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TEMPERATURE-JUMP KINETICS OF THE dC-G-T-G-A-A-T-T-C-G-C-G DOUBLE HELIX WHICH CONTAINS A G.T BASE PAIR AND THE dC-G-C-A-G-A-A-T-T-C-G-C-G DOUBLE HELIX WHICH CONTAINS AN EXTRA DENINE

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**Publication Date**

1982-06-01

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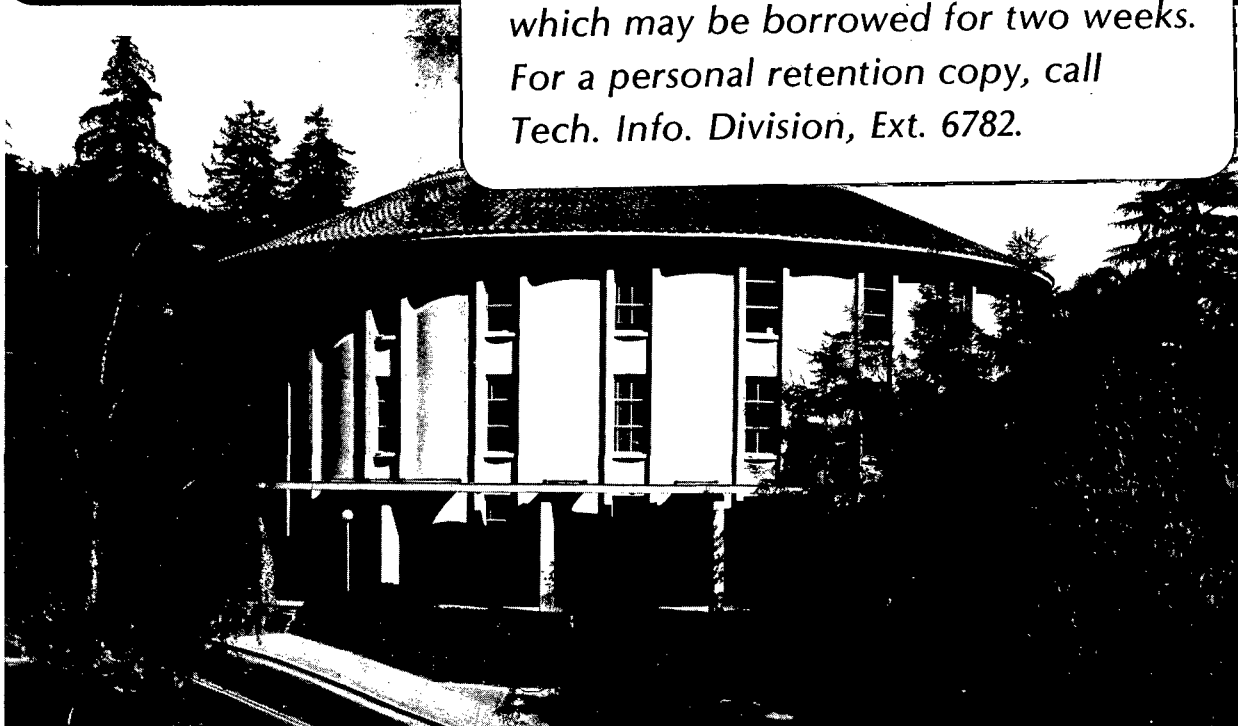## UNIVERSITY OF CALIFORNIA

## CHEMICAL BIODYNAMICS DIVISION

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DOUBLE HELIX WHICH CONTAINS AN EXTRA ADENINE

Y. Gloria Chu and Ignacio Tinoco, Jr.

June 1982

## DISCLAIMER

Temperature-Jump Kinetics of the dC-G-T-G-A-A-T-T-C-G-C-G

Double Helix which Contains a G·T Base Pair and the

dC-G-C-A-G-A-A-T-T-C-G-C-G Double Helix which Contains

an Extra Adenine

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## Synopsis

The kinetics of helix formation were investigated using the temperature-jump technique for the following two molecules: dC-G-T-G-A-A-T-T-C-G-C-G, which forms a double helix containing a G•T base pair (the G•T 12-mer), and dC-G-C-A-G-A-A-T-T-C-G-C-G, which forms a double helix containing an extra adenine (the 13-mer). When data were analyzed in an all-or-none model, the activation energy for the helix association process was 22 ± 4 kcal/mole for the G•T 12-mer and 16 ± 7 kcal/mole for the 13-mer. The activation energy for the helix dissociation process was 68 ± 2 kcal/mol for the G•T 12-mer and 74 ± 3 kcal/mole for the 13-mer. Rate constants for the helix association process were near $10^5$ sec$^{-1}$ M$^{-1}$ in the temperature range from 32°C to 47°C; for the helix dissociation process the rate constants varied from 1 sec$^{-1}$ near 32°C to 130 sec$^{-1}$ near 47°C. Possible effects of hairpin loops and fraying ends on the above data are discussed.

