Structural Biology (LS5648)

March 8, 2006

The UV and CD spectroscopic studies on nucleic acids and proteins

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Biophysical Structural Program

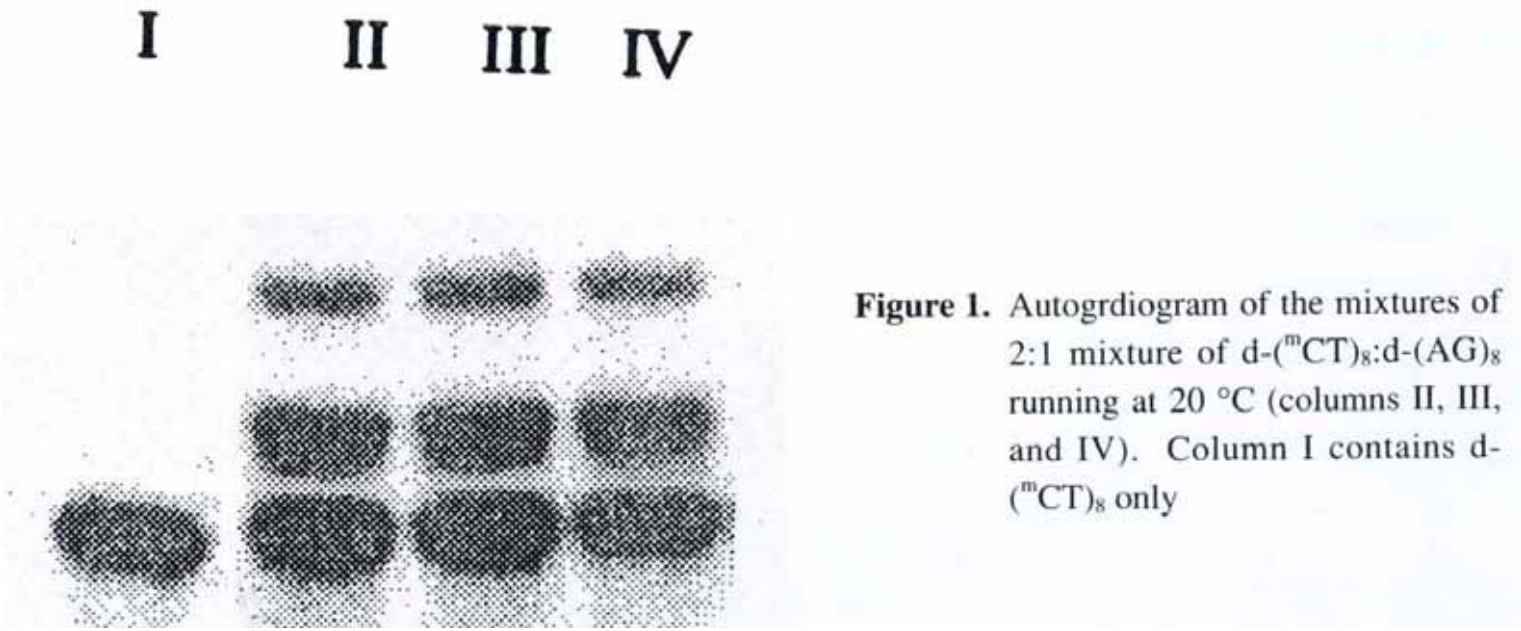

Figure 1. Autogrdiogram of the mixtures of 2:1 mixture of d-(^{m}CT)$_8$:d-(AG)$_8$ running at 20 °C (columns II, III, and IV). Column I contains d-(^{m}CT)$_8$ only

$$\text{d-}(^{m}\text{CT})_8 + \text{d-(AG)}_8 \text{-----> d-(AG)}_8\text{·d-}(^{m}\text{CT})_8 \quad (1)$$

$$\text{d-(AG)}_8\text{·d-}(^{m}\text{CT})_8 + \text{d-}(^{m}\text{C}^{+}\text{T})_8 \text{-----> d-}(^{m}\text{C}^{+}\text{T})_8\text{·d-(AG)}_8\text{·d-}(^{m}\text{CT})_8 \quad (2)$$

Thus, the ratio of concentration of the single stranded d-(^{m}CT)$_8$ + d-(^{m}C^{+}T)$_8$ (ss) to double stranded complex d-(AG)$_8$·d-(^{m}CT)$_8$ (ds) and to triplex d-(^{m}C^{+}T)$_8$·d-(AG)$_8$·d-(^{m}CT)$_8$ (ts) equals to CMP_{ss} to CMP_{ds} to (1/2)CMP_{ts} (CPM is counted per minute measured by scitillation counter). Only one half of the CMP_{ts} is counted due to two strands of d-(^{m}CT)$_8$ in the triplex. The concentrations of these species can then be calculated if the total initial concentration of d-(^{m}CT)$_8$(C_o) is known.

$$[ts] = (1/2)(CMP_{ts}/CMP_{total})C_o \quad (3)$$

$$[ds] = (CMP_{ds}/CMP_{total})C_o \quad (4)$$

$$[ss] = (CMP_{ss}/CMP_{total})C_o \quad (5)$$

Table I. The counts of ss, ds, and ts of d-($^{m}C^{+}T)_8$:d-$(AG)_8$ (2:1) measured at 10, 20, and 25 °C, respectively. The K_t's are also calculated based on $C_o = 6.0 \times 10^{-8}$ M.

temp.(°C)	ts	ds	ss	$K_t \times 10^{-6}$	Ave. $K_t \times 10^{-6}$
10	39,128	102,707	135,638	6.5	
10	47,985	152,223	161,822	6.5	
10	37,684	115,106	148,247	5.5	
					6.2
20	58,651	161,258	333,179	5.0	
20	60,865	162,981	334,439	5.0	
20	50,317	130,927	235,908	5.5	
					5.2
25	3,540	9,053	21,433	5.0	
25	3,527	9,080	25,954	4.8	
25	7,869	20,410	31,334	4.9	
					4.9

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$$\Delta H = -R[d(\ln K)/d(1/T)]$$

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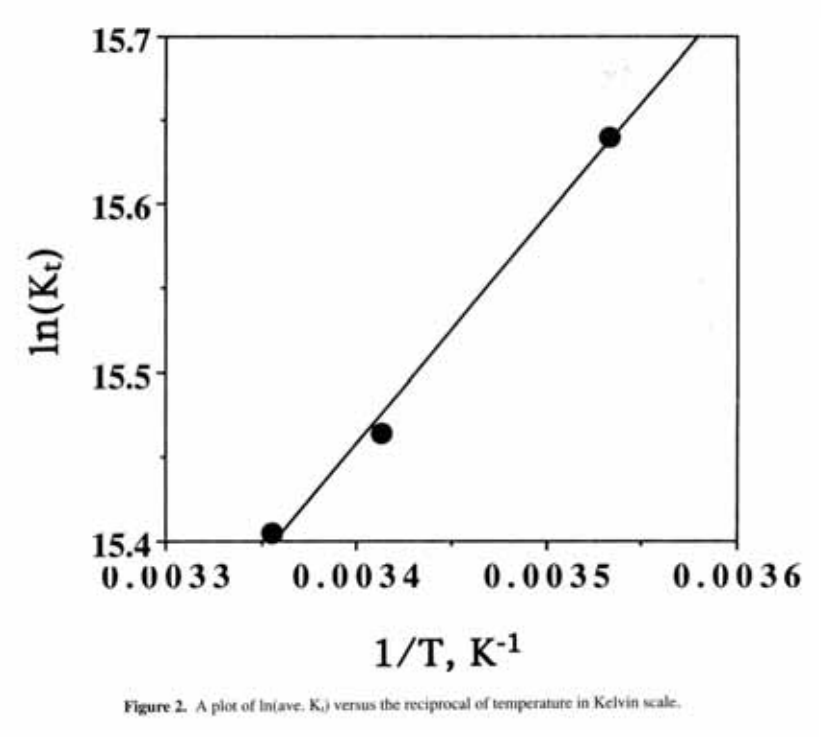
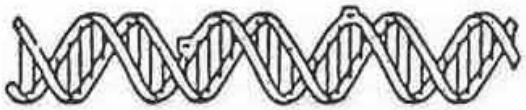


Figure 2. A plot of ln(ave. K_t) versus the reciprocal of temperature in Kelvin scale.

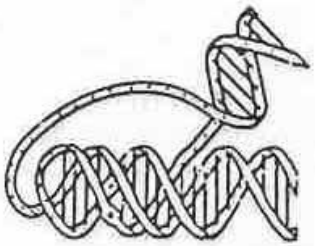
$$\Delta H = -R[d(\ln K)/d(1/T)]$$

$$\Delta H = -R \text{ slope} = -12 \text{ KJ/mole}$$



UV
---------->
stoichiometric ratio
stability
secondary structure
thermodynamic properties

CD
---------->
secondary structure
stability



IR
---------->
local configuration

gel electrophoresis
---------->
formation
association constant

NMR

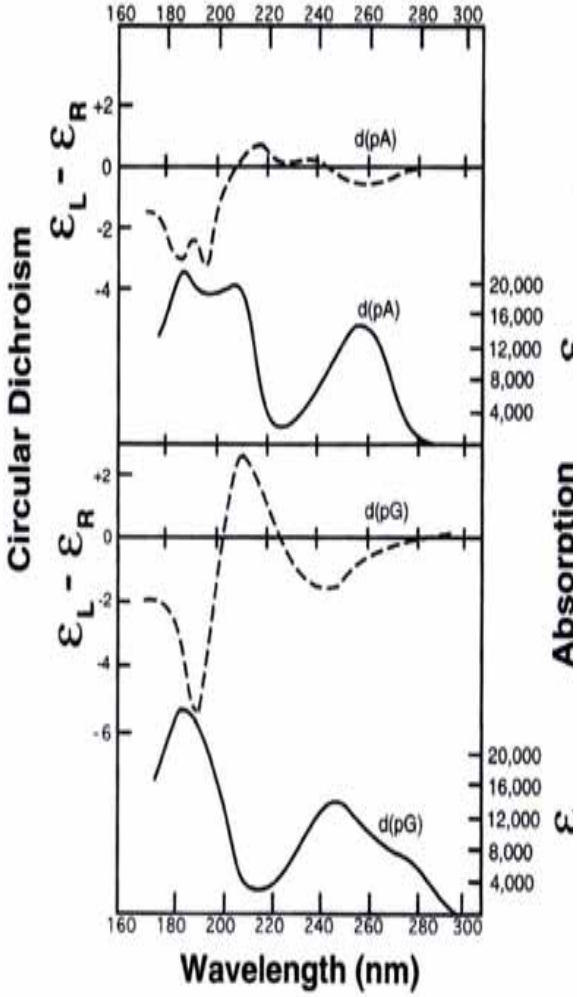


Figure 6-1
Circular dichroism (- - - -) and absorption (—) spectra of purine deoxyribonucleotides; molar extinction coefficients in units of M^{-1} cm^{-1}, ε and $\varepsilon_L - \varepsilon_R$, are plotted versus wavelength. The upper curves are for 5′-deoxyriboadenylic acid; the lower curves are for 5′-deoxyguanylic acid. [Data are from Sprecher and Johnson, 1977.]

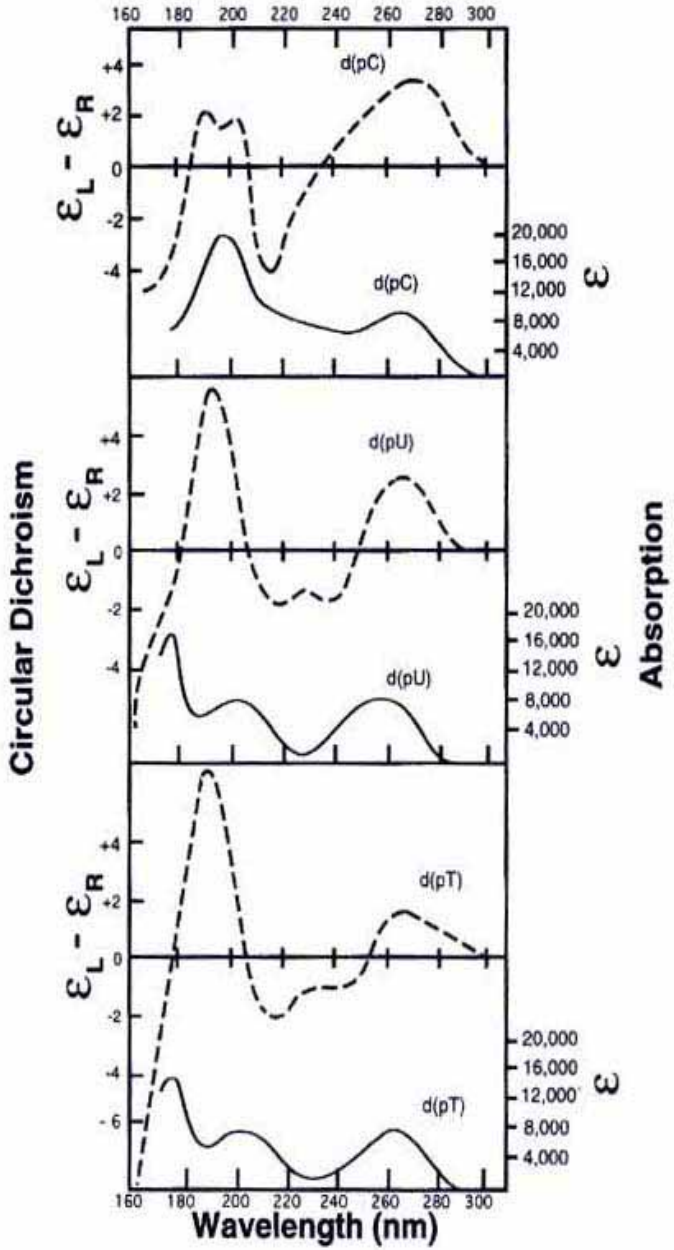


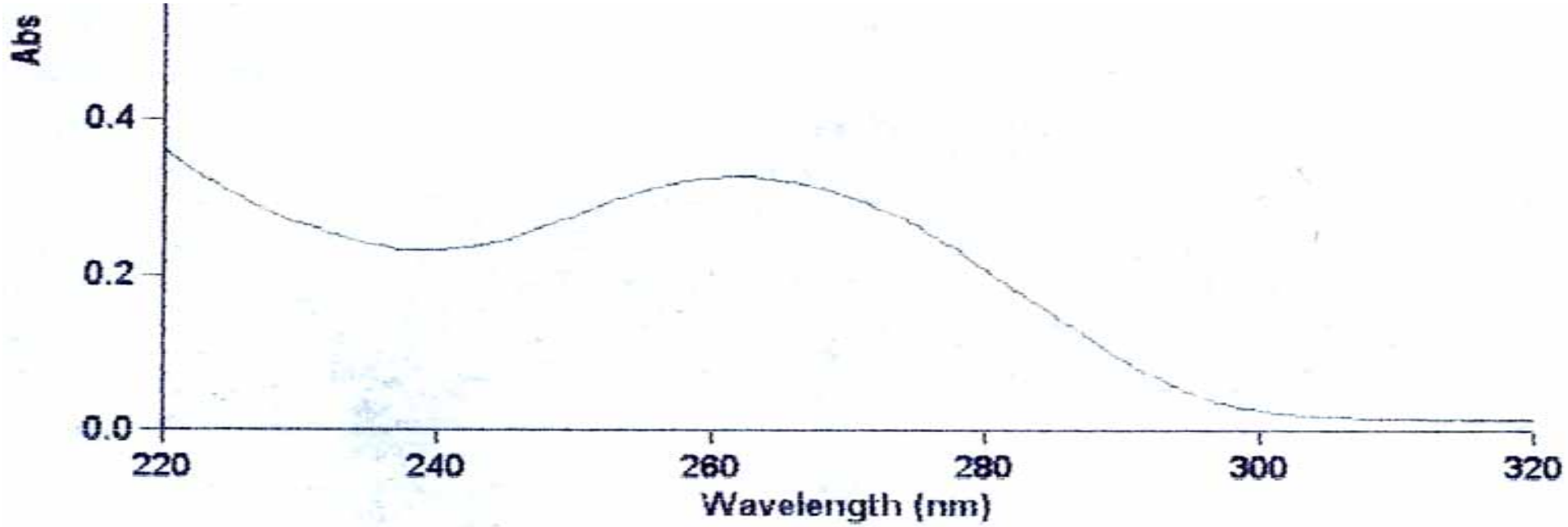
Figure 6-2
Circular dichroism and absorption spectra of pyrimidine deoxyribonucleotides plotted as in Figure 6-1. The curves are from top to bottom: 5′-deoxyribocytidylic acid, 5′-deoxyribouridylic acid, and thymidylic acid. [Data are from Sprecher and Johnson, 1977.]

