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Topography of Nucleic Acid Helices in Solutions. I. The Nonidentity of Polyadenylic–Polyuridylic and Polyinosinic–Polycytidylic Acid Helices[*]

Edmond J. Gabbay

ABSTRACT: The effect of diquaternary ammonium salts of the general structure, $R_1R_2R_3N^+(CH_2)_nN^+R_1R_2R_3 \cdot 2Br^-$, I, on the helix–coil transition of polyadenylic–polyuridylic (poly-A–poly-U) and polyinosinic–polycytidylic acid (poly-I–poly-C) helices are reported. Helix stability was measured by absorbancy–temperature profiles.

The results may be summarized. (1) Maximum stabilization by I occurs at $n = 3$. The observed stabilization appears to be due to electrostatic interactions between adjacent phosphate anions, and the results are consistent with the Watson–Crick model. (2) Increasing the hydrophobic nature of the substituted salts, I, results in a decreased stability of the helices, and the effects are dependent on n for a given change in R_3, where $R_1 = R_2 = Me$. (3) Polar substituents stabilize both helices. (4) Finally, differences in the behavior of poly-I–poly-C and poly-A–poly-U toward I strongly suggest that in the former the bases are packed more closely.

It has been generally accepted that double-stranded nucleic acids have a uniform and unique structure, *i.e.*, the Watson–Crick double helix (1953). Although the dimensions of the Watson–Crick model are well known, the full physical consequences of such a structure in solutions have not yet been exploited. For example, the following stereochemical and physical characteristics of the double-stranded helix are predicted: (1) a highly polar surface, (2) a 7-A separation of adjacent negatively charged oxygen atoms on the same chain, and (3) the identity of all nucleic acid helices.

In an attempt to elucidate the structure and/or topography of nucleic acid helices in solution we have studied the effect of diquaternary ammonium salts of the general structure $R_1R_2R_2N^+(CH_2)_nN^+R_1R_2R_3 \cdot 2Br^-$ on the helix–coil transition of two model helices, polyadenylic–polyuridylic (rA–rU) and polyinosinic–polycytidylic (rI–rC) complexes. In this paper we report the effect of constant concentration of I on T_m, the transition temperature for the conformational changes helix–coil, as measured by absorbancy–temperature profiles.

Experimental Section

Polyadenylic (ϵ_p^{257} 8730),[1] polyuridylic (ϵ_p^{260} 8310),[1] polycytidylic (ϵ_p^{268} 4860),[1] and polyinosinic acids (ϵ_p^{258} 7980)[1] were obtained from Calbiochem, lot no. 52830, 52995, 46113, and 46114, respectively. Stock solutions of the homopolymers were made in 0.1 M Tris–HCl buffer, pH 7.25, and stored at 0–4° at 1.10–1.30 × 10^{-3} mole of P/l. Aliquots were removed and diluted with 0.1 M Tris–HCl buffer or with a solution of the diquaternary ammonium salts, I, in 0.1 M Tris–

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[1] In 0.1 M Tris-HCl buffer, pH 7.25, at 25°.

TABLE I: $R_1R_2R_3N^+(CH_2)_nN^+R_1R_2R_3 \cdot 2Br^-[\cdot \frac{1}{2}H_2O$ or $\cdot H_2O]$.[a,b]