## INTRODUCTION

Mutations occur when DNA double strands contain non-complementary bases opposite each other, or when one strand contains extra nucleotides. We are thus interested in the kinetic effect of mismatched bases and extra bases on the helix opening and closing process. Here we report the temperature-jump kinetics of two molecules: dC-G-T-G-A-A-T-T-C-G-C-G, which forms a double helix containing a G•T base pair, and dC-G-C-A-G-A-A-T-T-C-G-C-G, which forms a double helix containing an extra adenine. Extensive nuclear magnetic resonance (NMR) studies on these two molecules have been reported. The G•T base pair in the G•T 12-mer resulted in a small decrease in stacking of the thymidine residue with adjacent base pairs; the phosphorus NMR spectra of the G•T 12-mer were different from those obtained for the perfect 12-mer, suggesting that the G•T interaction perturbs the phosphodiester backbone in the G•T 12-mer double helix.[1] The extra adenine in the 13-mer was stacked into the duplex.[2] Lifetime measurements of the exchangeable imino protons in the double helix showed that the presence of the G•T base pair in the G•T 12-mer affected only the opening rate of the nearest-neighbor base pairs, but the presence of the extra adenine in the 13-mer affected the opening rates of all the base pairs. Temperature dependence studies of lifetimes also suggested that in the G•T 12-mer and 13-mer several base pairs exchanged together, whereas in the dC-G-C-G-A-A-T-T-C-G-C-G double helix (henceforth called the 12-mer) one base pair could exchange independently of the others.[3] Calorimetric measurements and NMR studies also showed that melting temperatures of the G•T 12-mer double helix and 13-mer double helix were lowered by about 20° C when compared to the 12-mer double helix.[1,2]

## MATERIALS

dC-G-T-G-A-A-T-T-C-G-C-G (G·T 12-mer) and dC-G-C-A-G-A-A-T-T-C-G-C-G (13-mer) were synthesized as described previously.[1,2] The buffer used for our studies contained 33 mM $NaH_2PO_4$, 67 mM $Na_2HPO_4$ and 2.5 mM EDTA at a final pH of 7.0. For each molecule, solutions of five different concentrations were prepared by diluting weighed aliquots of the concentrated sample. The concentration of one solution was determined by absorbance measurement;[1,2] concentrations of the other solutions were calculated from their relative dilution factors. Concentrations of the G·T 12-mer solution varied from $4.8 \times 10^{-6}$ M to $1.8 \times 10^{-5}$ M and of the 13-mer from $8.0 \times 10^{-6}$ M to $2.0 \times 10^{-5}$ M.

## TEMPERATURE-JUMP KINETICS

The temperature-jump instrument used for our experiments has been described previously.[4,5] Data were collected digitally and analyzed with a VAX 11/780 computer.[6] Kinetics of the G·T 12-mer were followed over a time range of 0.1 to 2 sec for all samples except that a range of 0.1 to 4 sec was used for the sample of the lowest concentration. Kinetics of the 13-mer were followed over a time range of 0.04 to 2 sec. The amplitude of the temperature-jump was 1.8°C for a 20 nF capacitor at 20 KV.[5] For both molecules, initial temperatures of 30.0, 35.0, 40.0 and 44.9°C were used. This temperature range covered the helix-to-coil transition for both molecules. The kinetics at 25°C were too slow to measure. Data at temperatures above 50°C were not collected because our temperature-jump cell was not designed to be heated above 50.0°C. Samples were deaerated immediately before use by bubbling helium gas into the sample. Melting curves were measured on a Gilford Model 250 spectrophotometer.[7]

## DATA ANALYSIS

Temperature-jump data were analyzed by using the program DISCRETE written by S. W. Provencher[8,9] to obtain the relaxation time, $\tau$. Data were analyzed by a two-state model. For a reaction of the following form

$$2A \; \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \; B$$
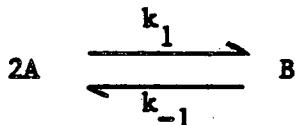
the rate constants $k_1$ for the forward process and $k_{-1}$ for the backward process were obtained from a plot of $1/\tau^2$ vs. total strand concentration, $C_T$, from the following equation[10]

$$\frac{1}{\tau^2} = 8 \, k_1 \, k_{-1} \, C_T + k_{-1}^2 \qquad (1)$$

Every $\tau$ reported was the average of at least five measurements. Standard deviations of the average relaxation times were used in a weighted linear regression to obtain $k_1$ and $k_{-1}$ from the best fit of the data to Equation 1. Activation energies were obtained from the temperature dependence of the rate constants; errors for activation energies were obtained as described earlier.[6]