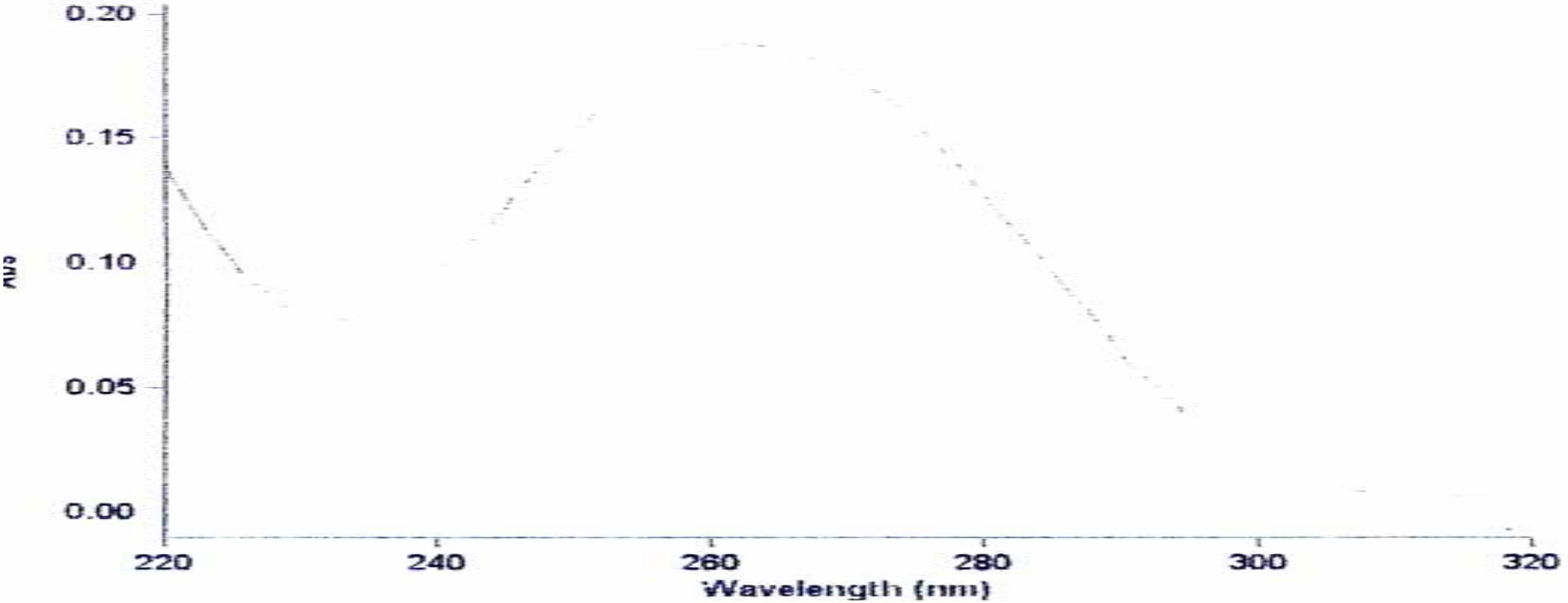
Advantages:

High sensitivity

Easy to use

Multiple parameters


Abs
0.4
0.2
0.0
220
240
260
280
300
320
Wavelength (nm)


0.20
0.15
0.10
0.05
0.00
220
240
260
280
300
320
Wavelength (nm)

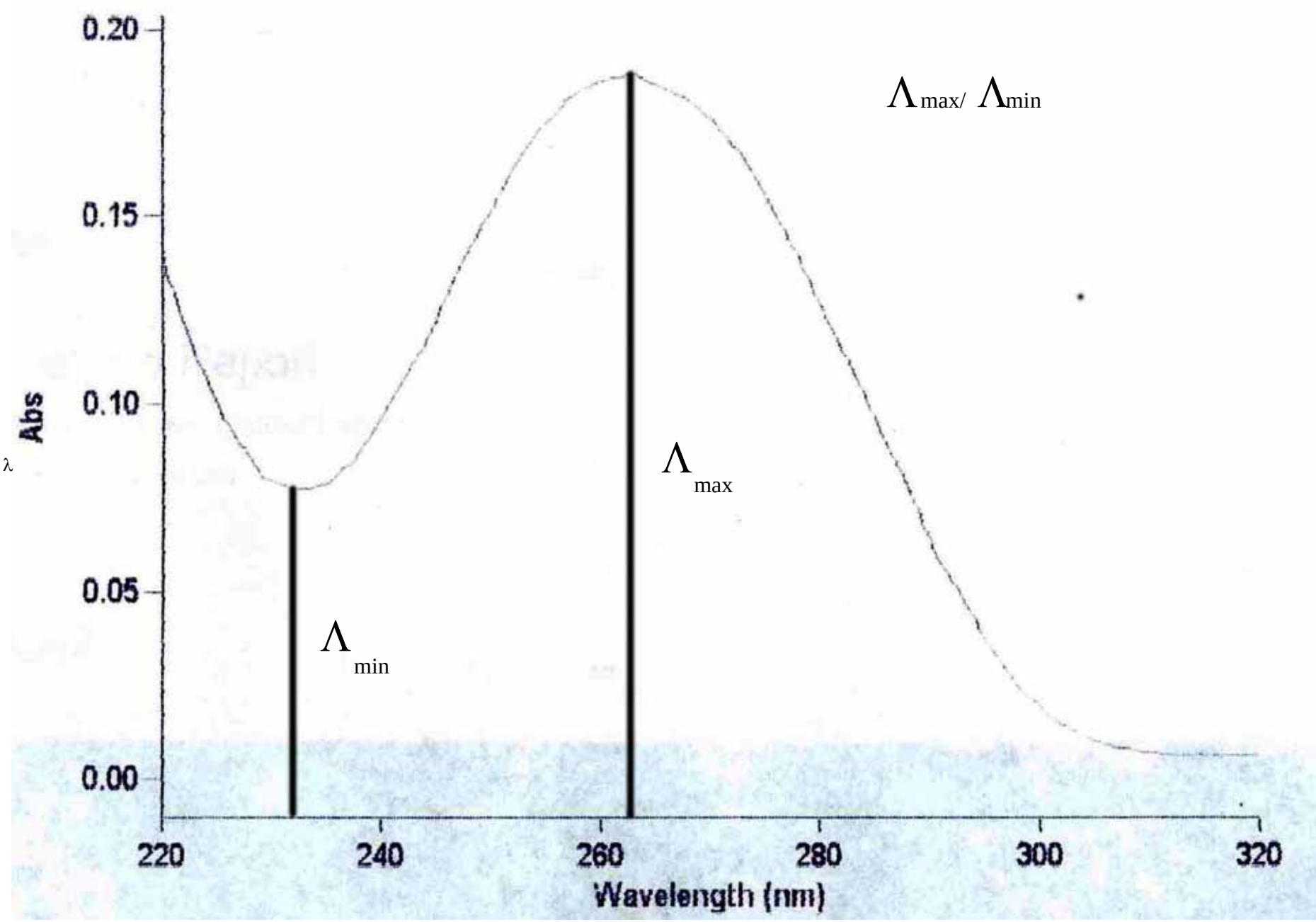

0.20
0.15
0.10
0.05
0.00
Abs
λ
220
240
260
280
300
320
Wavelength (nm)
Λmax/ Λmin
Λmax
Λmin

TABLE I

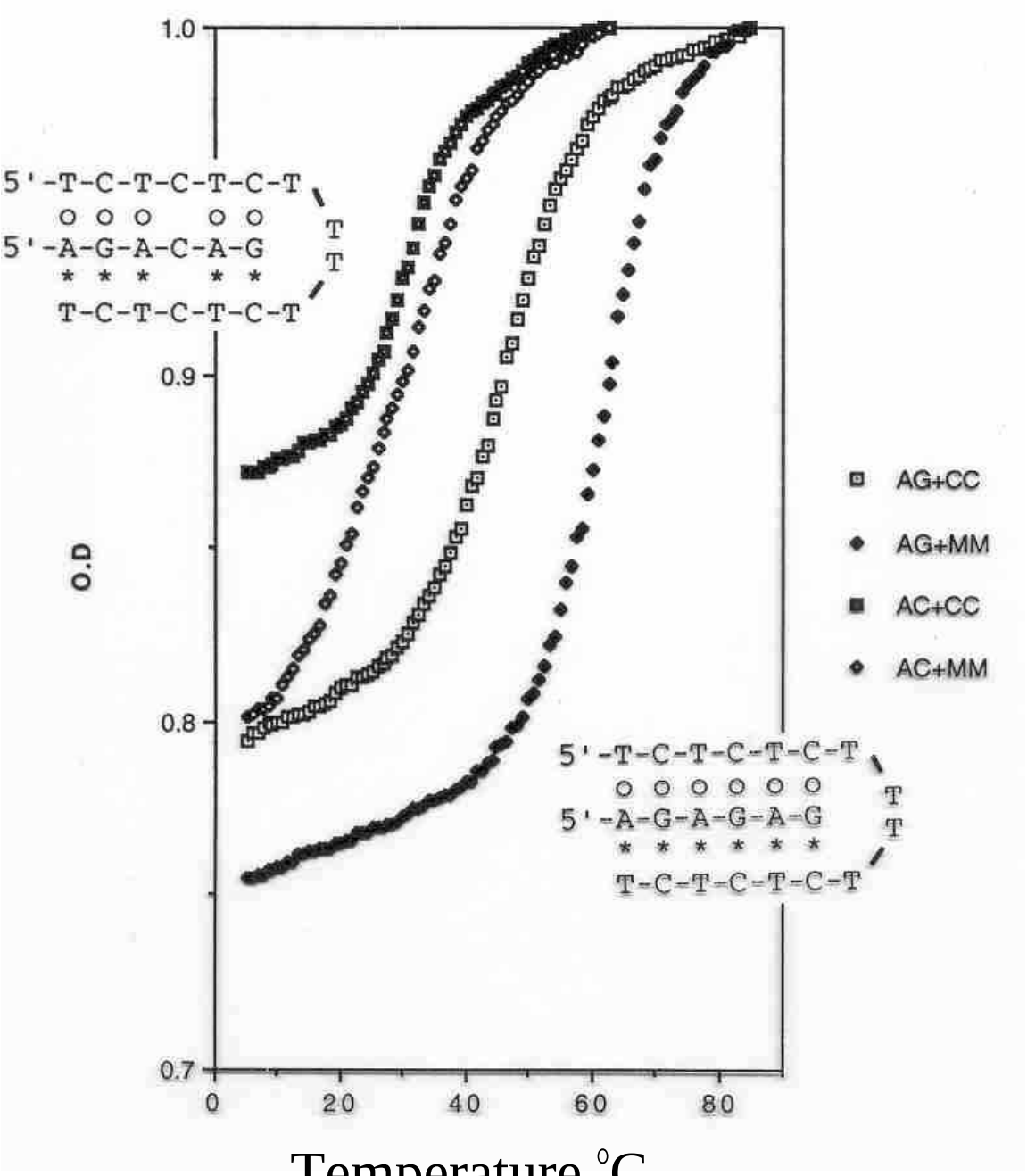
MOLAR EXTINCTION COEFFICIENTS OF NUCLEOTIDES AND DINUCLEOSIDE PHOSPHATES[a,b,c]

Phosphates	ε (260), M^{-1} cm^{-1} RNA	DNA
Monomer		
Ap	15,340	15,340
Cp	7,600	7,600
Gp	12,160	12,160
Up (dT)	10,210	8,700
Dimer		
ApA	13,650	13,650
ApC	10,670	10,670
ApG	12,790	12,790
ApU (ApT)	12,140	11,420
CpA	10,670	10,670
CpC	7,520	7,520
CpG	9,390	9,390
CpU (CpT)	8,370	7,660
GpA	12,920	12,920
GpC	9,190	9,190
GpG	11,430	11,430
GpU (GpT)	10,960	10,220
UpA (TpA)	12,520	11,780
UpC (TpC)	8,900	8,150
UpG (TpG)	10,400	9,700
UpU (TpT)	10,110	8,610