						Calcd		Found[d]	
Compd	R_1	R_2	R_3	*n*	Mp (°C)[c]	C	H	C	H
1	H	H	H	2	>350				
2	H	H	H	3	248				
3	H	H	H	4	320				
4	H	H	H	5	247				
5	Me	Me	H	2	281				
6	Me	Me	H	3	289				
7	Me	Me	H	4	265	31.39	7.19	31 50	7.59
8	Me	Me	H	5	243	33.78	7.50	33.53	7.62
9	Me	Me	H	6	228				
10	Me	Me	Me	3	293				
11	Me	Me	Me	4	320	35.95	7.80	35.62	8.68
12	Me	Me	Me	5	303	37.92	8.05	37.80	8.37
13	Me	Me	Me	6	285	39.80	8.29	39.69	8.94
14	CH_3	CH_3	Et	2	255	35.90	7.80	35.70	8.08
15	CH_3	CH_3	Et	3	234	37.93	8.05	37.76	8.07
16[a]	CH_3	CH_3	Et	4	312	37.93	8.42	38.29	8.78
17[b]	CH_3	CH_3	Et	5	283	40.51	8.58	40.53	8.39
18[b]	CH_3	CH_3	Et	6	230	42.10	8.78	42.24	9.63
19	CH_3	CH_3	Pr	2	235	39.80	8.31	40.08	8.43
20	CH_3	CH_3	Pr	3	210	41.50	8.52	40.92	8.10
21	CH_3	CH_3	Pr	4	224	43.07	8.72	42.86	8.95
22[b]	CH_3	CH_3	Pr	5	200	43.53	8.95	43.50	9.01
23[b]	CH_3	CH_3	Pr	6	224	45.00	9.15	45.20	9.50
24	Me	Me	Bu	2	210	43.05	8.72	42.97	9.06
25	Me	Me	Pentyl	2	124	45.95	9.10	45.75	9.14
26[a]	Me	Me	Benzyl	2	217	51.60	6.87	51.53	6.81
27	Me	Me	Benzyl	3	135	52.50	7.09	51.80	7.31
28	Me	Me	Benzyl	4	230	53.35	6.99	54.03	7.13
29	Me	Me	Benzyl	5	290	55.20	7.20	55.29	7.36
30[a]	Me	Me	Benzyl	6	230	55.15	7.46	55.19	7.62
31	Et	Et	Et	2	252				
32	Et	Et	Et	3	245				
33	Et	Et	Et	4	225	45.90	9.08	45.72	9.21
34	Et	Et	Et	5	270	47.20	9.26	46.92	9.62
35	Me	Me	CH_2CH_2OH	3	125	34.75	7.37	34.33	7.80
36	Me	Me	CH_2CH_2OH	4	264	36.60	7.62	36.35	7.56
37	Me	Me	CH_2CH_2OH	5	256	38.18	7.84	38.26	7.88
38	Me	Me	CH_2CH_2OH	6	175	39.90	8.06	40.23	8.41
39	Me	H	H	2	184				
40	Me	H	Pr	2	185				

[a] Analyzes for monohydrate. [b] Analyzes for hemihydrate. [c] Melting points are uncorrected. [d] Only new compounds were analyzed.

HCl buffer (prepared by addition of HCl to 0.1 M Tris–base), pH 7.25, to a final concentration of 3.0–3.5 × 10^{-5} mole of P/l. in each polynucleotide. The thermal transition was followed at 257 mμ for the rA–rU helix[2] and at 250 mμ for the rC–rI helix.[2] All values of T_m were obtained in duplicate, and standard T_m curves in the absence of I were obtained before each determination. Spectrophotometric measurements were made with a Beckman DU spectrophotometer. The cell compartment was thermostated by a Haake constant-temperature circulator and the temperature inside the compartment was checked by a copper *vs.* constantan thermocouple. Under these conditions the melting temperature for the rA–rU helix is found to be 57.0

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[2] Wavelength at which the hyperchromicity upon helix–coil transition for rI–rC and rA–rU was found to be 48 and 36%, respectively, at 0.1 M Tris–HCl buffer, pH 7.25.

TABLE II: Variation of ΔT_m of rA–rU and rI–rC with I, $R_1R_2R_3N^+(CH_2)_nN^+R_1R_2R_3 \cdot 2Br^-$.

					ΔT_m rA–rU[a]		ΔT_m rI–rC[a]	
Compd	n	R_1	R_2	R_3	2×10^{-3} M	2×10^{-2} M	2×10^{-3} M	2×10^{-2} M
1	2	H	H	H	11.5	27.8	9.3	
2	3	H	H	H	13.1	28.1	10.4	
3	4	H	H	H	10.6	23.7	8.3	
4	5	H	H	H	8.3	20.5	4.9	
5	2	Me	Me	H	1.1	16.9		8.8
6	3	Me	Me	H	5.5	18.0		8.5
7	4	Me	Me	H	2.7	14.4		5.3
8	5	Me	Me	H	1.7	10.2		2.4
9	6	Me	Me	H	0.5	6.5		0.2
10	3	Me	Me	Me	6.5	13.7		4.6
11	4	Me	Me	Me	1.7	9.2		2.6
12	5	Me	Me	Me		6.9		1.0
13	6	Me	Me	Me	0.4	3.7		−0.5
14	2	Me	Me	Et	1.0	11.5		4.4
15	3	Me	Me	Et	3.2	10.8		2.9
16	4	Me	Me	Et	1.0	6.7		1.0
17	5	Me	Me	Et	0.4	3.0		−0.3
18	6	Me	Me	Et	0.0	0.0		−1.9
19	2	Me	Me	Pr		7.0		1.7
20	3	Me	Me	Pr		6.0		0.0
21	4	Me	Me	Pr		1.8		−1.6
22	5	Me	Me	Pr		−0.3		−2.1
23	6	Me	Me	Pr		−1.2		−3.0
24	2	Me	Me	Bu		5.2		−0.2
25	2	Me	Me	Pentyl		−1.0		−2.7
26	2	Me	Me	Benzyl	−2.0		−2.7	
27	3	Me	Me	Benzyl	−2.3		−2.9	
28	4	Me	Me	Benzyl	−2.1		−2.9	
29	5	Me	Me	Benzyl	−2.7		−2.8	
30	6	Me	Me	Benzyl	−3.3		−3.0	
31	2	Et	Et	Et		−0.9 (−2.0)[b]		−1.8 (−3.1)[b]
32	3	Et	Et	Et		1.4 (2.4)[b]		−0.6 (−1.6)[b]
33	4	Et	Et	Et		−0.3		−1.9
34	5	Et	Et	Et		−1.4		−3.6
35	3	Me	Me	CH_2CH_2OH		14.7		5.2
36	4	Me	Me	CH_2CH_2OH		11.4		2.4
37	5	Me	Me	CH_2CH_2OH		7.2		1.0
38	6	Me	Me	CH_2CH_2OH		4.4		0.0
39	2	Me	H	H		22.0		15.1
40	2	Me	H	Pr		8.7		8.8