## RESULTS

Because of the small amount of G·T 12-mer and 13-mer available, the same sample solution was used for temperature-jump experiments done at all four temperatures of 31.8°C, 36.8°C, 41.8°C and 46.7°C. Measurements were started at the lowest temperature, and at the end of data collection, measurements were repeated at the lowest temperature. The relaxation time changed less than 20%. For each solution prepared for temperature-jump experiments, a portion was put aside as a comparison sample to test for degradation. The melting curve of the sample used in the temperature-jump experiments was then

compared to the melting curve of the comparison sample. Using the 260 nm absorbance at 60°C as representing single strands, and the absorbance at 10°C for the double strand helix, we found a maximum change in hypochromicity of 13% for samples which had been used in the temperature-jump measurements. Some samples had no change in hypochromicity. The maximum change in absorbance at 60°C for the samples used in the temperature-jump studies was 5%.

Analysis of temperature-jump data for the G•T 12-mer at 31.8 and 36.8°C gave two exponential terms, except that only one exponential term was obtained for the sample of the lowest concentration at 36.8°C. Because of the small amplitude of the minor exponential term, the main relaxation time was within 5% of that obtained by one exponential fitting. The main relaxation time was used in the calculation of rate constants. Data collected at 41.8 and 46.7°C gave only one relaxation time. Exponential decay curves of the G•T 12-mer at a concentration of $1.2 \times 10^{-5}$ M obtained at 31.8 and 46.7°C are given in Fig. 1 as examples.

Rate constants were obtained from a plot of $1/\tau^2$ vs. total concentration as described in DATA ANALYSIS section. Plots of $1/\tau^2$ vs. total concentration for the G•T 12-mer are shown in Fig. 2. Rate constants obtained are given in Table 1. The activation energy, $E_a$, was obtained from an Arrhenius plot; plots of the rate constants of the G•T 12-mer are shown in Fig. 3. The activation entropy $\Delta S^{\ddagger}$ was calculated from the pre-exponential term from the Eyring equation. Values of the thermodynamic enthalpy, $\Delta H$, and entropy, $\Delta S$, were calculated from $\Delta E_{a,1} - \Delta E_{a,-1}$ and $\Delta S_1^{\ddagger} - \Delta S_{-1}^{\ddagger}$, respectively. The results are summarized in Table 2.

The amplitude of the minor exponential term was at most 15% of the total amplitude and decreased as the temperature increased. The minor relaxation time obtained either in the case of the G•T 12-mer or 13-mer was one order of

magnitude smaller than the corresponding main relaxation time. It did not show any strong concentration dependence. Similar observations have been reported before.[6,11] Because of its small amplitude and large error, we will not try to explain the cause of the minor relaxation time.

## DISCUSSION

The activation energies for the helix formation process of the G·T 12-mer (22 ± 4 kcal/mole) and 13-mer (16 ± 7 kcal/mole) are very large compared to activation energies reported for other oligonucleotides. The activation energies for dG-C-G-C-G-C, dA-T-G-C-A-T, $dA_8 + dT_8$ and $dCA_5G + dCT_5G$ range from +1 kcal/mole to -4 kcal/mole.[5,6,11] The activation energies for ribo-oligonucleotides containing only A·U base pairs are about -9 kcal/mole, and for sequences containing G·C base pairs, they are about +6 to +9 kcal/mole. The formation of a nucleus containing one-to-three base pairs was suggested to be required in the mechanism of helix formation. In this mechanism, the formation of the next base pair was the rate-limiting step. Once the nucleus formed, the rest of the base pairs zipped up very quickly. For the G·T 12-mer, base pairs 1 and 2 (See Chart 1) can probably form quickly, but since the formation of the G·T base pair, and perhaps also base pair 4, may involve some unusual conformational changes, formation of these two base pairs could require a high activation energy. In the case of the 13-mer, NMR studies showed that the extra adenine is stacked inside the double helix and that the phosphodiester linkage opposite the extra adenine may be extended. Thus the formation of base pairs 1, 2 and 3 (See Chart 2) should be fast, but the formation of base pair 4 may be slow and require a high activation energy. However, we do not know if the energies required for these conformational changes would be large enough to account for the high activation energies we measured for the G·T 12-mer and 13-mer. Since the measured activation energy

for the helix association process decreases as the size of the nucleus increases, it is reasonable that the 13-mer have a smaller activation energy than the G·T 12-mer. This seems to be the case.