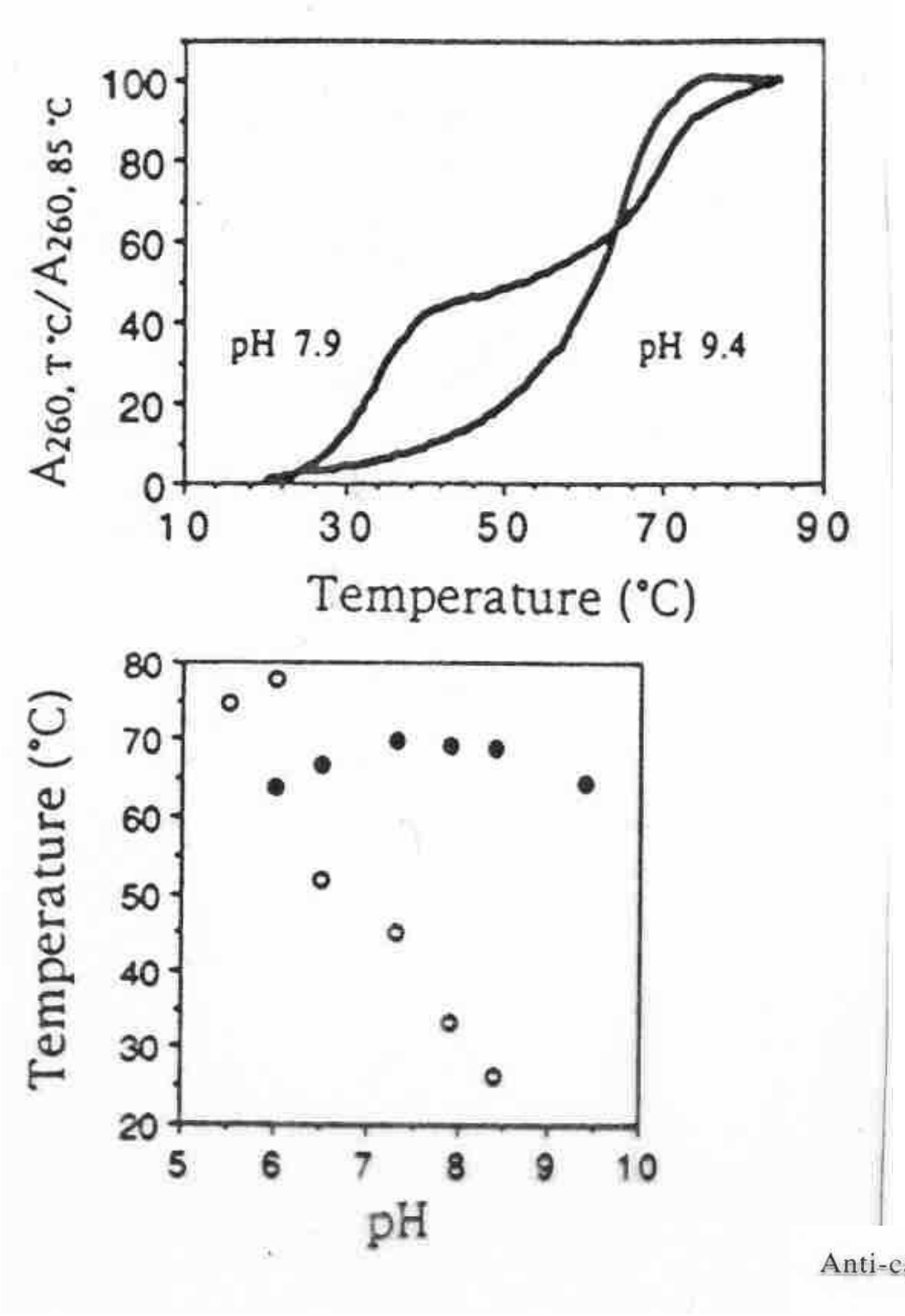
[a] At 260 nm. The values are needed to calculate the molar extinction coefficients of single-stranded DNA and RNA. All units are per molar concentration of *monomer*.

[b] Values were derived from the extinction coefficients of the 3′-monophosphate ribonucleotides (M. Alexis, Ph.D. Thesis, Univ. of London, 1978) at the wavelength maxima, absorption spectra of the 3′-monophosphates to convert peak values to those at 260 nm, and hypochromicity values for the RNA dimers (M. M. Warshaw, Ph.D. Thesis, Univ. of California, Berkeley, 1966). Monomer Ap, Cp, and Gp extinction coefficients and dimer hyperchromicity values were assumed to be the same for DNA and RNA; the extinction coefficient for dTMP was taken to be the same as that for dT [C. R. Cantor, M. M. Warshaw, and H. Shapiro, *Biopolymers* **9**, 1059 (1970)]. Values are for 0.1 *M* ionic strength, pH 7.0, 25°.

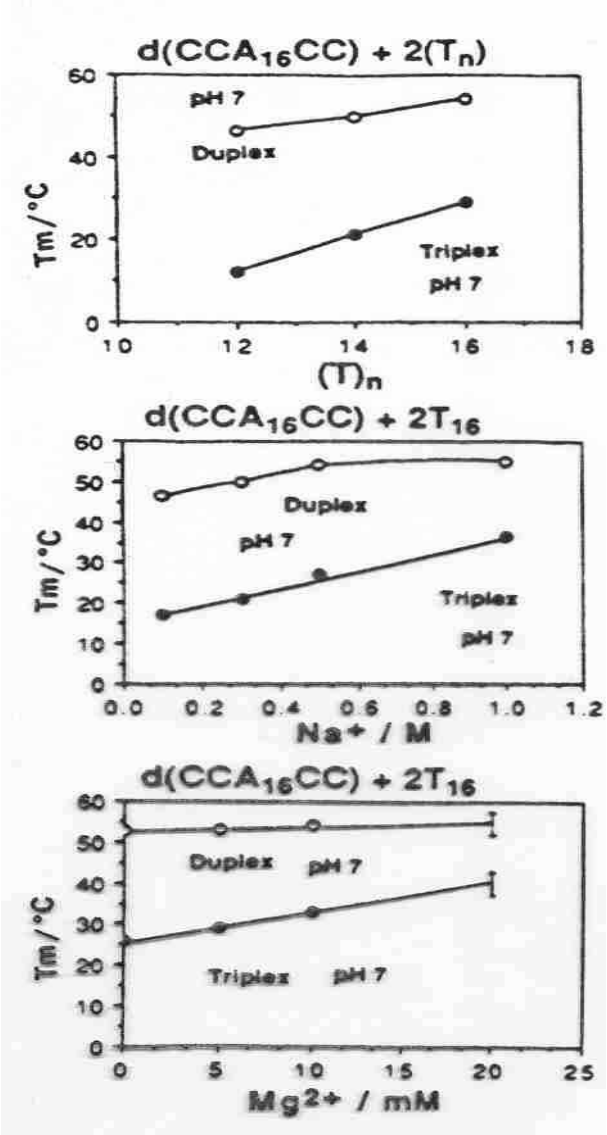
[c] Monomer and dimer extinction coefficients are estimated to be accurate to ±100 M^{-1} cm^{-1} and ±4%, respectively.



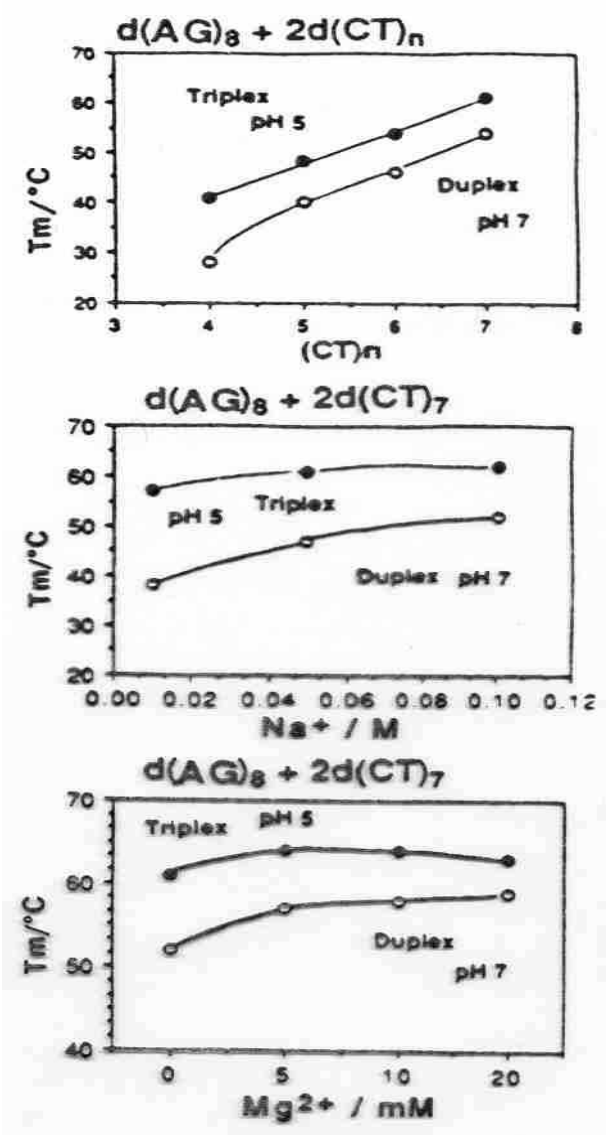
Chemistry (Taipei), A**54**, 24-32 (1996).

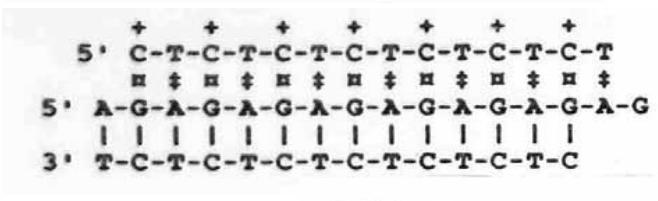


Anti-cancer Drug Design, 9, 1 (1994).


d(CCA16CC) + 2(Tn)
pH 7
Duplex
Triplex
pH 7
Tm/°C
(T)n
d(CCA16CC) + 2T16
Duplex
pH 7
Triplex
pH 7
Tm/°C
Na+ / M
d(CCA16CC) + 2T16
Duplex pH 7
Triplex pH 7
Tm/°C
Mg2+ / mM


5' T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T
5' C-C-A-A-A-A-A-A-A-A-A-A-A-A-A-A-A-C-C
3' T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T


d(AG)8 + 2d(CT)n
Triplex
pH 5
Duplex
pH 7
Tm/°C
(CT)n
d(AG)8 + 2d(CT)7
pH 5
Triplex
Duplex pH 7
Tm/°C
Na+ / M
d(AG)8 + 2d(CT)7
Triplex pH 5
Duplex
pH 7
Tm/°C
Mg2+ / mM


5' C-T-C-T-C-T-C-T-C-T-C-T-C-T
5' A-G-A-G-A-G-A-G-A-G-A-G-A-G-A-G
3' T-C-T-C-T-C-T-C-T-C-T-C-T-C

Conclusions:

1. The stabilities of triplexes is proportional to

   The chainlength of oligonucleotides;

   The concentration of NaCl;

   The concentration of $MgCl_2$.

2. The stability of C^+GC base triad is higher than TAT at pH 5.

3. Triplex is a unique molecular. It is not a complex of a single strand and a double strand DNA's.

Self-complementary $2A \xrightarrow{\text{------}} A_2$

$K = [A_2]/[A]^2 = \alpha/[2(1-\alpha)^2 C_T]$

Nonself-complementary $A + B \xrightarrow{\text{----}} AB$

$K = [AB]/[A][B] = 2\alpha/[(1-\alpha)^2 C_T]$

when $[A_o] = [B_o] = C_T/2$

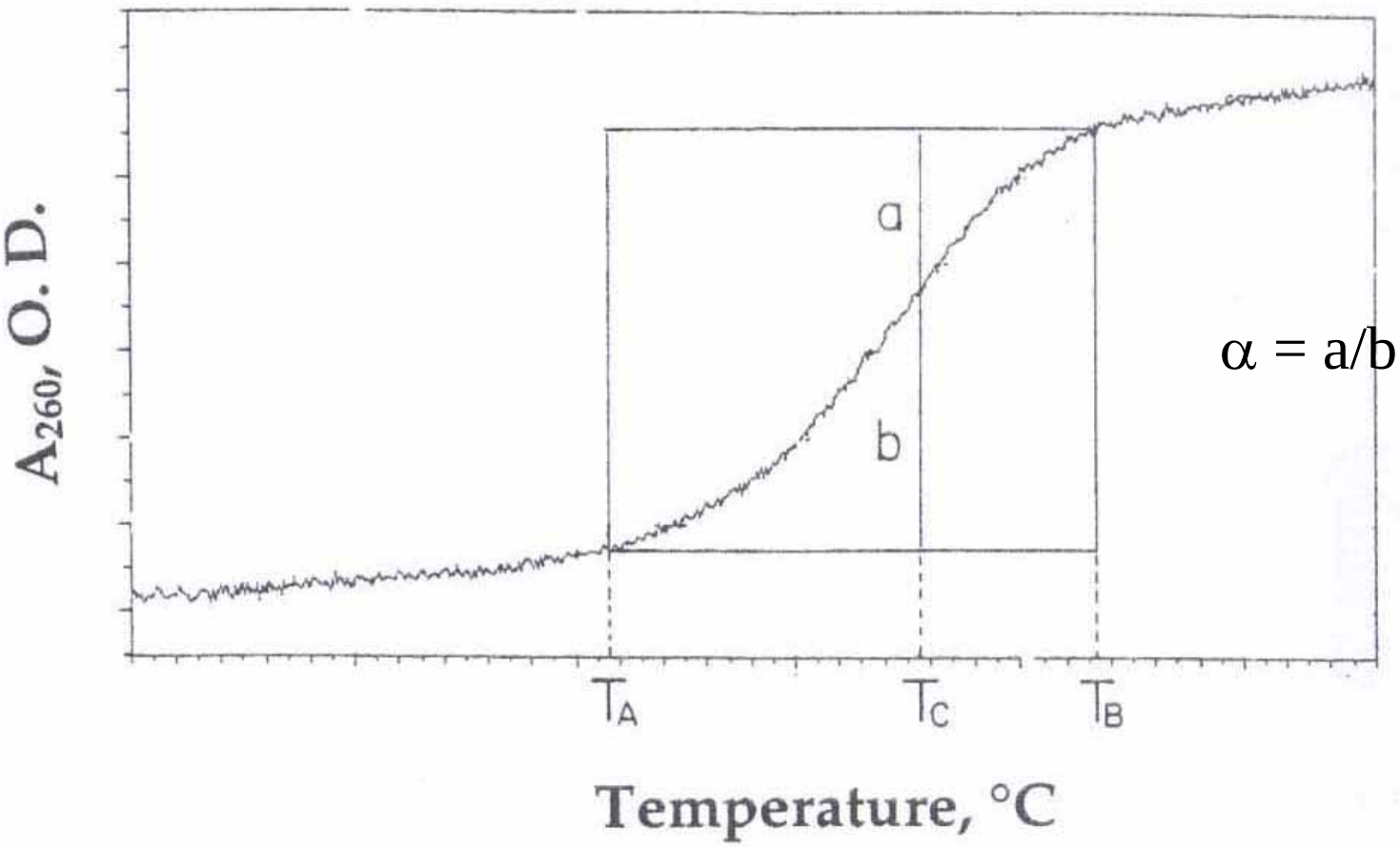


Figure 6. The analysis UV melting curve. T_A and T_B are temperatures of the beginning and ending of the dissociation. The quantities of a and b represent the fractions of unmelted and melted oligonucleotides base at T_C based on the none-or-all hypothesis. T_m is defined as the temperature where a = b.