[a] The reported value of ΔT_m is obtained from the difference of the average T_m, obtained from two separate melting temperature curves (±0.2° average deviation), and T_m of blank, *i.e.*, 57.0 ± 0.2 and 62.0 ± 0.3° for rA–rU and rI–rC, respectively. The value of ΔT_m given above has therefore an average deviation of ±0.4 and ±0.5° for rA–rU and rI–rC, respectively. [b] At 6×10^{-2} M concentration of I.

± 0.2° (average of 20 determinations) and for rI–rC the T_m is 62.0 ± 0.3° (average of 16 determinations). The values of T_m reported by Chamberlin *et al.* (1963) of rA–rU, and Chamberlin and Patterson (1965) of rI–rC, in 0.1 M sodium citrate buffer, pH 7.5, are 56.8 and 60.2°, respectively.

The simple diamines, $NH_2(CH_2)_nNH_2$ (n = 2–5) (Aldrich Chemical Co., Milwaukee, Wis.), were converted to the hydrobromide salts, II, and recrystallized. Diquaternary ammonium salts of the general structure $R_1R_2R_3N^+(CH_2)_nN^+R_1R_2R_3 \cdot 2Br^-$ (n = 2–6) (where $R_1 = R_2 = R_3$ = Me; $R_1 = R_2 = R_3$ = Et; $R_1 = R_2$

= Me, R_3 = benzyl; and $R_1 = R_2$ = Me, $R_2 = CH_2CH_2OH$) were synthesized from the corresponding dibromides, $Br(CH_2)_nBr$ (Aldrich Chemical Co., Milwaukee, Wis.) by refluxing for 16–24 hr with an excess of the appropriate tertiary amine in ethanol.[3]

Diamines of the general structure $Me_2N(CH_2)_nNMe_2$, III, were obtained as follows: n = 2 and 6 (Aldrich Chemical Co.) and n = 3–5 were synthesized from the corresponding unsubstituted diamines by treatment with formaldehyde and formic acid according to the procedure of Clarke *et al.* (1933). Diamines III were converted to the diquaternary ammonium salts IV (where $R_1 = R_2$ = Me and R_3 = ethyl, propyl, butyl, and pentyl) by refluxing for 16–24 hr with an excess of the appropriate alkyl bromide in ethanol.

A typical synthesis aimed for 10–20 mmoles of product and was carried out in 15–20 ml of ethanol. The work-up procedure involved evaporation of the solvent until a crystalline solid was obtained. The solid was collected, washed with acetone, and recrystallized from either water–ethanol or ethanol–acetone mixtures. The yields of products ranged from 70 to 95%. The nuclear magnetic resonance spectra of all new derivatives were taken in D_2O and found to be consistent with their assigned structure. Table I contains a listing of these diquaternary ammonium salts along with their physical characteristics, *i.e.*, melting point and C and H analyses. Melting points were taken in thin-walled capillaries on a Mel-Temp apparatus by Mr. Armen S. Casparian. Elemental analyses were performed by George I. Robertson, Florham Park, N. J.

Results

The results of the addition of salts I on the melting temperature of rA–rU and rI–rC are recorded in Table II. In all cases, 0.1 M Tris-HCl buffer (prepared by addition of HCl to 0.1 M Tris–base), pH 7.25, was used.