The melting curve of the G·T 12-mer showed a sharp transition, but the melting curve of the perfect 12-mer helix showed a less cooperative transition, especially at temperatures above $T_m$ (data not shown). This is probably due to hairpin loop formation in the perfect 12-mer[12]. Melting curves of the 13-mer also showed a relatively broad transition. However, this effect may be due to the presence of the extra adenine in the double helix. NMR studies of these three molecules did not indicate the existence of hairpin loops. However, NMR studies were done at concentrations much higher than those used for melting curve and temperature-jump studies. The formation of a hairpin loop is much less favored in the case of G·T 12-mer and 13-mer than in the case of the perfect 12-mer.

Let us consider the following mechanism:

$$D.S. \underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}} 2 \ S.S.$$

$$S.S. \underset{k_{32}}{\overset{k_{23}}{\rightleftarrows}} H.L.$$
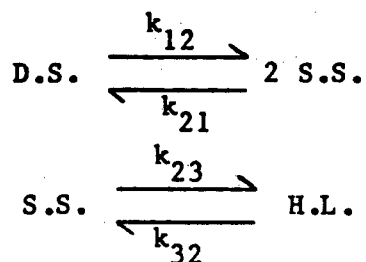
where S.S. is the single-stranded species, H.L. is the hairpin loop and D.S. is the double-stranded helix. We have assumed that the double-stranded helix cannot be formed directly from the hairpin loop; a hairpin loop can be formed only from a single-stranded helix (direct transition between double helices and hairpin loops has been suggested, however[13]). If the equilibrium between the single-stranded helix and the hairpin loop is a fast equilibrium, then two relaxation times occur; the fast one ($\tau_1$) depends only on $k_{32}$ and $k_{23}$, the

slow one ($\tau_2$) depends on the bimolecular process.

$$\frac{1}{\tau_1} = k_{23} + k_{32}$$

$$\frac{1}{\tau_2} = 4 \frac{k_{21} k_{32}}{k_{23} + k_{32}} C_S + k_{12}$$

where $C_S$ is the concentration of single-stranded species at equilibrium. $\tau_1$ is in the μsec range[14] and would not be measurable in our experiments. $\tau_2$ corresponds to the main relaxation time we obtained. If we square both sides of the above equation and introduce $C_T$, the total strand concentration, we obtain

$$\left(\frac{1}{\tau_2}\right)^2 = 8 \, k_{12} k_{21} \left(\frac{k_{32}}{k_{23} + k_{32}}\right)^2 C_T + k_{12}^2$$

Thus rate constants $k_1$ and $k_{-1}$ obtained from the plot of $1/\tau^2$ vs. $C_T$ in the two-state model in Equation 1 are

$$k_1 = k_{21} \left(\frac{k_{32}}{k_{23} + k_{32}}\right)^2 = k_{21} \left(\frac{1}{K + 1}\right)^2$$

where K is the equilibrium constant for the formation of hairpin loops from a single-stranded helix, and

$$k_{-1} = k_{12}$$

Thus if a hairpin loop is interfering with the double helix association and dissociation process, the $k_{-1}$ we reported in Table 1 is still the rate constant for the double helix dissociation step, but $k_1$ may be smaller than the real rate constant for the helix recombination step. For the extreme case where $K + 1 \simeq K$, the real activation energy $\Delta E_{a,21}$ and activation entropy

$\Delta S_{21}^{\ddagger}$ for the helix association step becomes $\Delta E_{a,21} = \Delta E_{a,1} + 2\Delta H$, and $\Delta S_{21}^{\ddagger} = \Delta S_1^{\ddagger} + 2\Delta S$ where $\Delta H$ and $\Delta S$ are the enthalpy and entropy change for the formation of hairpin loops from the single-stranded helix. Although unlikely, we cannot exclude the possibility that the measured high activation energy for the helix association process was due to the energy needed to open the hairpin loops before association. If we use thermodynamic parameters for RNA double helices,[15] RNA hairpin loops and G·U base pairs[14] to calculate K for the G·T 12-mer, assuming that $\Delta H$ for forming the first base pair in a hairpin loop of size 4 is zero, then the calculated $\Delta E_{a,21}$ is 21 kcal/mole and $\Delta S_{21}^{\ddagger}$ is 32 e.u. If the $\Delta H$ for the first base pair formation is not zero[16] then $\Delta E_{a,21} > \Delta E_{a,1}$ and $\Delta S_{21}^{\ddagger} > \Delta S_1^{\ddagger}$ ($\Delta H$ and $\Delta S$ are both positive).

NMR studies of the line width of the exchangeable imino protons have indicated the existence of fraying ends which extend to base pair 3 in the G·T 12-mer and 13-mer.[1,2] If the concentration of the partially-opened helix is not negligible, then the temperature-jump data cannot be analyzed by a two-state model.