Molecule†	T_m (°C) at‡ 10 μM	100 μM	Concn (μM) at T_m = 25°C	Total ΔH^0 (kcal/mol)	Total ΔG^0 (kcal/mol) at 25°C	Total ΔS^0 (kcal/mol deg.)
			Helix length = 6			
$A_4CG + CGU_4$	−13·9	−1·3	13500	−26	−3·4	−0·0758
U_2CGA_2	1·6	11.3	2040	−37	−3·7	−0·112
A_2CGU_2	10·8	22·1	174	−34	−5·1	−0·0969
$A_4G_2 + C_2U_4$	14·0	22·8	347	−44	−5·5	−0·129
A_2CGU_2	19·6	28·3	40·7	−46	−6·0	−0·134
			Helix length = 7			
$A_3CU_3 + A_3GU_3$	7·1	16·4	1510	−41	−4·7	−0·122
			Helix length = 8			
A_4U_4	5·3	11·5	4070	−51	−3·3	−0·160
$A_4CU_3 + A_3GU_4$	14·6	22·1	436	−52	−5·4	−0·156
A_3CGU_3	28·3	35·1	2·75	−66	−7·6	−0·196
A_3GCU_3	34·7	42·3	0·794	−53	−8·3	−0·150
			Helix length = 9			
$A_4CU_4 + A_4GU_4$	19·1	26·0	135	−58	−6·1	−0·174
			Helix length = 10			
A_5U_5	18·2	24·3	129	−67	−5·3	−0·207
A_4UAU_4	21·9	27·7	32·5	−72	−6·1	−0·221
$A_5CU_4 + A_4GU_5$	25·8	32·6	14·1	−63	−7·4	−0·187
A_4CGU_4	37·0	41·9	0·0977	−76	−9·6	−0·223
A_4GCU_4	40·7	46·8	0·0123	−78	−10·8	−0·225
			Helix length = 11			
$A_5CU_5 + A_5GU_5$	30·6	35·4	1·17	−89	−8·9	−0·269
			Helix length = 12			
A_6U_6	26·4	31·5	4·79	−84	−7·3	−0·257
			Helix length = 14			
A_7U_7	36·2	39·3	0·0219	−107	−10·4	−0·324

† None of the oligomers in this work has terminal phosphates and the phosphodiester linkage has been omitted. Thus A_nU_n is identical to $(Ap)_n(Up)$.
‡ The T_m values for the non self-complementary helices refer to concentrations of 20 μM and 200 μM for each oligomer. ΔH^0 is obtained $\Delta H^0 = R\,\mathrm{d}\ln c/\mathrm{d}(1/T_m)$. For self-complementary molecules $\Delta G^0 = RT_m \ln c$; for non self-complementary molecules $\Delta G^0 = RT_m \ln (c/4)$. c = the concentration in mol/l of the oligomers.

The nearest neighbor stacking effect

AA AC AG AT

CA CC CG CT

GA GC GG GT

TA TC TG TT

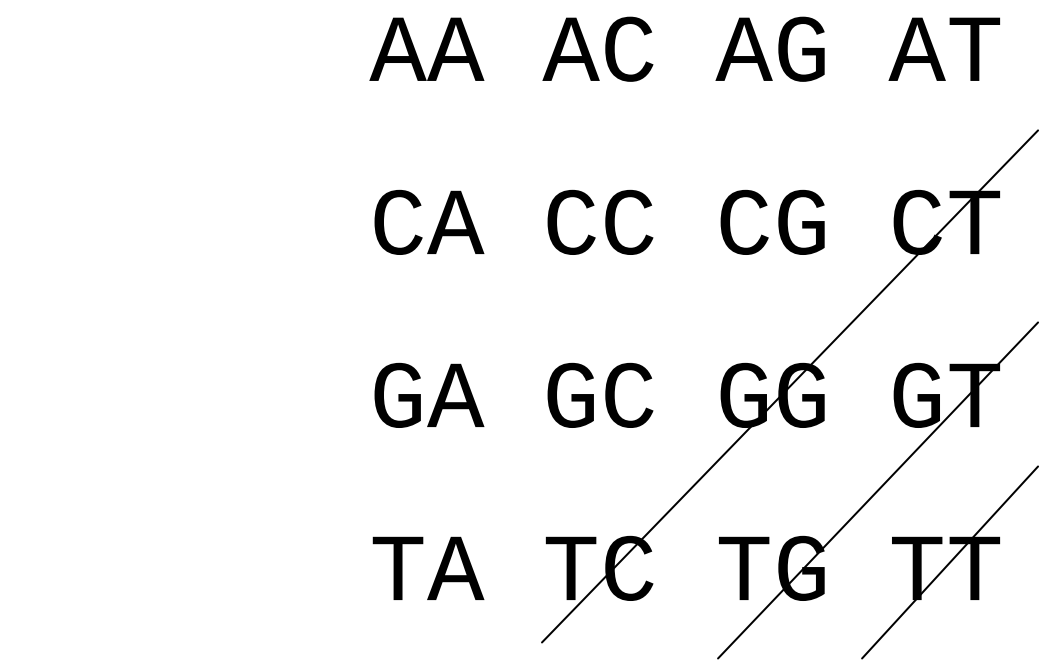
in RNA, U=T

ΔH = sum of the nearest neighbors ΔH

ΔH of AAGCUU = AA + AG + GC + CU + UU = 2 AA + 2 AG + GC

ΔH of UUCGAA = UU + UC + CG + GA + AA = 2 AA + 2 GA + CG

The nearest neighbor stacking effect



in RNA, U=T

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$A_3CU_3 + A_3GU_3$	7·1	16·4	1510	−41	−4·7	−0·122
			Helix length = 8			
A_4U_4	5·3	11·5	4070	−51	−3·3	−0·160
$A_4CU_3 + A_3GU_4$	14·6	22·1	436	−52	−5·4	−0·156
A_3CGU_3	28·3	35·1	2·75	−66	−7·6	−0·196
A_3GCU_3	34·7	42·3	0·794	−53	−8·3	−0·150
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$A_4CU_4 + A_4GU_4$	19·1	26·0	135	−58	−6·1	−0·174
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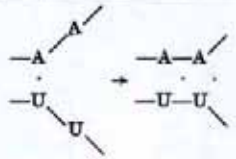
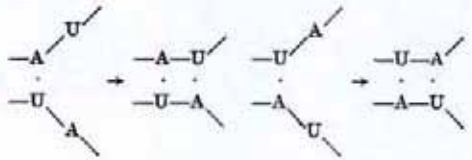
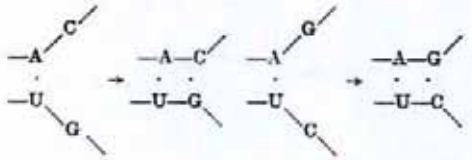
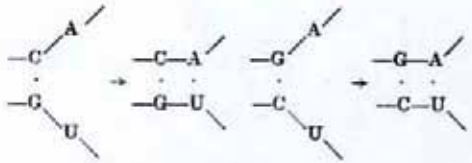
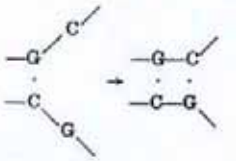
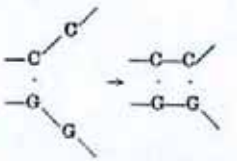
Thermodynamics of adding a base pair to a double-stranded helix

Reaction	ΔH (kcal)	ΔS (kcal/deg.)	ΔG (kcal) at 25°C
	−8·2	−0·0235	−1·2
	−6·5	−0·0164	−1·6
	−5·9	−0·0127	−2·1
	−13·0	−0·0335	−3·0
	−14·7	−0·0349	−4·3
	−13·7	−0·0298	−4·8

A value for the standard (strand concn equals 1 M) free energy of initiation at 25°C of +6·0 kcal for an A·U base pair and + 5·0 kcal for a G·C base pair is assumed.

Table 8.4
Thermodynamic Parameters for Helix Initiation and Propagation in 1*M* NaCl

Propagation Sequence	RNA[a] ΔH° (kcal mol^{-1})	RNA[a] ΔS° (eu)	RNA[a] ΔG°_{37} (kcal mol^{-1})	DNA[b] ΔH° (kcal mol^{-1})	DNA[b] ΔS° (eu)	DNA[b] ΔG°_{37} (kcal mol^{-1})	RNA/DNA[c] ΔH° (kcal mol^{-1})	RNA/DNA[c] ΔS° (eu)	RNA/DNA[c] ΔG°_{37} (kcal mol^{-1})
RNA → GC / CG ← DNA	−14.88	−36.9	−3.42	−9.8	−24.4	−2.24	−8.0	−17.1	−2.7
→ GG / CC ←	−13.39	−32.7	−3.26	−8.0	−19.9	−1.84	−12.8	−31.9	−2.9
							−9.3	−23.2	−2.1
→ CG / GC ←	−10.64	−26.7	−2.36	−10.6	−27.2	−2.17	−16.3	−47.1	−1.7
→ GA / CU ← → GA / CT ←	−12.44	−32.5	−2.35	−8.2	−22.2	−1.30	−5.5	−13.5	−1.3
							−8.6	−22.9	−1.5
→ GU / CA ← → GT / CA ←	−11.40	−29.5	−2.24	−8.4	−22.4	−1.44	−7.8	−21.6	−1.1
							−5.9	−12.3	−2.1
→ CA / GU ← → CA / GT ←	−10.44	−26.9	−2.11	−8.5	−22.7	−1.45	−9.0	−26.1	−0.9
							−10.4	−28.4	−1.6
GA ← GA ←									
							−9.1	−23.5	−1.8
→ UA / AU ← → TA / AT ←	−7.69	−20.5	−1.33	−7.2	−21.3	−0.58	−7.8	−23.2	−0.6
→ AU / UA ← → AT / TA ←	−9.38	−26.7	−1.10	−7.2	−20.4	−0.88	−8.3	−23.9	−0.9
→ AA / UU ← → AA / TT ←	−6.82	−19.0	−0.93	−7.9	−22.2	−1.00	−7.8	−21.9	−1.0
							−11.5	−36.4	−0.2
For Bimolecular Associations									
Initiation	3.61	−1.5	4.09	0.2	−5.6	1.96	1.9	−3.9	3.1
Each terminal AU or AT	3.72	10.5	0.45	2.2	6.9	0.05			
Symmetry correction (self-complementary)	0	−1.4	0.43	0	−1.4	0.43			
Symmetry correction (nonself-complementary)	0	0	0	0	0	0			

[a]Xia et al., (1998).

[b]Allawi and SantaLucia (1997).

[c]Sugimoto et al., (1995). For RNA/DNA hybrids with two sets of parameters, the top set corresponds to the top strand as RNA and the bottom set corresponds to the botto strand as RNA. For example, $\Delta H^\circ \begin{smallmatrix} 5'\text{rGG3}' \\ 3'\text{dCC5}' \end{smallmatrix} = -12.8$ kcal mol^{-1} and $\Delta H^\circ \begin{smallmatrix} 5'\text{dGG3}' \\ 3'\text{rCC5}' \end{smallmatrix} = -9.3$ kcal mol^{-1}. Alternative analyses of the RNA/DNA data base have also beer

Self-complementary $2A \text{ ------} \rightarrow A_2$

$K = [A_2]/[A]^2 = \alpha/[2(1- \alpha)^2 C_T]$

At Tm, $\alpha = ½$, $K = 1/C_T$

$-RTmlnK = \Delta G = \Delta H – Tm\Delta S = RTmlnC_T$

Nonself-complementary $A + B \text{ ----} \rightarrow AB$

$K = [AB]/[A][B] = 2\ \alpha\ /[(1- \alpha)^2 C_T]$

At Tm, $\alpha = ½$, $K = 4/C_T$

$-RTmlnK = \Delta G = \Delta H – Tm\Delta S = RTmlnC_T/4$

Calculated values of melting temperatures for double-stranded oligonucleotide helices

Molecule	Calculated T_m at 100 μM (°C)	Experimental T_m at 100 μM (°C)
A_3U_3	−8	−13
A_5G+CU_5	0	
$A_3GU_2+A_2CU_3$	5	
$A_3CUG+CAGU_3$	11	
$A_4CG+CGU_4$	14	
A_2CGU_2	19	22
A_2GCU_2	28	28
$A_2G_2U_2+A_2C_2U_2$	32	
ACGUCG+CGACGU	39	
ACGCGU	50	
GCGCGC	66	
G_3C_3	94	
G_6+C_6	99	

The T_m values for the non self-complementary helices refer to concentrations of 200 μM for each oligomer.

