGraw-Hill, New York, chapter 6.

Breslauer, K. J. & Bina-Stein, M. (1977) Biophys. Chem. 7, 211-216.

Craig, M. E., Crothers, D. M. & Doty, P. (1971) J. Mol. Biol. 62, 383-401.

Dewey, T. G. & Turner, D. H. (1979) Biochemistry 18, 5757-5762.

Drobnies, A. E. (1979) Ph.D. Thesis, University of California, Berkeley.

Freier, S. M., Albergo, D. D. & Turner, D. H. (1982) Biopolymers, submitted.

Hoggett, J. G. & Maass, G. (1971) Ber. Bunsenges. 75, 45-54.

Khorana, H. G. (1968) Pure Appl. Chem. 17, 349-381.

Martin, F. H. & Tinoco, I., Jr. (1980) Nucl. Acids Res. 8, 2295-2299.

Nelson, J. W., Martin, F. H. & Tinoco, I., Jr. (1981) Biopolymers 20, 2509-2531.

Pardi, A., Martin, F. H. & Tinoco, I., Jr. (1981) Biochemistry 20, 3986-3996.

Pardi, A. & Tinoco, I., Jr. (1982) Biochemistry, submitted.

Podder, S. K. (1971) Eur. J. Biochem. 22, 467-477.

Pörschke, D. & Eigen, M. (1971) J. Mol. Biol. 62, 361-381.

Pörschke, D., Uhlenbeck, O. C. & Martin, F. H. (1973) Biopolymers 12, 1313-1335.

Provencher, S. W. (1976a) Biophys. J. 16, 27-41.

Provencher, S. W. (1976b) J. Chem. Phys. 64, 2772-2777.

Ravetch, J., Gralla, J. & Crothers, D. M. (1974) Nucl. Acids Res. 1, 109-127.

Rigler, R., Rabl, C. & Jovin, T. M. (1974) Rev. Sci. Instrum. 45, 580-588.

Uhlenbeck, O. C., Martin, F. H. & Doty, P. (1971) J. Mol. Biol. 57, 217-229.

Table I: Kinetic results for $rCA_5G + rCU_5G$ using different analytical methods.

	Recombination Rate Constant (x 10^{-6}) (ℓ mol^{-1} sec^{-1})			Dissociation Rate Constant (sec^{-1})		
	$1/\tau$ vs. C_s	iterating $1/\tau$ vs. C_s	$1/\tau^2$ vs. C_{tot}	$1/\tau$ vs. C_s	iterating $1/\tau$ vs. C_s	$1/\tau^2$ vs. C_{tot}
6.7°C	6.2±0.7	5.2±0.6	5.6±6.4	11±4	12±4	11±1
12.0°C	6.4±0.4	6.8±0.4	6.8±1.9	38±4	37±4	37±8
16.4°C	5.9±0.6	6.0±0.6	6.0±1.3	108±9	107±9	107±12
21.1°C	4.6±1.1	4.4±1.0	4.5±1.3	330±20	330±20	330±20
k (12.0°C)	6.2 x 10^6	6.2 x 10^6	6.7 x 10^6	38	38	37
E_a (kcal/mol)	-2±2	0±2	-6±6	39±2	39±2	40±3
$\Delta S^{\ddagger}$ (e.u.)	-36±7	-29±7	-50±20	84±6	84±6	87±10

Table II: Kinetic Results for $rCA_5G + rCU_5G$, $dCA_5G + dCT_5G$ and $rCA_5G + dCT_5G$

	$rCA_5G + rCU_5G$	$dCA_5G + dCT_5G$	$rCA_5G + dCT_5G$
k_r (12.0°C, $\ell\ mol^{-1}\ sec^{-1}$)	6.2×10^6	8.3×10^6	6.6×10^6
$E_{a,r}$ (kcal/mol)	0±2	-0.5±2	---
$\Delta S^{\ddagger}_r$ (e.u.)	-29±7	-30±4	---
k_d (12.0°C, sec^{-1})	38	9.6	50
$E_{a,d}$ (kcal/mol)	39±2	43±3	---
$\Delta S^{\ddagger}_d$ (e.u.)	84±6	95±9	---
ΔH° (kcal/mol)[a]	-41	-49	
ΔS° (e.u.)[a]	-120	-145	

[a] Thermodynamic results from Nelson et al. (1981).