The enthalpies calculated by the difference between $\Delta E_{a,1}$ and $\Delta E_{a,-1}$ for the G·T 12-mer and 13-mer, 46 kcal/mole and 60 kcal/mole, respectively, were smaller than those measured by the calorimetric method in a buffer containing 0.1 M NaCl. The enthalpies obtained by integrating the areas under the calorimetric heat capacity curves for the G·T 12-mer and 13-mer are 106 kcal/mole and 104 kcal/mole, respectively; the Van't Hoff enthalpies obtained from the analysis of the shapes of the calorimetric heat capacity curves for the G·T 12-mer and 13-mer are 76 kcal/mole and 70 kcal/mole, respectively. The size of the cooperative melting unit as determined from the ratio of the Van't Hoff enthalpy to the calorimetric enthalpy was 9 ± 1 base pairs for both the G·T 12-mer and 13-mer.[1,2] Using the same method as above, but substituting our $\Delta H$

for the Van't Hoff $\Delta H$ obtained from the calorimetric curve, we calculate a cooperative unit of $5.2 \pm 0.5$ base pairs for the G·T 12-mer and $6.7 \pm 0.9$ base pairs for the 13-mer. Therefore, the helix dissociation process may involve partial opening of the helix. The entropy calculated from the difference between $\Delta S_1^{\ddagger}$ and $\Delta S_{-1}^{\ddagger}$ for the G·T 12-mer and 13-mer also seemed small. Rate constants for the helix association process seemed low although direct comparison with data of other oligodeoxynucleotides cannot be made because different buffers were used. The transition state for the helix association process was entropy-favored in contrast to results obtained for other oligodeoxynucleotides.

In order to further understand the kinetic results obtained for G·T 12-mer and 13-mer, we tried to do temperature-jump experiments on the perfect 12-mer double helix. However, it has a melting temperature of about 60°C and our temperature-jump cell was not designed for experiments above 50°C. We tried to collect data for the 12-mer at temperatures between 43 and 49°C. The signal was too low and the relaxation process was too slow. Decay curves obtained at 4 sec time range gave sloping base lines; presumably this is due to convection and diffusion effect. When 10 mole % ethanol was added to the solution the melting temperature decreased about 12°C. However, at 40°C, where the rate constant for the G·T 12-mer in 10 mole % ethanol can be measured for comparison, the signal was still too low and the relaxation process was still too slow.

In summary, we measured the temperature-jump kinetics of two imperfect double helices: one contained a mismatched base pair and one contained an extra base. They both had a high activation energy for the helix association process compared to normal helices. Further studies on other imperfect helices may allow the determination of the effect of errors in replication on the kinetics of double strand formation.

## ACKNOWLEDGEMENTS

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Chart 1

```
        1 2 3 4 5 6 6 5 4 3 2 1
        ─────────────────────────
        C-G-T-G-A-A-T-T-C-G-C-G
        | | | | | | | | | | | |
        G-C-G-C-T-T-A-A-G-T-G-C
        ─────────────────────────
```

Chart 2

```
        1 2 3    4 5 6 6 5 4    3 2 1
        ─────────────────────────────
        C-G-C-A-G-A-A-T-T-C---G-C-G
        | | |    | | | | | |    | | |
        G-C-G---C-T-T-A-A-G-A-C-G-C
        ─────────────────────────────
```

Table 1        Rate Constants for the Helix Association and Dissociation Process

| Temperature °C | $k_1 \times 10^{-5}$ (sec$^{-1}$, M$^{-1}$) | | $k_{-1}$ (sec$^{-1}$) | |
|---|---|---|---|---|
| | G·T 12-mer | 13-mer | G·T 12-mer | 13-mer |
| 31.8 | 0.8±0.4 | 0.7±0.6 | 1.0±0.4 | 0.7±0.5 |
| 36.8 | 2.3±0.5 | 2.2±0.5 | 3.2±0.5 | 3.1±0.5 |
| 41.8 | 3.5±0.2 | 2.6±0.6 | 15.4±0.3 | 22±2 |
| 46.7 | 6±1 | 5±2 | 87±4 | 130±10 |

Table 2          Summary of Kinetic Results

|  |  | G·T 12-mer | 13-mer |
|---|---|---|---|
| Activation Energy (kcal/mole) | $\Delta E_{a,1}$ | 22±4 | 16±7 |
|  | $\Delta E_{a,-1}$ | 68±2 | 74±3 |
| Activation Energy (e.u.) | $\Delta S_1^{\ddagger}$ | 40±10 | 20±20 |
|  | $\Delta S_{-1}^{\ddagger}$ | 159±6 | 180±10 |
| Enthalpy (kcal/mole) | $\Delta H$ | 46±6 | 60±10 |
| Entropy (e.u.) | $\Delta S$ | 120±20 | 160±30 |