CTCTCTAGAGAG ----→ (CTCTCTAGAGAG)|

CT + TC + CT + TC + CT + TA + AG + GA + AG + GA + AG = 6AG + 4GA + TA

	ΔH	ΔS
AG	-7.8	-0.021
GA	-8.2	-0.0222
TA	-7.2	-0.0213
Total	-86.8	-0.236

$$Tm = (\Delta H/(R\ln C_T + \Delta S)$$

C_T	$R\ln C_T$	$R\ln C_T + \Delta S$	Tm (K)	Tm (C)
0.002	-12.4	-0.249	349	76
0.0002	-16.9	-0.253	243	70
0.00002	-21.5	-0.258	336	63
0.000002	-26.1	-0.262	331	58

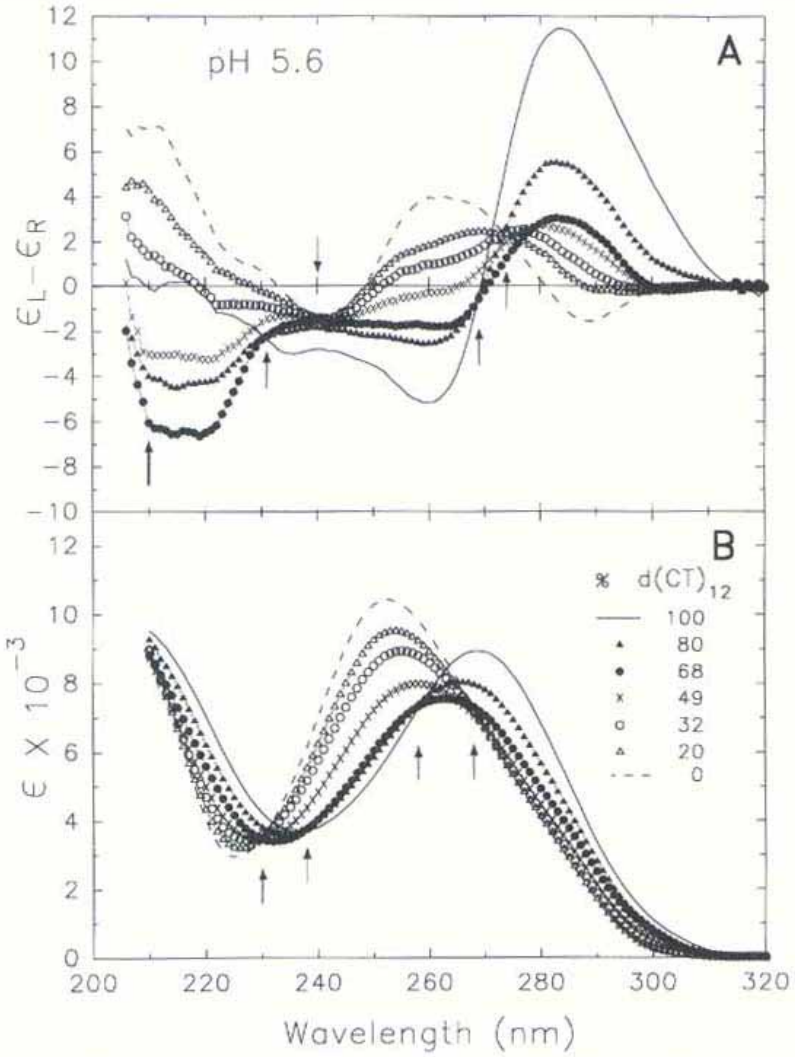


FIG. 4. (A) CD spectra and (B) absorption spectra of $d(AG)_{12}:d(CT)_{12}$ mixtures in the molar proportions of 0 : 100 (—), 20 : 80 (▲), 32 : 68 (●), 51 : 49 (×), 68 : 32 (○), 80 : 20 (△), and 100 : 0 (---). Solution conditions (50 m*M* sodium phosphate buffer, pH 5.6) were such that a $d(C^+T)_{12} \cdot d(AG)_{12} \cdot d(CT)_{12}$ triplex formed. The long arrow in (A) at 210 nm marks the maximum deviation in the CD spectrum of the triplex from the weighted average of the spectra of the individual strands. Short arrows in (A) and (B) indicate isodichroic points in the CD spectra and isoabsorptive points in the absorption spectra.

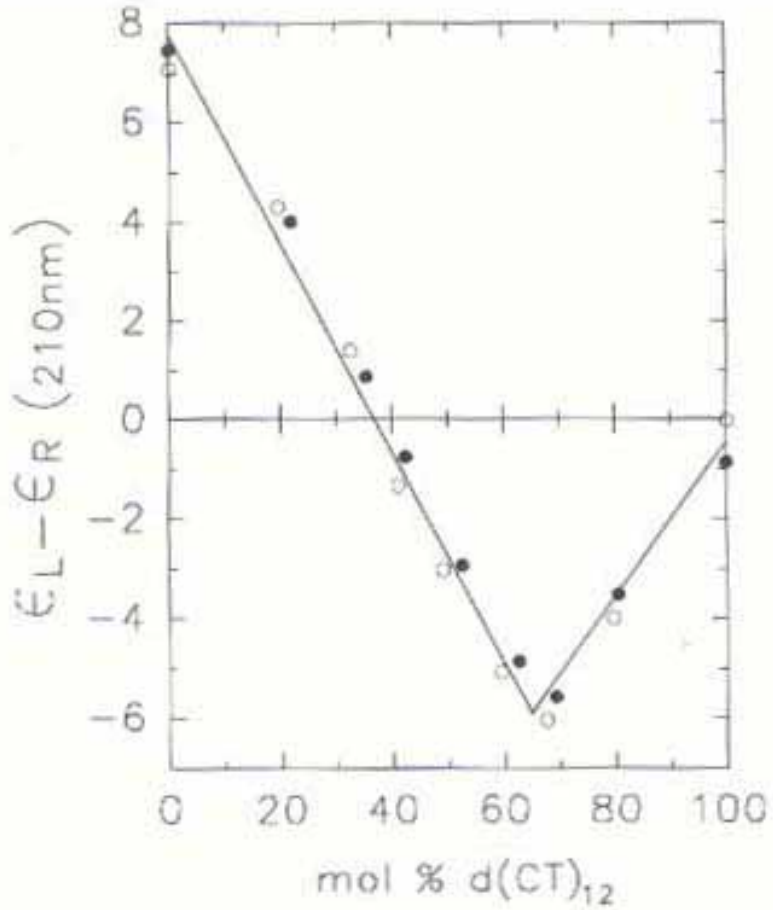


FIG. 5. CD mixing curve at 210 nm for mixtures of d(AG)$_{12}$ and d(CT)$_{12}$ under the same conditions used to obtain data for Fig. 4A. Data from Fig. 4A (○) were included plus data (●) from a duplicate experiment. The break point near 67 mol % of d(CT)$_{12}$ showed that a triplex was formed that contained two strands of d(CT)$_{12}$ and one strand of d(AG)$_{12}$.

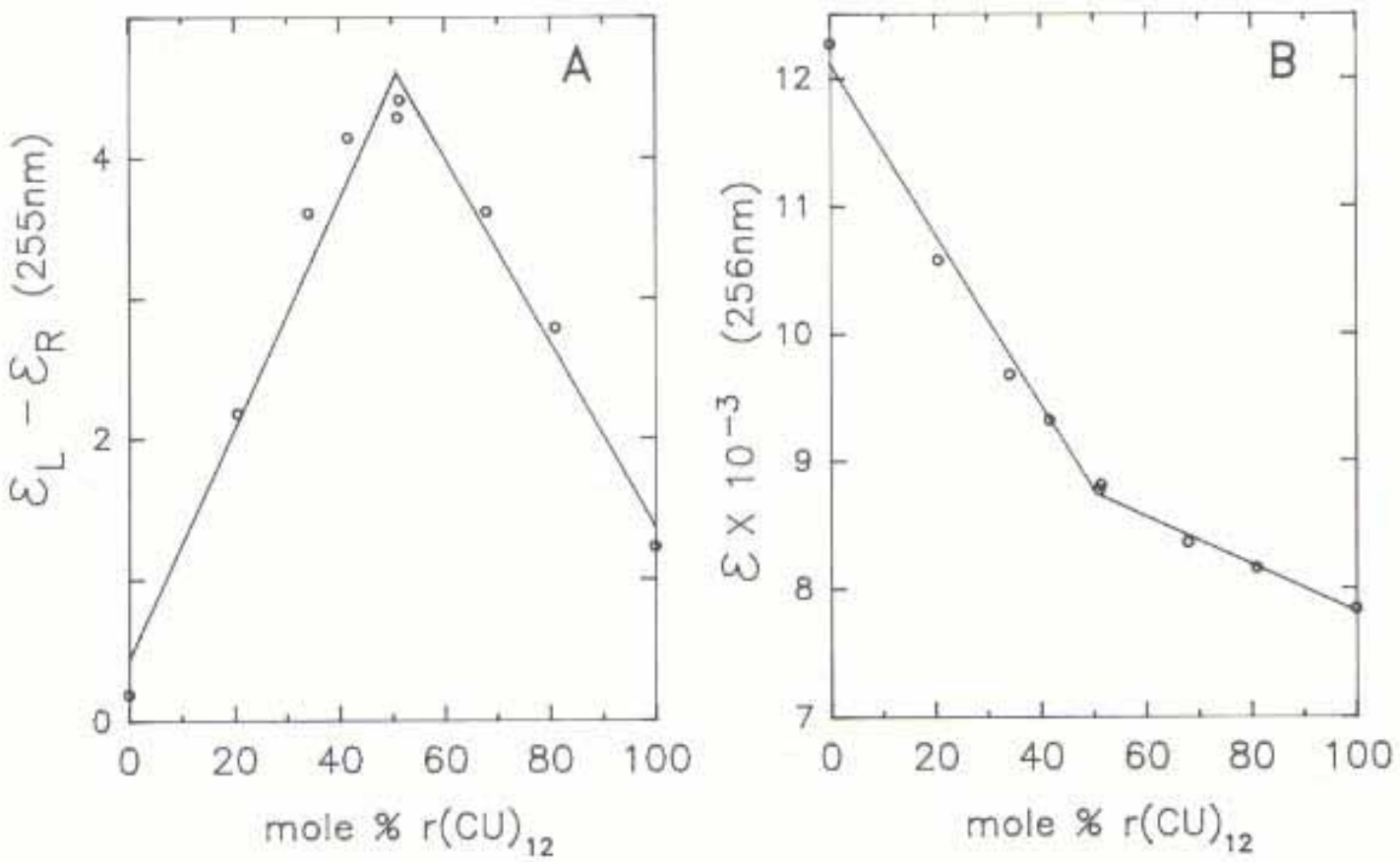


FIG. 3. (A) CD and (B) absorption mixing curves at 255 and 256 nm, respectively, for mixtures of r(AG)$_{12}$ and r(CU)$_{12}$. Data are plotted as a function of the mole percent of r(CU)$_{12}$. The mole percent of r(AG)$_{12}$ is 100 minus the percentage of r(CU)$_{12}$ in each mixture. Data were taken from Fig. 1, plus a mixture containing about 40 mol % of r(CU)$_{12}$ and an additional mixture with approximately 50 mol % of each oligomer. The break points at about 50 mol % of r(CU)$_{12}$ showed that a duplex was formed.

鏈沒有上述的困難，但它們乾燥時常以粉末狀存在，所以它含對應陽離子及結晶水的數目均爲不定，加之核酸具有強烈的吸水性，所以核酸(除少數核酸鹼基、單一核醣(nucleoside)單一核醣磷酸(nucleotide))不能以稱重的方法來定濃度。

以往是用滴定法，由於核酸含磷酸，磷酸的數目是一定的，或者乾脆以磷酸的濃度來表示核酸的濃度。它的步驟是將核酸分解，這時候磷酸根就釋出來了，再經氧化作用以保証所有的磷原子都以PO_4的離子形式存在。如此可以用鹼滴定之。這個方法要用強酸、高溫等激烈手段，危險性高，所需實驗時間較長，所用試料要多，而且最後是將它們破壞了，也不經濟。如果用紫外光譜法就簡單和經濟多了。

如果用紫外光譜來定核酸鏈濃度的原理只要看看附錄中的Beer氏定理就知道了。問題是我們不知道公式中ε值。所以還是要來諸以稱重法，好在單一核醣和單一核醣磷酸是可以稱得很準的。所以單一核醣和單一核醣磷酸的ε值可以附錄公式(1)中求得(表一)。有了這個基礎就可以求得核酸鏈的濃度，如果這一條核酸鏈有五個腺核酸鹼基，它的ε值應該是單一腺核酸鹼基的五倍。這是在鹼基之間沒有作用的假設下才能成立。但是大多數核酸鹼基之間相互之間或多或少有些作用，由於hypochromism(見下一段)之作用，核酸鏈的ε值要比各成份之總和要低。低多少沒有定論，要看核酸鏈中各單一核酸鹼基的比例而定。要克服這個困難也不難，方法就是將核酸鏈以酵素分解成單一核酸磷酸之後再量一次紫外光譜，此時ε的值就是各成份單一核酸鹼基之ε的總和了。所以（寡）核酸鏈的ε值可由下式求得。

$$\varepsilon_{\text{oligomer}} = A_{\text{oligomer}}\, \varepsilon_{\text{sum of monomer}} / A_{\text{sum of monomer}}$$

式中A是測出之吸收值，$\varepsilon_{\text{sum of monomer}}$可由表一中查得，所以（寡）核酸鏈的ε值就可以計算出來了。

方法：1. 將A（在260nm）=0.5 O. D.左右的寡核酸鏈溶在1 mL緩衝溶劑中。

（緩衝溶劑：20 mM Tris, pH 8.2, 10 mM $MgCl_2$）

2. 將溶液轉入一公分光徑分光器中量其紫外光譜，記下在260nm的吸收值。
3. 加入三微克的snake venom phosphodiesterase I。

（要領：加入容積要在10微升以下，則對溶液的體積的改變可忽略不計。）

4. 將溶液加熱至攝氏37度，放置二小時。
5. 重複步驟一。

（要領：可在分光器中行之。）

6. 步驟一至五至少做三次，取平均值。

每做一種新的寡核酸鏈就得將上述的步驟做一次。實驗所需的寡核酸鏈非常多，它們有不同的長短和鹼基順序。所以不可能把所有的ε都收在一張報表之內，實驗去查查就行

了。但是做實驗終究很麻煩，而且不是每間實驗室具有做酵素反應的設備與技能，那又該怎麼辦？那麼計算法是一個答案。

計算法有一個假設。它就是只算最近一個核酸鹼基的作用，隔一個作用就是零。所以我們可以把一寡核酸鏈依鹼基順序分成許多雙核酸磷酸(dinucleotide)。這方法得以實施是除了知道單一核酸磷酸的ε值外還要知道雙核酸磷酸的ε值，核酸鹼基有四種，所以一共有十六種雙核酸磷酸，這數字不算大所以它們的ε值可由其他方法求出（見表一）。有了表一之後我們可以以一個實例來說明計算法。

設想有一寡去氧核酸鏈TCTCTCCTCTCTAGAGAG (L)，它有十八個鹼基。可以把它拆成TC, CT, TC, CT, TC, CC, CT, TC, CT, TC, CT, TA, AG, GA, AG, GA, AG等十七個雙核酸磷酸。歸納一下是TC和CT各有五個，三個AG，二個GA，而CC和TA卻各有一個。那麼寡去氧核酸鏈L的ε值本應是這十七個雙核酸磷酸的ε值之和。可是除了L首尾兩個鹼基之外，其他都重複了一次，所以要把這些單一核酸磷酸（共有六個C，五個T，三個A及二個G）之ε值減去就得到了整個L之ε值。以下式表之。

$$\varepsilon_L = 5\varepsilon_{TC} + 5\varepsilon_{CT} + 3\varepsilon_{AG} + 2\varepsilon_{GA} + \varepsilon_{CC} + \varepsilon_{TA} - 3\varepsilon_A - 2\varepsilon_G - 6\varepsilon_C - 5\varepsilon_T$$

核酸鏈的ε值也可以以平均值表之，只要將前述之值除以鹼基之總數（在此例是十八）即可。

$$\varepsilon_{L'} = (1/18)\varepsilon_L$$

有了L的ε值，就可以以A值由的Beer氏定理來求它的濃度。單位是莫耳。

五、混合滴定曲線

這是用來測定形成結構各成份之間的當量比，通常核酸結構之形成須兩條或兩條以上核酸鏈。爲了免除有多餘的成份，它們的組成當量比是必須知曉的。

用紫外光譜來測這當量比是相當方便的。它是利用核酸的hypochromism。也就是混合物中有正確的當量比時所呈現的吸收爲最小值。舉一例來說明。假設寡核酸鏈A和B可形成核酸雙螺旋，所以它們正確的當量濃度比是一比一。如果以不同之濃度混合時其各種成份如下。