Table III: Apparent Rate Constants and Activation Energies for the Second Exponential Component.

	$k_{app} = 1/\tau_2$ (sec^{-1})	
	$rCA_5G + rCU_5G$	$rCA_5G + dCT_5G$
6.7°C	440 ± 230	470 ± 150
12.0°C	2000 ± 1500	1900 ± 1100
$E_{a(app)}$	45 kcal/mol	41 kcal/mol

Table IV: Recombination Activation Energies for Several Oligonucleotides

Oligonucleotide	(NaCl)	E_a (kcal/mol)	Reference
$rA_n + rU_n$, n=8-18	0.05M	-9	1
rA_nU_n, n=4-7	0.25M, 1M	-4 to -6	2,3
rA_nGCU_n, n=2-4	0.05M,		
$rA_nCG + rCGU_n$, n=6,8		+6 to +9	4
$rA_nG_2 + rC_2U_n$, n=4,5			
rCG_3+rG_3C	0.18M	+4.5	5
dG-C-G-C-G-C	1M	+1	6
dA-T-G-C-A-T	1M	-4	7
$dA_8 + dT_8$	0.05M	-2	7
$rA_n + dT_n$, n=7-9	1M	-1	8

(1) Pörschke & Eigen (1971)
(2) Breslauer & Bina-Stein (1977)
(3) Craig et al. (1971)
(4) Pörschke et al. (1973)
(5) Podder (1971)
(6) Freier et al. (1982)
(7) Drobnies (1979)
(8) Hoggett & Maass (1971)