FIGURE CAPTIONS

Fig. 1   The trace of a temperature-jump experiment on $1.2 \times 10^{-5}$ M G·T 12-mer at a final temperature of (a) 31.8°C and (b) 46.7°C. The original signal was 5 volts. The flat part of the trace near time zero is the signal prior to the temperature-jump. (c) is the semi-log plot of (a). $V_\infty$ is determined from the two exponential fit. The solid line shows the two exponential fit which yields $\tau_1$ = 0.306 sec, $a_1$ = 21.7 mvolts; $\tau_2$ = 21.9 msec, $a_2$ = 1.75 mvolts. Dashed line shows the one exponential fit which yields $\tau$ = 0.302 sec, a = 22.0 mvolts. (d) is the semi-log plot of (b). One exponential fit gives $\tau$ = 8.26 msec, a = 45.6 mvolts.

Fig. 2   The plot of $\tau^{-2}$ vs. total strand concentration for the G·T 12-mer at (a) 31.8°C, (b) 36.8°C, (c) 41.8°C, and (d) 46.7°C.

Fig. 3   The Arrhenius plot for the (a) helix association process and (b) helix dissociation process for the G·T 12-mer.
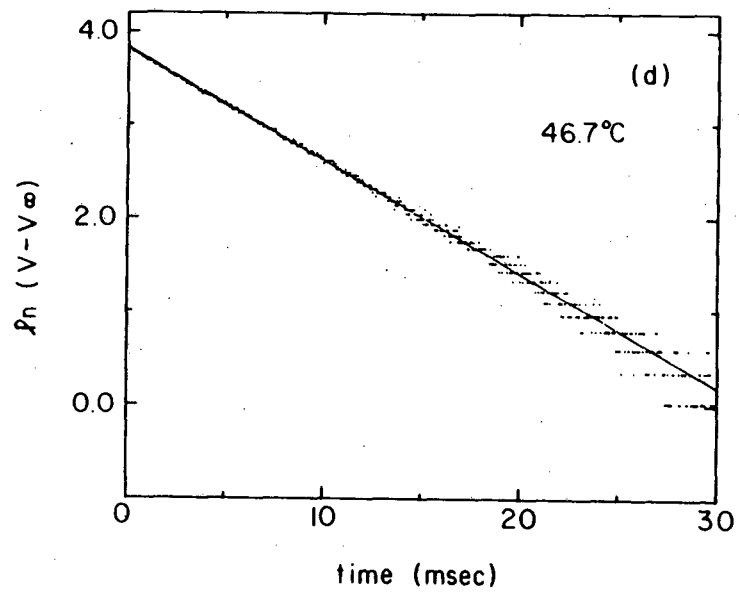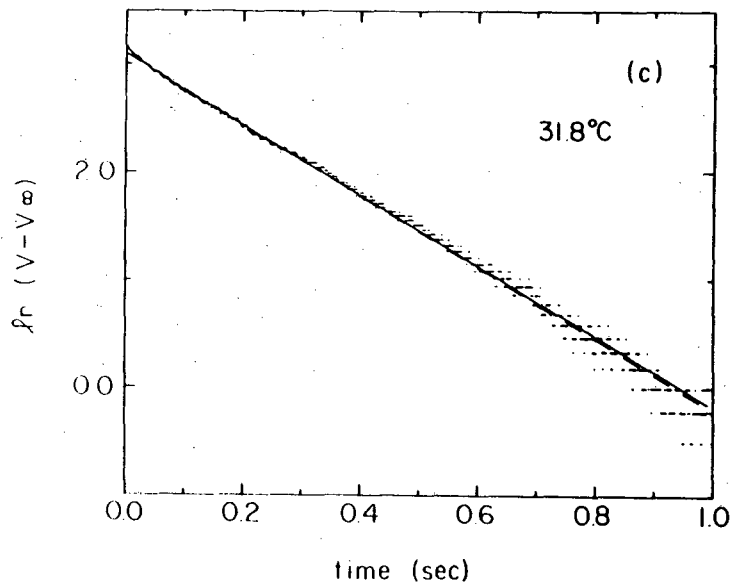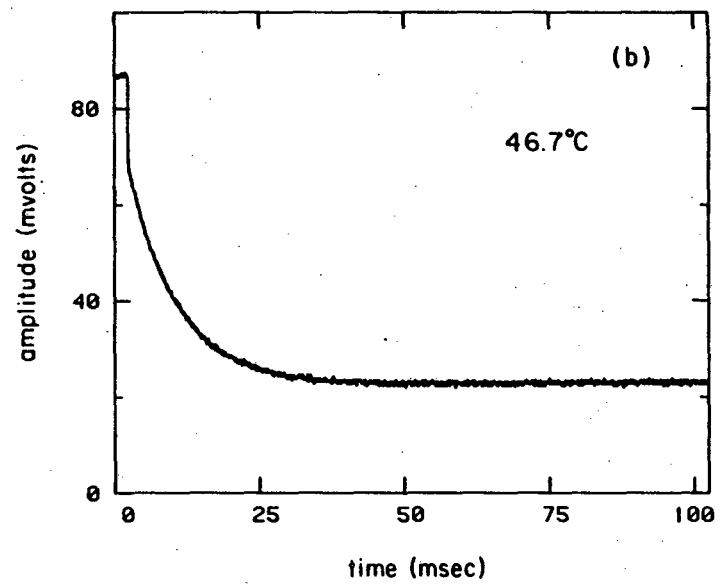