混合之前		混合之後		
%A	%B	%A	%B	%AB
100	0	100	0	0
90	10	80	0	10
80	20	60	0	20
70	30	40	0	30
60	40	20	0	40
50	50	0	0	50

九、紫外光譜的缺點

1. 不能觀察沒有紫外吸收的化合物。
2. 解析度低，通常全部光譜才一個峰，使理論工作印証十分困難，
3. 只能得到宏觀的結果。

十、附錄

光譜學是研究電磁波和原子、分子交互作用，這些作用包括吸收、發射和散射光子是和分子或原子的性質與構造有密切關係。電磁波的範圍很廣，由波長極短的x-光到調幅無線電波。不過它們都有下列之通性。

波長(λ) x 頻率(f) = 光速 (C)

電磁攜帶的能量是和頻率成正比。

能量(E) = 常數(h) x f

所以波長愈短的波能量愈大。另外一個單位，波數(wave number)也爲我們所愛用。

波數(σ) = 1/λ

所以σ愈大者，能量也愈大。

當電磁波通過一介質時結果有三：通過、吸收和折射。如果通過則介質毫不起作用所以我們不感興趣，折射則不在此章討論。電磁波被吸收量是和入射光成比例，和介質的厚度與濃度成正比。

$-dI/I_o = \varepsilon' \times C \times dl$

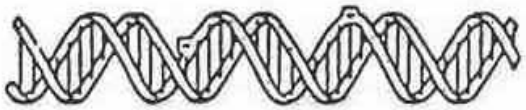
I是光（電磁波）的強度，l是介質長度，負號是減少之意。

積分之後是 $\ln(I_o/I) = \varepsilon' Cl$

如果將自然對數換成以十爲底之後就成了有名的Beer氏定律。

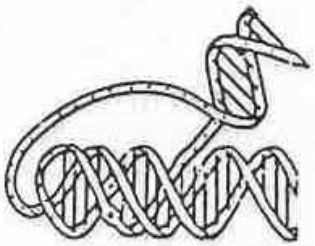
吸收質(A) = $\log(I_o/I) = \varepsilon Cl$

紫外光譜是電磁波一小部份，常用的波長爲350到200十億分之一米(nm)。但有時會觀察到約170 nm。



UV
---------->
stoichiometric ratio
stability
secondary structure
thermodynamic properties

CD
---------->
secondary structure
stability



IR
---------->
local configuration

gel electrophoresis
---------->
formation
association constant

NMR

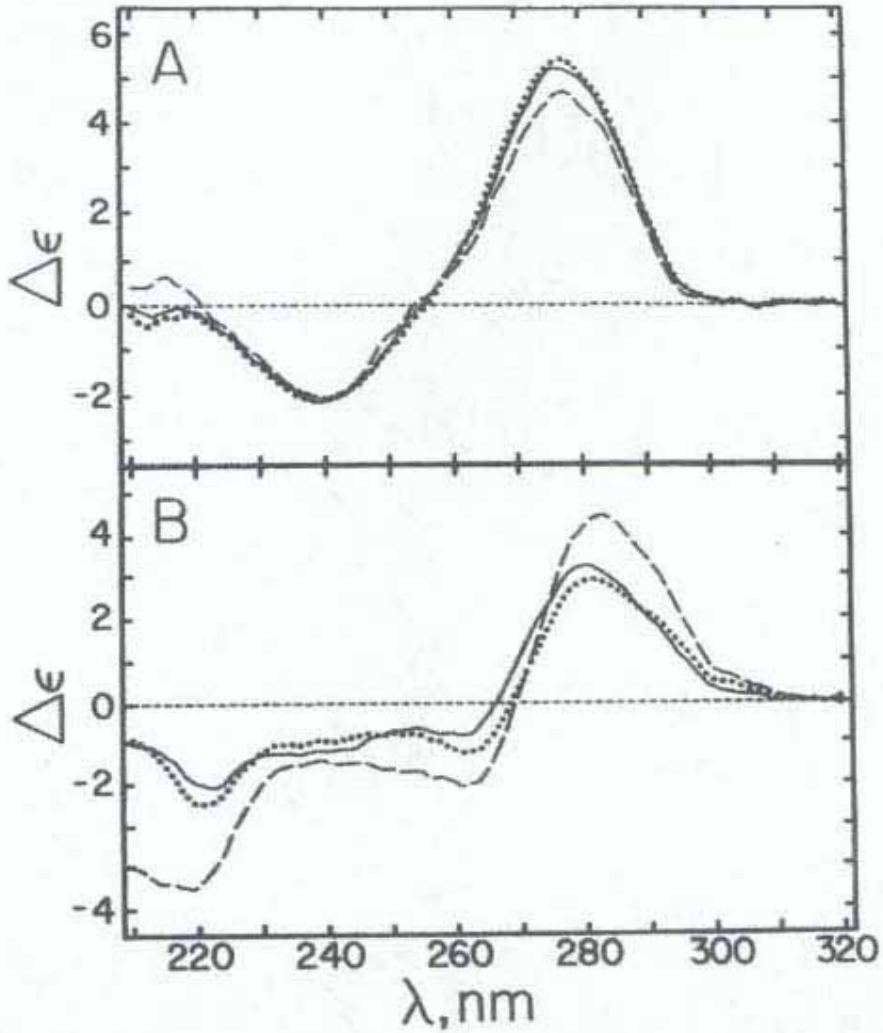
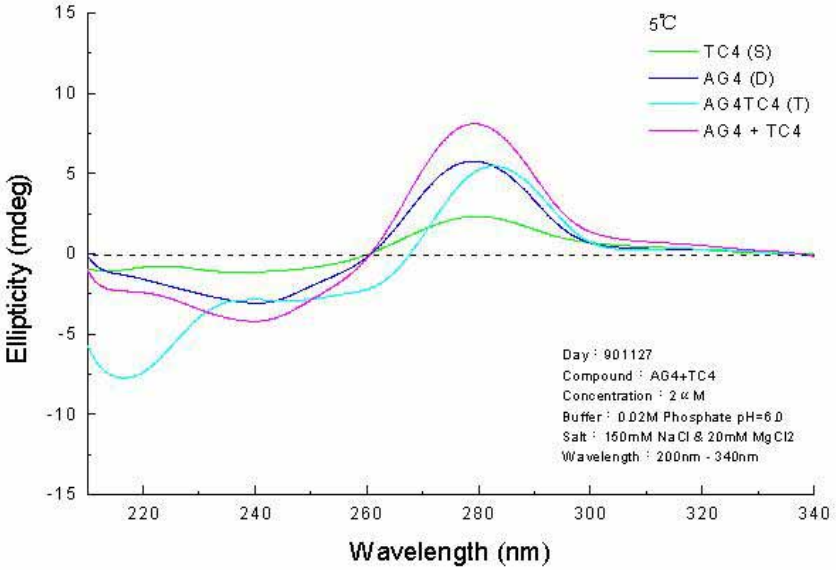
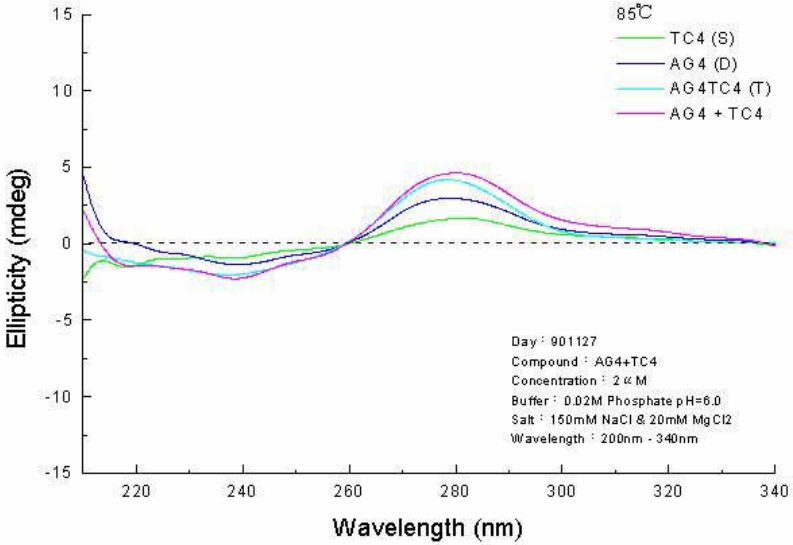
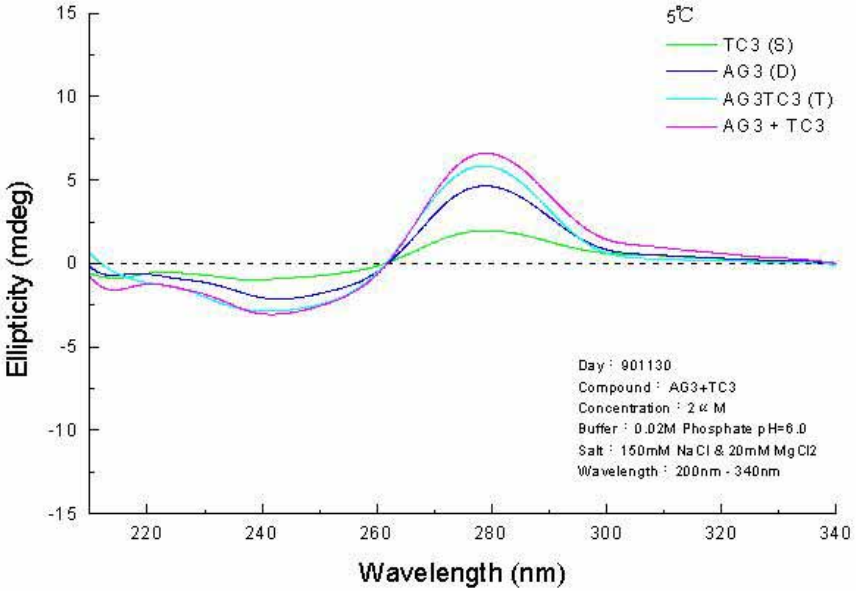


FIGURE 5: Comparison of calculated and observed CD spectra for 2:1 and 1:1 $d(CT)_8$:$d(AG)_8$. (A) 1:1 experimental spectrum at pH 7.0 (—); 2:1 experimental spectrum at pH 7.0 (– – –); spectrum calculated by taking the weighted average of spectra of single-stranded $d(CT)_8$ and duplex, both at pH 7.0 (···). (B) 1:1 experimental spectrum at pH 5.5 (—); 2:1 experimental spectrum at pH 5.5 (– – –); spectrum calculated by taking the weighted average of spectra of single-stranded $d(AG)_8$ and triplex, both at pH 5.5 (···).


5℃
TC4 (S)
AG4 (D)
AG4TC4 (T)
AG4 + TC4
Day : 901127
Compound : AG4+TC4
Concentration : 2 μM
Buffer : 0.02M Phosphate pH=6.0
Salt : 150mM NaCl & 20mM MgCl2
Wavelength : 200nm - 340nm
Ellipticity (mdeg)
Wavelength (nm)


85℃
TC4 (S)
AG4 (D)
AG4TC4 (T)
AG4 + TC4
Day : 901127
Compound : AG4+TC4
Concentration : 2 μM
Buffer : 0.02M Phosphate pH=6.0
Salt : 150mM NaCl & 20mM MgCl2
Wavelength : 200nm - 340nm
Ellipticity (mdeg)
Wavelength (nm)


5℃
TC3 (S)
AG3 (D)
AG3TC3 (T)
AG3 + TC3
Day : 901130
Compound : AG3+TC3
Concentration : 2 μM
Buffer : 0.02M Phosphate pH=6.0
Salt : 150mM NaCl & 20mM MgCl2
Wavelength : 200nm - 340nm
Ellipticity (mdeg)
Wavelength (nm)

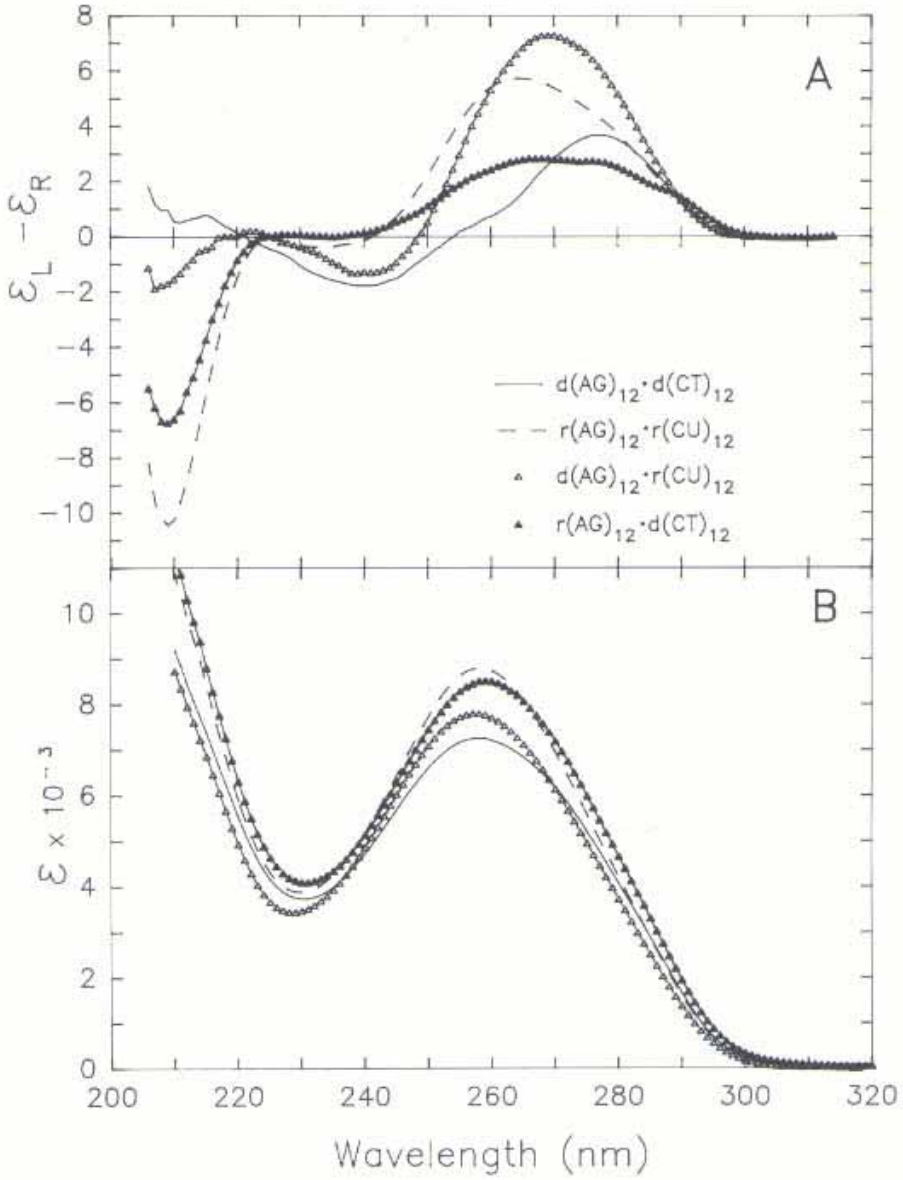


FIG. 6. (A) CD and (B) absorption spectra of four oligomer duplexes, the DNA–DNA duplex $d(AG)_{12} \cdot d(CT)_{12}$ (—), the RNA–RNA duplex $r(AG)_{12} \cdot r(CU)_{12}$ (– – –), and the two analogous hybrid duplexes $d(AG)_{12} \cdot r(CU)_{12}$ (△) and $r(AG)_{12} \cdot d(CT)_{12}$ (▲).