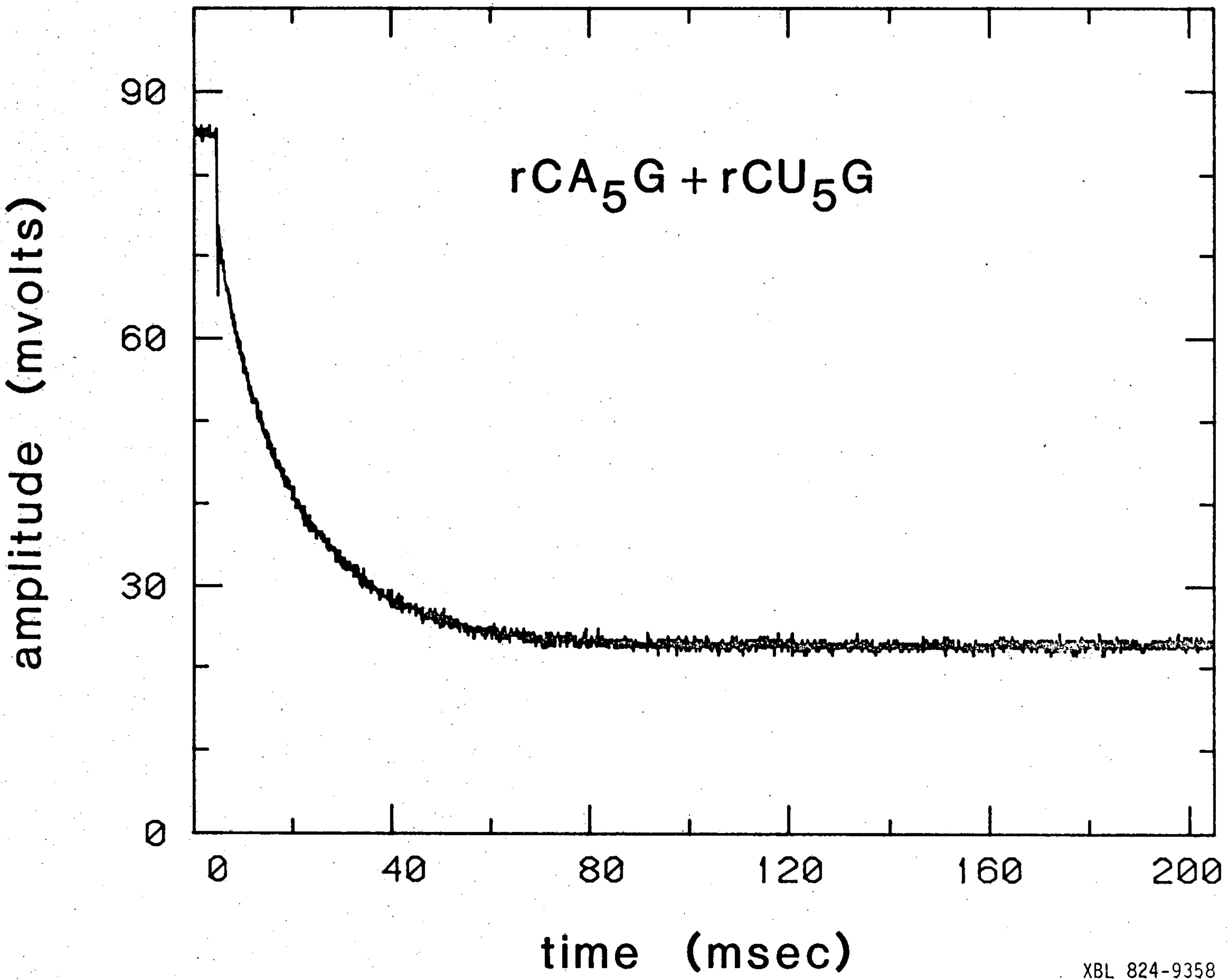
Figure Captions

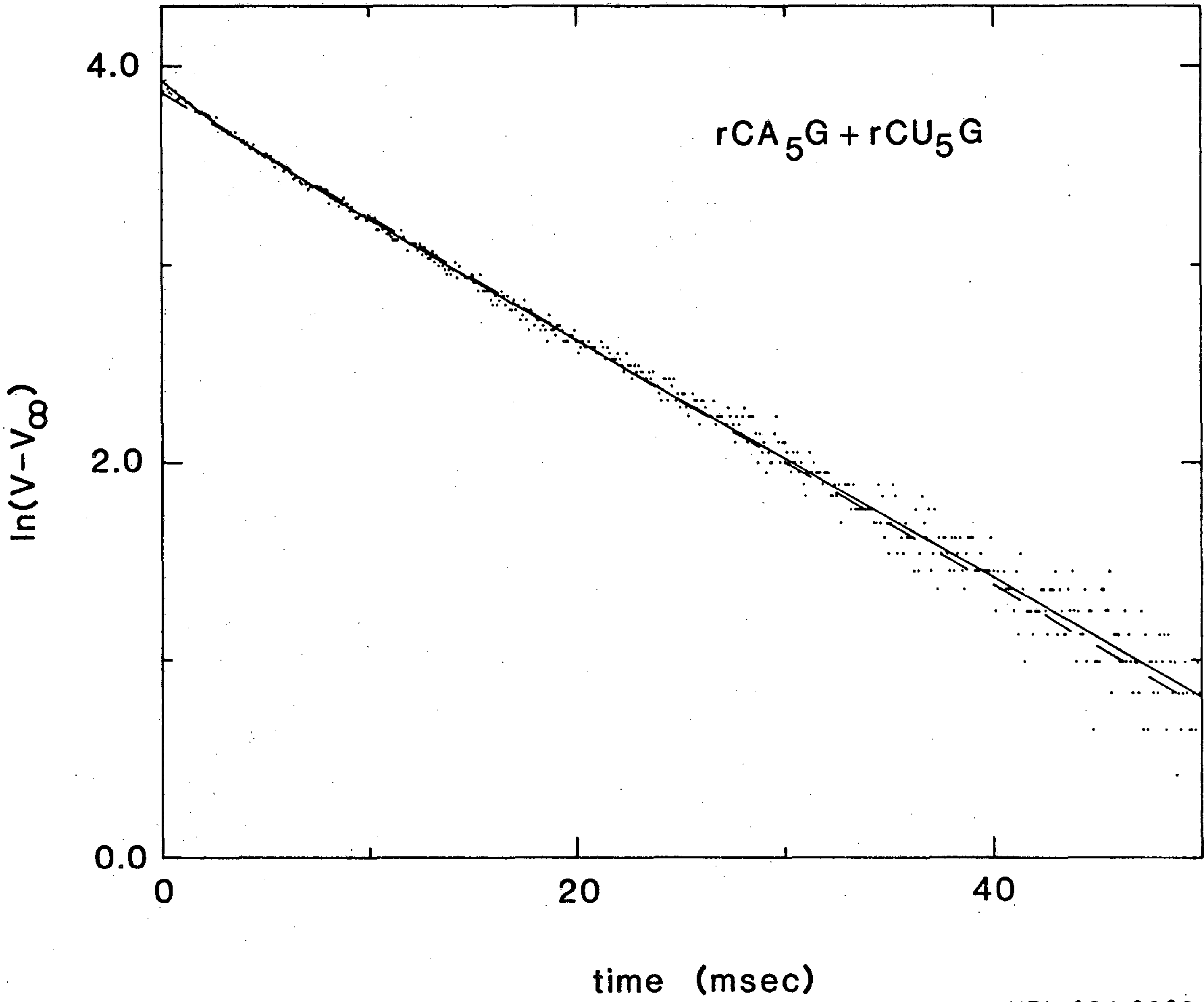
Figure 1. A trace of a temperature-jump experiment on 14.2μM rCA_5G + rCU_5G in 1M NaCl, 0.01M phosphate buffer, pH=7, 0.1mM EDTA, at a final temperature of 6.7°C. The temperature jump was 1.8°C; λ = 267nm. The initial signal was 5 volts. The first 5 msec shows the signal prior to the temperature jump.