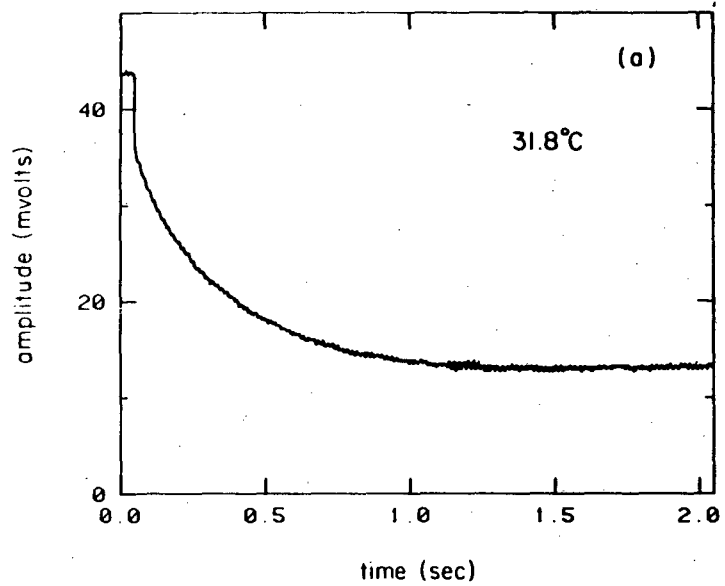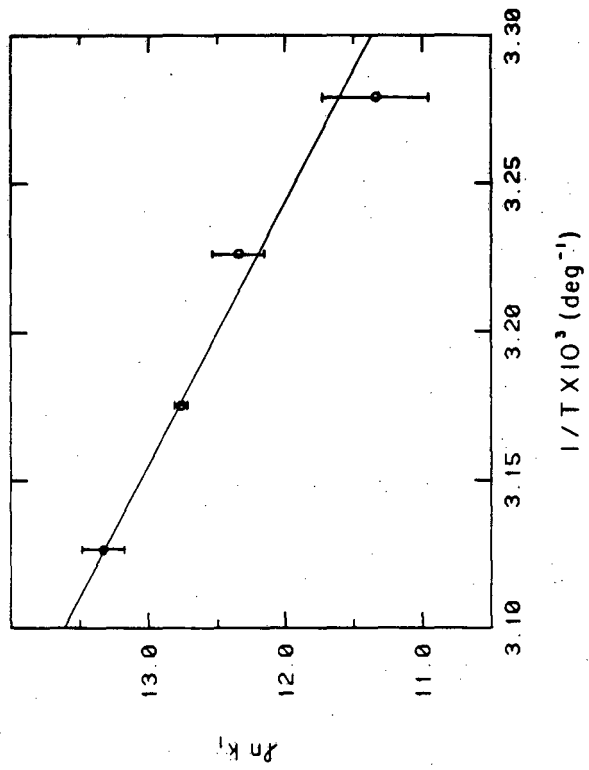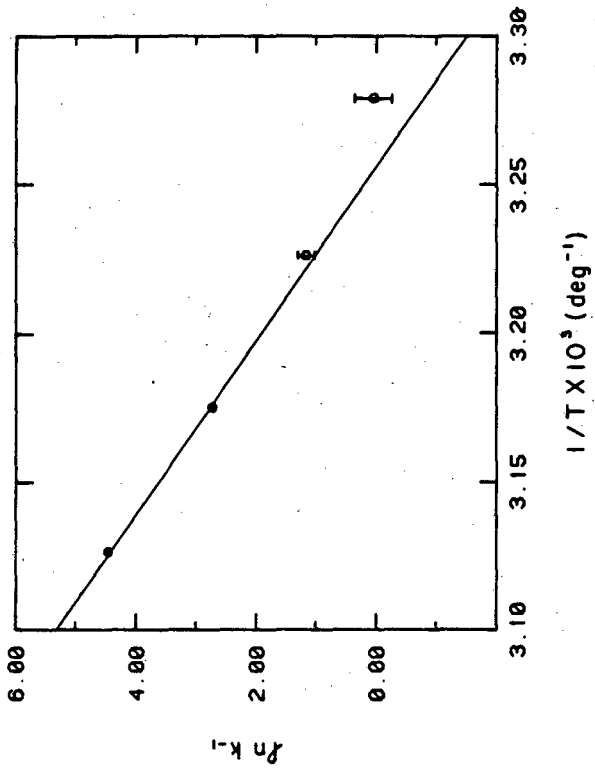
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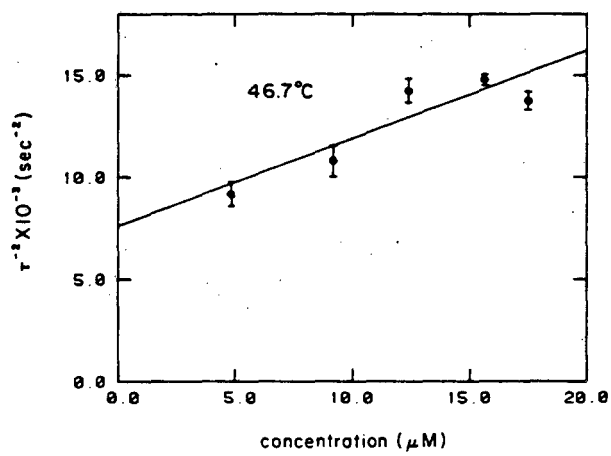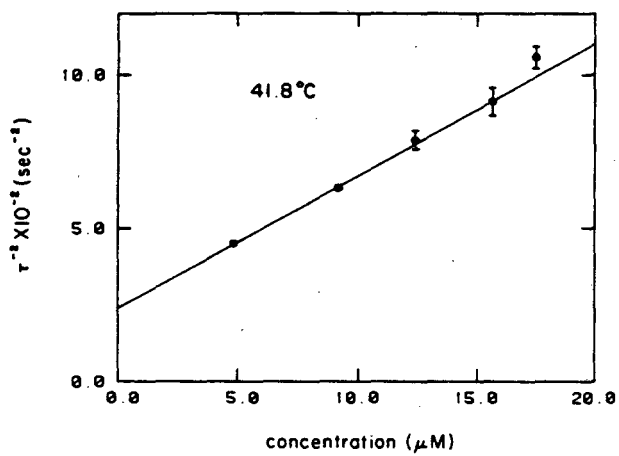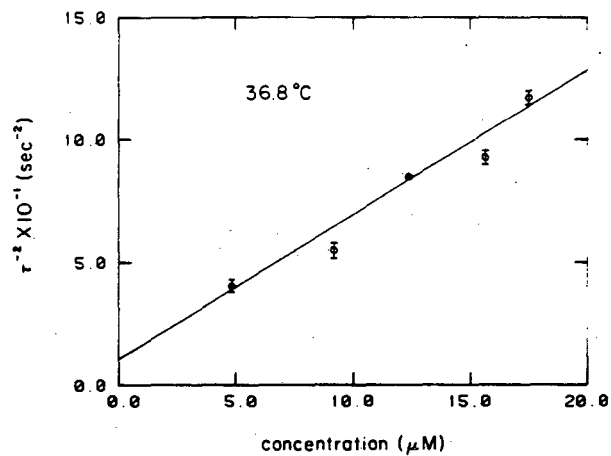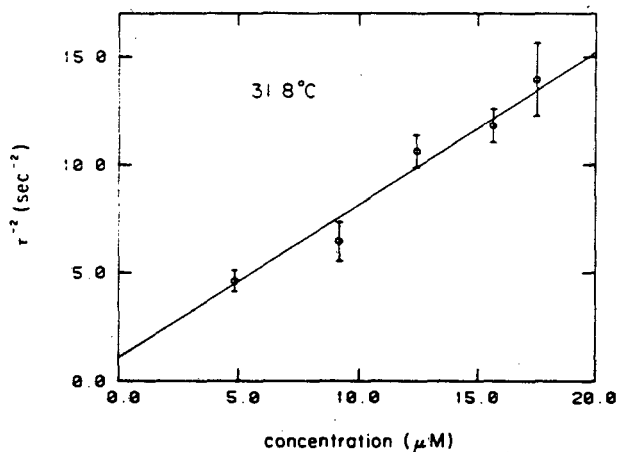
$\ell n\ k_{-1}$

$1/T \times 10^3\ (\text{deg}^{-1})$

XBL 826-10316

$\ell n\ k_1$

$1/T \times 10^3\ (\text{deg}^{-1})$

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