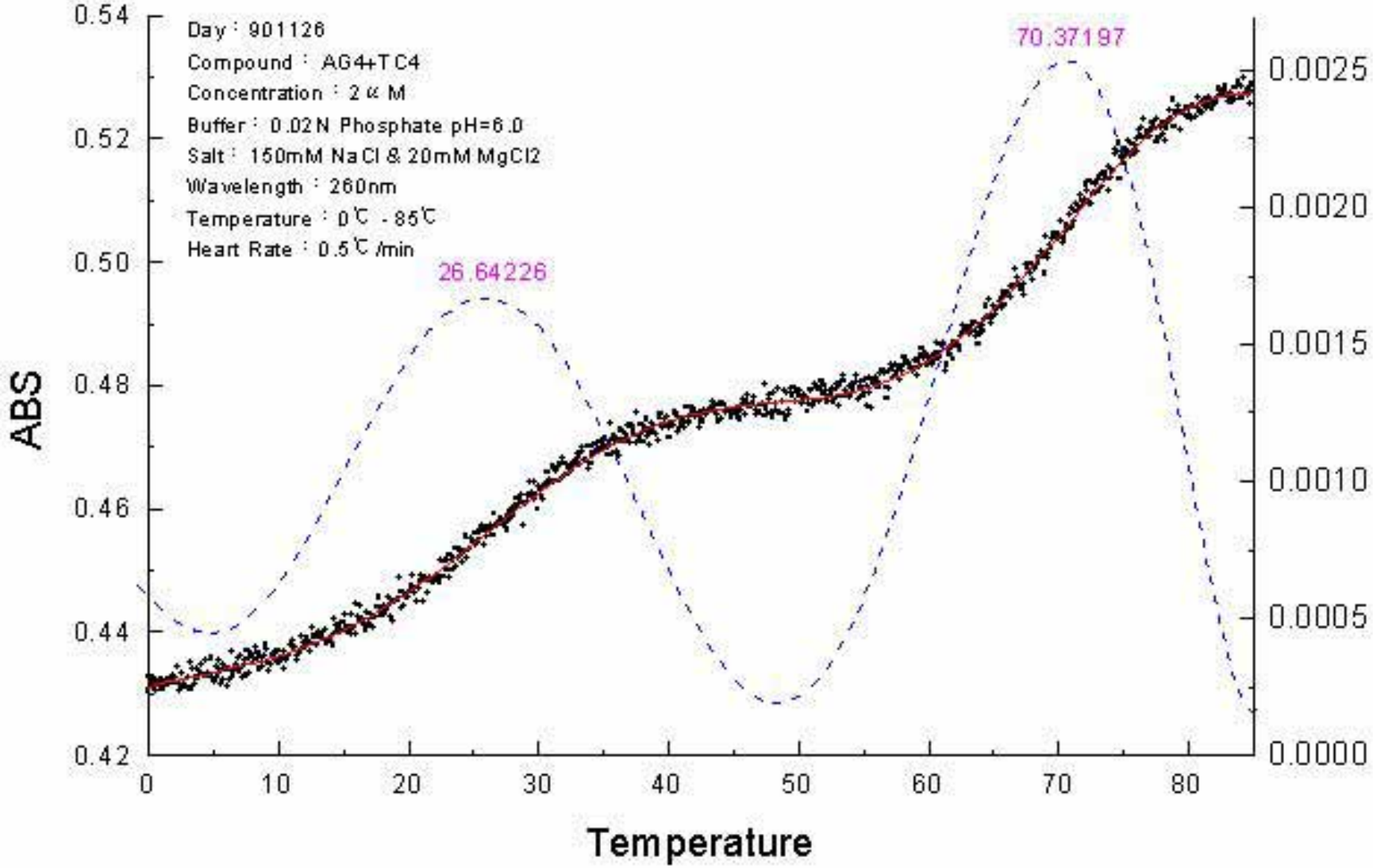

Day：901126
Compound：AG4+TC4
Concentration：2 μ M
Buffer：0.02N Phosphate pH=6.0
Salt：150mM NaCl & 20mM MgCl2
Wavelength：260nm
Temperature：0℃ - 85℃
Heart Rate：0.5℃ /min
26.64226
70.37197
ABS
Temperature
0.54
0.52
0.50
0.48
0.46
0.44
0.42
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
0
10
20
30
40
50
60
70
80

Figure4. Plot of the CD spectra intensity at 217nm and 240nm against temperature for TCA,CAC and ACT at pH= 6.5

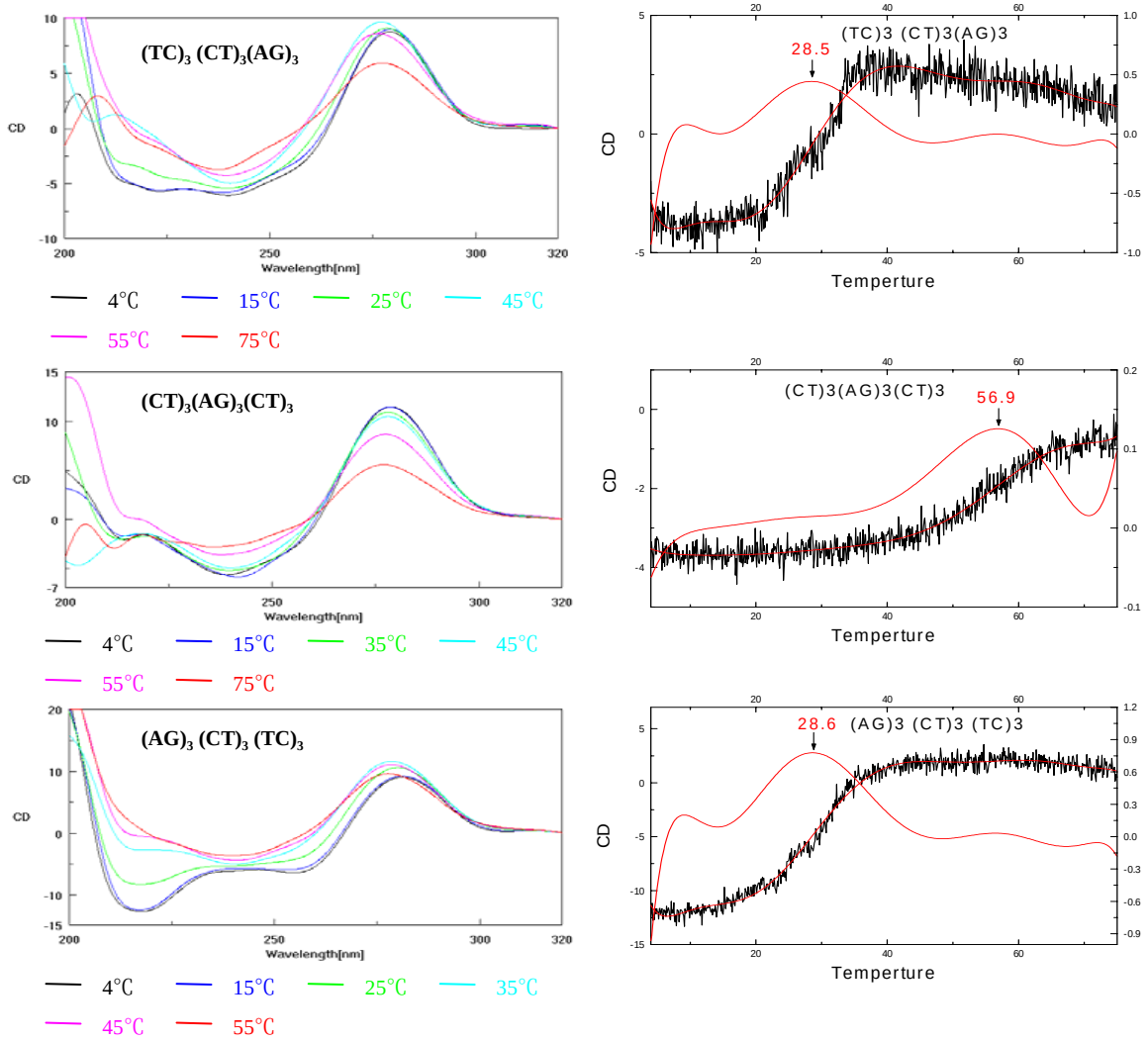


Figure5. Plot of the CD spectra intensity at 217nm and 240nm against temperature for TCA,CAC and ACT at pH= 7.5

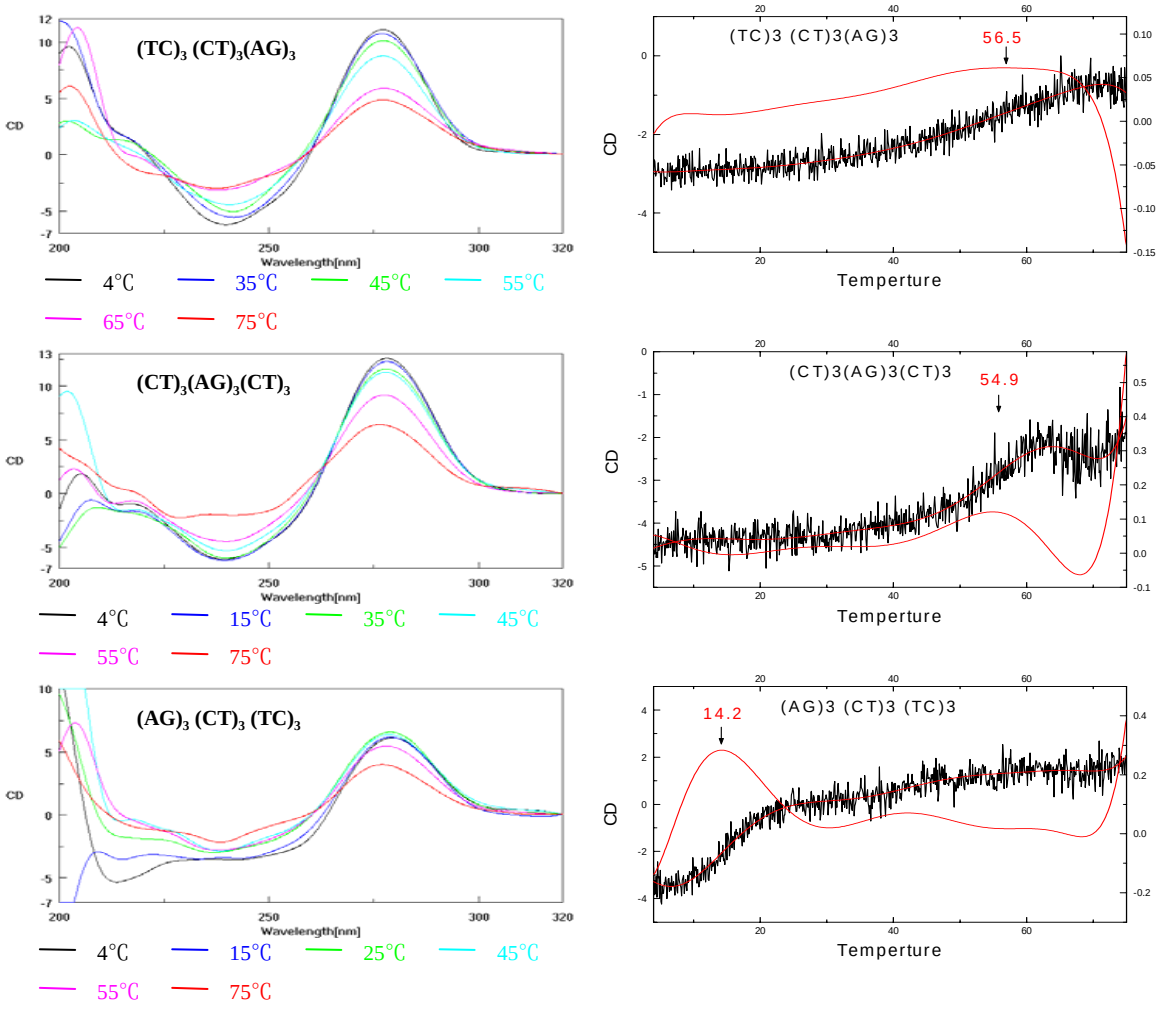


Table3.The melting temperture (ºC) of the six oligonulectides with CD spectra at 217nm and 240nm as a function of pH

217nm/240nm	pH=4	pH=5	pH=6	pH=(6.5)	pH=7	pH=(7.5)	pH=8
(TC)3(CT)3(AG)3	66.9	64.6	48.3	28.5	50.2	56.5	48.8
(CT)3(AG)3(CT)3	54.9	55.6	45.8	56.9	50.6	54.9	53.7
(AG)3(CT)3(TC)3	57.9	68.5	51.3	28.6	15.5 59.7	14.2 60.3	56.8
(CT)3(TC)3(GA)3	62.8	69.4	57.1	38.6	25.2 49.3	13.9 50.8	45.8
(TC)3(GA)3(TC)3	47.1	49.2	54.9	51.8	49.5	50.2	48.5
(GA)3(TC)3(CT)3	71.7	60.2	51.1	29.9	13.4 55.9	51.9	49.8

Tsay et al. J. Chin. Chem. Soc. 42, 493-498 (1995)

Table 1. The Sequence of the C-Terminal Fragment (10 kDa) of HSC70 from Rat and Deduced Secondary Structures According to the CF[16] and GOR[17] Methods

residue #	1	11	21	31	41	
	AFNMKATVED	EKLGGKINDE	DKQKILDKCN	EIISWLDKNQ	TAEKEEFEHQ	
CF	HHHHHHHHHH	HHHTT...HH	HHHHHHTT.	.BBBBB.TTH	HHHHHHHHHH	
GOR	HHHHHHHHHH	HHHH.HHHHH	HHHHHHHTT	TTTTT.....	.HHHHHHHHH	
residue #	51	61	71	81	91	101
	QKELEKVCNP	IITKLYQSAG	GMPGGMPGGF	PGGGAPPSGG	ASSGPTIEEV	D
CF	HHHHHHHH.B	BBBBBBB.TT	..TTT.TTT.	TTTT..TTTT	TTTTHHHHHH	H
GOR	HHHHHHH.BB	BBBBBBB.TT	.....	.TT....TTT	.....TT	.

secondary structure	fraction of total/%	
	CF	GOR
α helix	47.5	42.5
β sheet	12.9	8.9
turns	25.7	15.8
unordered	13.9	32.7

H, B, T, and represent α helix, β sheet, turn, and disordered structures, respectively. The proportion of each fraction is listed in the lower part. Amino acid residues are specified according to one-letter symbols. Identification #1 corresponds to amino acid 546 and #101 is the C-terminus of hsp70.