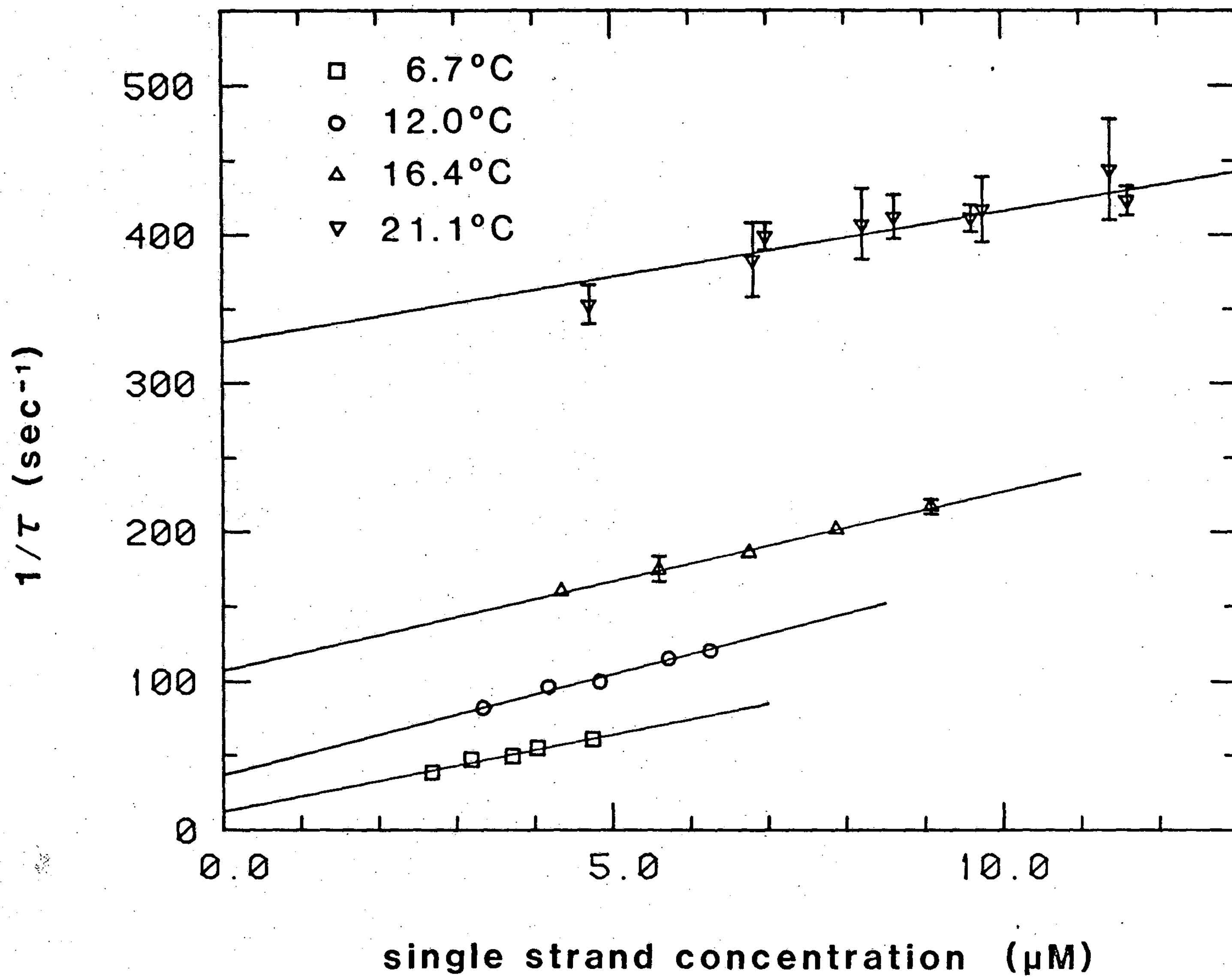
Figure 2. Semi-log plot of the trace in Figure 1. The amplitude at long times, v_∞, was determined from the two component fit of program DISCRETE. The two lines show the two component fit: (———), τ_1 = 16.6msec, a_1 = 45.8mV; τ_2 = 2.87msec, a_2 = 4.92mV; and the one component fit (------), τ_1 = 16.1msec, a_1 = 47.8mV.

Figure 3. $1/\tau$ as a function of equilibrium single strand concentration of rCA_5G + rCU_5G, determined by the second method (see text). The four temperatures are: □ = 6.7°C, ○ = 12.0°C, △ = 16.4°C, ▽ = 21.1°C. The error bars not shown are on the order of the symbol size. The results are tabulated in Table I.