Table 2. Predicted Secondary Structures of the C-Terminal Fragment (10 kDa) of HSC70 from the Self-consistent Analysis of CD Spectra

		Secondary Structures, %				
pH	Na^+, M.	α helix	β sheet	turn	disordered	total[a]
4	0.055	24.8	36.4	24.3	13.6	102.0
	0.130	30.1	24.2	27.0	19.1	100.0
	0.180	32.5	17.5	24.8	23.7	98.5
5	0.055	32.6	15.8	22.7	28.4	99.5
	0.130	32.5	19.1	26.1	23.2	100.9
	0.180	32.6	18.3	25.8	23.6	100.3
6	0.055	32.4	17.2	24.4	23.6	97.6
	0.130	33.7	18.8	24.5	23.8	100.8
	0.180	34.3	17.0	25.3	23.4	100.0
7	0.055	30.8	19.2	25.5	24.9	100.4
	0.130	32.1	18.9	25.6	23.9	100.5
	0.180	38.1	13.4	24.2	23.9	99.5
8	0.055	33.3	20.5	23.8	22.9	100.5
	0.130	32.8	19.5	26.0	22.6	100.9
	0.180	33.7	17.6	23.8	24.7	99.8
9	0.055	32.7	18.5	24.3	24.9	100.4
	0.130	33.2	18.7	24.4	24.0	100.3
	0.180	34.3	16.7	24.7	24.5	100.2

[a] ± 2.5% is allowed to save computing time to attain convergence.

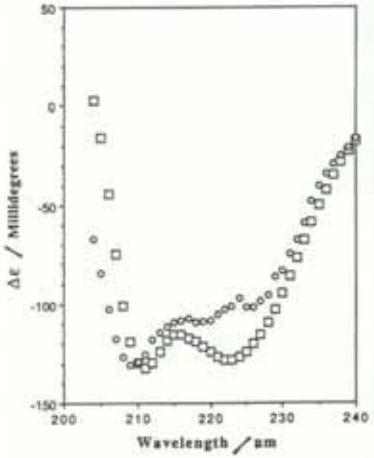


Fig. 1. CD spectrum of the C-terminal fragment (10 kDa) of hsc70 in solution at [Na^+] 0.055 M, pH 6 and 20 °C. The observed spectrum (with square symbol) was reconstructed with a 1 nm interval from 240 to 204 nm. The calculated spectrum (with circle symbol) in the same wavelength region was done according to the method of Yang and Doty in reference 14.

Tsay et al. J. Chin. Chem. Soc. 42 493-498 (1995)

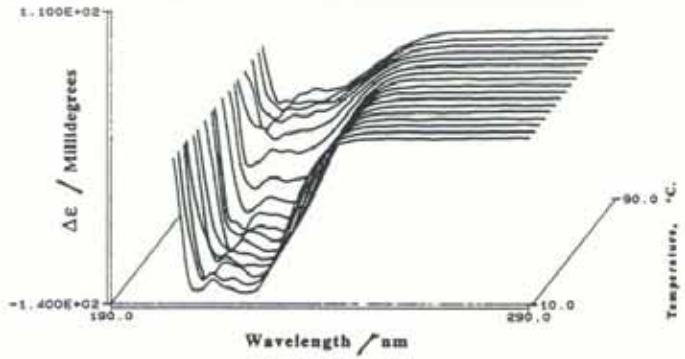


Fig. 2. CD profiles of the C-terminal fragment (10 kDa) of hsc70 in solution at [Na^+] 0.055 M and pH 6 as a function of temperature. The melting temperature experiment started at 10 °C (at the front) and finished at 90 °C with interval 5 °C. In total 17 CD traces appear in the figure.

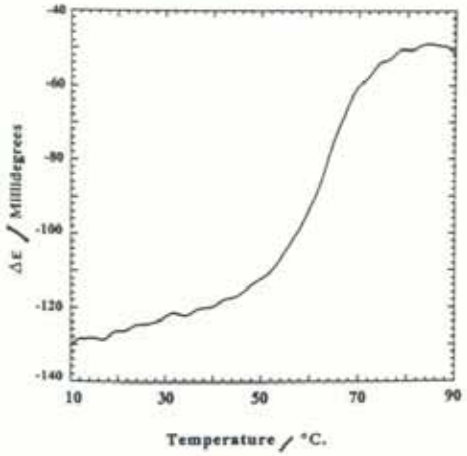
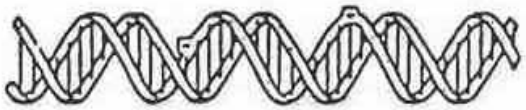


Fig. 3. Plot of Δε at 220 nm in Fig. 2 versus temperature.

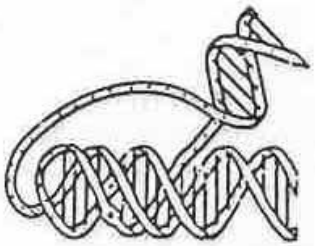
Melting Temperature T_m of the C-Terminal Fragment (10 kDa) of HSC70 at pH 4-9 and Varied Concentrations of Salt

[Na^+]/M pH	0.055 T_m/°C	0.13 T_m/°C	0.18 T_m/°C
4	48.0	47.0	48.0
5	59.0	57.0	58.0
6	63.0	62.0	62.0
7	63.0	62.0	61.0
8	60.0	59.0	58.0
9	55.0	52.0	49.0



UV
---------->
stoichiometric ratio
stability
secondary structure
thermodynamic properties

CD
---------->
secondary structure
stability



IR
---------->
local configuration

gel electrophoresis
---------->
formation
association constant

NMR

紅外線及Raman光譜

一、理論基礎

紅外線吸收及Raman散射光譜都是因分子內原子振動而起的。所以它們的光譜都能透露分子內振動能量的訊息。這些能量是和構造息息相關，是吾人追求的目標。

雖然紅外線吸收及Raman散射光譜能量大小相近，但發生的機構並不相同，也遵循不同的量子選擇規則(selection rule)。試簡述如下。

甲：紅外線吸收光譜

如果分子內有N個原子，在三度空間中有3N個自由度，但其中有三個平移、三個(非線性)或二個(線性)旋轉自由度，剩下來的才是振動的自由度。所以一般分子內振動的自由度是3N-6個，線性分子則多一個(3N-5)。能產生一個永久偶距的振動才能產生紅外吸收光譜。

乙：Raman散射光譜

Raman散射光譜是由入射光的震盪電位能(E)所引發的偶距所引起的。由於入射光的電位能把正電荷中心和負電荷中心分開產生所謂極化現象。

現在用二氧化碳來說明。二氧化碳是一直線分子共有三個原子，所以一共有四個振動自由度。包括兩個伸展振動，兩個彎曲振動。其中一個伸展振動是兩個氧原子同時向外伸展或收縮，它不產生偶距，所以沒有紅外吸收。但是這種振動改變了分子的大小所以它的極化是隨時間變化，因之它有Raman散射。

另外一個伸展振動是一邊氧原子伸展，另一邊氧原子收縮。這樣的不對稱的振動有一永久偶距，但沒有極化現象，所以有紅外吸收而沒有Raman散射。

彎曲的振動是以碳原子爲中心，兩氧原子向同邊接近，如一組發生在二氧化碳分子的平面上，另一組則在它的垂直平面上。這兩組都產生偶距且能量相等，所以只有一條紅外吸收線。這樣的彎曲振動也沒有極化現象，所以也沒有Raman散射。

上述的現象也說明直線分子的紅外吸收及Raman散射互斥現象。也就是說有紅外吸收的振動就沒有Raman散射，反之亦然。但非線性分子則不在此限。

二、優點

甲、紅外線吸收比紫外線吸收能量更低，所以是試料沒有破壞性。

乙、試料可以相當多狀態存在。它可是溶液、懸浮液、沉澱物、膠狀物、薄膜、纖維狀物、單晶、多晶體及不定形固體。

丙、所需之試料很少，有時有一撮升就夠了。

丁、發生所需的時間極短。

戊、已供有大量的資料。

己、Raman的水分子及重水分子的散射要比紅外吸收線小很多，所以在水溶液中Raman要比紅外爲優。

三、缺點

甲、吸收線的解析度仍不高。

乙、Raman散射線非常弱，試料要仔細處理。

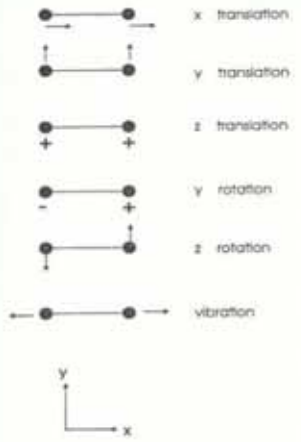


Table III
Vibrational Modes of Trans Peptide Group[a]

Description	Observed frequency	Calculated frequency	Potential energy distribution[b]
Amide A	3236	3254	NH s (100)
Amide I	1653	1646	CO a (83), CN s (15), C_aCN d (11)
Amide II	1567	1515	NH ib (49), CN s (33), CO ib (12)
Amide III	1299	1269	NH ib (52), C_aC s (18), CN s (14)
NC_N stretch	1096	1070	NC_N s (77), C_NC (17)
CN stretch	881	908	CN s (31), C_aC s (17), CO s (16)
Amide V	725	721	CN t (75), NH ob (38)
Amide IV	627	637	CO ib (44), C_aC s (34), CNC_N d (11)
Amide VI	600	655	CO ob (85), CN t (13)
C_aCN bend	436	498	C_aCN d (63), CO ib (11)
CNC_N bend	289	274	CNC_N (71), CO ib (19), C_aCN d (13)
Amide VII	206	226	NH ob (64), CN t (15), CO ob (12)

[a] Based on the observed and calculated frequencies (cm^{-1}) of *N*-methylacetamide, C_aCONHC_N, where C_a and C_N refer to the acetyl and amide methyl groups, respectively, as reported by Bandekar (1992).
[b] Relative (unnormalized) contributions to the potential energy; s, stretch; d, deformation; t, torsion; ib, in-plane bend; ob, out-of-plane bend.

Table IV
Ranges of Infrared and Raman Amide Modes for Different Protein Secondary Structures[a]

Secondary structure	Amide mode	Infrared (cm^{-1})	Raman (cm^{-1})
α-Helix	Amide I	1648–1655	1648–1655
	Amide II	1540–1545	n
	Amide III	1270–1320	1270–1320
	Amide V	≈660	≈660
β-Strand	Amide I	1630–1635(⊥), 1690–1695(∥)	1660–1680
	Amide II	1520–1525(∥), 1550–1555(⊥)	n
	Amide III	1220–1235(∥)	1225–1240
	Amide V	≈700	u
Irregular	Amide I	1655–1660	1655–1665
	Amide II	1550–1570	n
	Amide III	1240–1255	1240–1250
	Amide V	u	u

[a] Compiled from data in Carey (1982), Thomas (1987), Bandekar (1992), Arrondo *et al.* (1993), Miura and Thomas (1995), and references therein. n, Not observed in the off-resonance Raman effect; u, undetermined; (∥), parallel component; (⊥), perpendicular component. The UVRR-determined amide II modes in model compounds are discussed by Austin *et al.* (1993). Additional correlations for various types of turns are also discussed by Bandekar (1992).

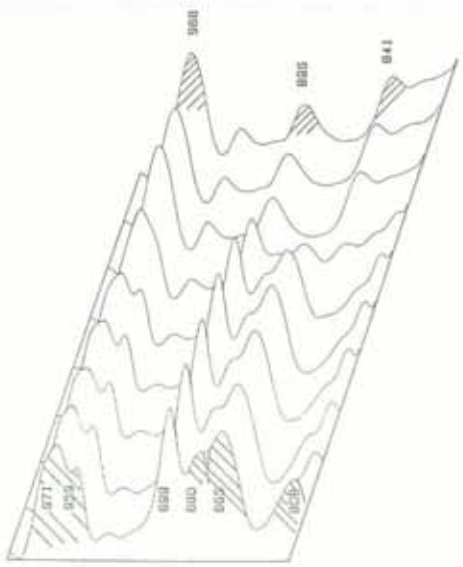


FIG. 4. B → A conformational transition of poly[d(A-T)] followed by FT-IR spectroscopy. The DNA film is exposed to decreasing relative humidities. (Top) B form, R.H. 100% (///). (Bottom) A form, R.H. 58% (\\\\). Bands characteristic of both geometries have been hatched.

TABLE I

SPECTROSCOPIC CHANGES FOR A,T-CONTAINING POLYMERS*

Assignment	Disordered form (cm^{-1})	Change B to disordered ←	B form (cm^{-1})	Change B to A →	A form (cm^{-1})
bk	—	—	644sh	inc	644
T	667	s.dec	670	dec	666
			n.r.	inc	706
A	728	inc	729	—	729
T	747	dec/shift	750	dec/shift	747
bk, T	794	s.dec	793	shift	779
C3′-*endo* bk	—	—	—	inc	807
C2′-*endo* bk	n.r.	dec	841	dec	n.r.
PO^{2-} bk	1096	—	1094	shift	1102
T	1186	inc	1186	—	—
T, A	1208	dec	1209	dec	n.r.
T	1237	inc	1240sh	inc	1239
T, A	1255sh	dec	1255	dec	1255sh
A	1307	s.inc	1303	s.inc	1301
A	1334	s.inc/shift	1341	inc/shift	1334
T, A	1374	No. change	1376	dec	1374
	—	—	n.r.	inc	1402
A, bk	1421	s.inc	1420	shift	(1415)
bk, A	1462		1462	inc	1462
A	1483	inc	1483	inc/shift	1478

* dec, Decrease in intensity; inc, increase in intensity; shift, change in band position; n.r., not resolvable; s., slight intensity change; sh, shoulder; A, adenine; T, thymine; and bk, deoxyribose phosphate backbone.