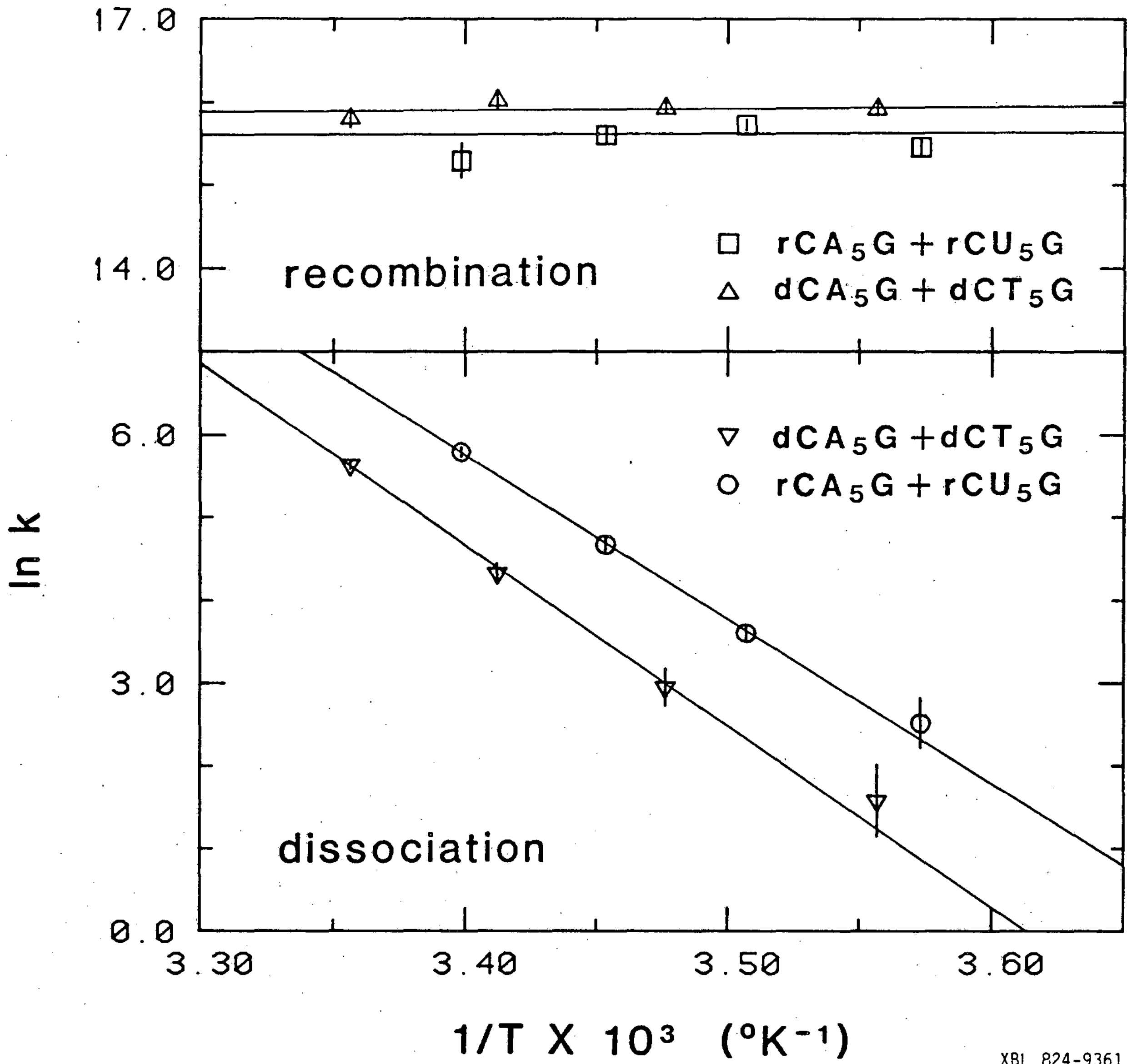
Figure 4. Arrhenius plot of the natural logarithm of the rate constants as a function of the inverse temperature for the recombination and dissociation rate constants. The slope of such a plot is $-E_a/R$. △ = dCA_5G + dCT_5G recombination rate; □ = rCA_5G + rCU_5G recombination rate; ▽ = dCA_5G + dCT_5G dissociation rate; ○ = rCA_5G + rCU_5G dissociation rate. The results are given in Table II.


rCA5G + rCU5G
amplitude (mvolts)
90
60
30
0
0
40
80
120
160
200
time (msec)
XBL 824-9358


rCA5G + rCU5G
4.0
2.0
0.0
ln(V-V∞)
0
20
40
time (msec)
XBL 824-9360



XBL 824-9359



XBL 824-9361

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.

TECHNICAL INFORMATION DEPARTMENT
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720