Effect of Charge Separation. The effect of varying n of I, $R_1R_2R_3N^+(CH_2)_nN^+R_1R_2R_3 \cdot 2Br^-$, on the stability of rA–rU helix appears at first glance to be similar to that of rI–rC helix. Considering **1–4**, $R_1 = R_2 = R_3$ = H, maximum value of ΔT_m occurs at n = 3 for both helices (see Table II). The nonsymmetrical diquaternary ammonium salts (I), $R_1 = R_2$ = Me, $R_3 \neq$ Me, exhibit a different effect. At 2×10^{-3} M concentration of **5–9**, $R_1 = R_2$ = Me and R_3 = H, and **14–18**, $R_1 = R_2$ = Me and R_3 = Et, maximum stabilization of rA–rU helix is seen at n = 3. In the presence of 2×10^{-2} M concentration of **5–9**, rA–rU helix is again maximally stabilized at n = 3; however, rI–rC helix appears to be equally and maximally stabilized at n = 2 and 3. At high concentrations of **14–18**, $R_1 = R_2$ = Me and R_3 = Et, and **19–23**, $R_1 = R_2$ = Me and R_3 = Pr; *i.e*, at 2×10^{-2} M, both helices show a maximum ΔT_m at n = 2 with decreasing ΔT_m as n varies from 2 to 6.

In the symmetrical diquaternary ammonium salts I, $R_1 = R_2 = R_3$ = Me, **10–13**, ΔT_m of rA–rU and rI–rC helices decreases as n increases from 3 to 6. All attempts to prepare the corresponding double salt I, as the bromide or chloride, for n = 2 failed. In this series, therefore, maximum stabilization as a function of n could not be obtained.

Effect of the Hydrophobic Nature of Substituent. Increasing the size of the alkyl substituent, R, of dipositive salts of the type $Me_2N^+(R)(CH_2)_nN^+(R)Me_2$, IV, results in a lowering of the melting temperature of rA–rU and rI–rC helices. At n = 2, and as R is varied from hydrogen (**5**), to ethyl (**14**), to propyl (**19**), to butyl (**24**), to pentyl (**25**), ΔT_m decreases for both helices (see Table II). Another way of looking at this effect is shown in Tables III and IV, where the values of the differences in ΔT_m values in going from hydrogen to methyl, methyl to ethyl, ethyl to propyl, etc., for different values of n are given. At n = 2, increasing R from ethyl to propyl results in a greater decrease in ΔT_m than increasing R from propyl to butyl for both helices. A dramatic effect is observed for n = 2 in rA–rU helix as one goes from butyl to pentyl substitution; *i.e.*, stabilization of the helix of

TABLE III: The Change, $\Delta\Delta T_m$,[a] for rA–rU as a Result of a Change in R in $Me_2N^+(R)(CH_2)_nN^+(R)Me_2$ (c 2×10^{-2} M).

n	H-Me	Me-Et	Et-Pr	Pr-Bu	Bu-Pentyl
2	...	...	−4.5	−1.8	−6.2
3	−4.3	−2.9	−4.8	...	...
4	−5.2	−2.5	−4.4	...	...
5	−3.3	−3.9	−3.3	...	...
6	−2.8	−3.7	−1.2	...	...

[a] $\Delta\Delta T_m$ (°C) values have compounded average deviation of ±0.8°.

TABLE IV: The Change, $\Delta\Delta T_m$,[a] for rI–rC as a Result of a Change in R in $Me_2N^+(R)(CH_2)_nN^+(R)Me_2$ (c 2×10^{-2} M).

n	H-Me	Me-Et	Et-Pr	Pr-Bu	Bu-Pentyl
2	...	...	−2.7	−1.9	−2.5
3	−3.9	−1.7	−2.9	...	...
4	−2.7	−1.6	−2.6	...	...
5	−1.4	−1.3	−1.8	...	...
6	−0.7	−1.4	−1.1	...	...

[a] The $\Delta\Delta T_m$ (°C) values have a compounded average deviation of ±1.0°.

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[3] In the case of trimethylamine, the reaction was carried out in a sealed tube at room temperature for 20 hr.

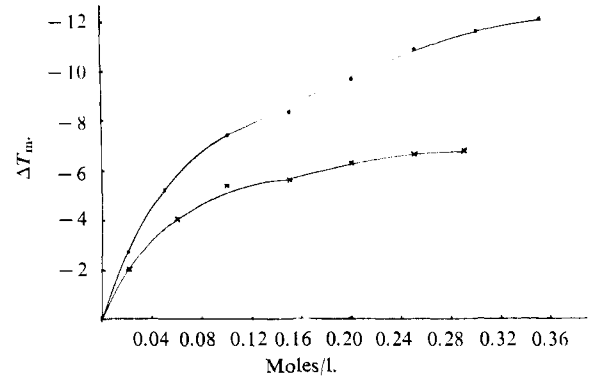

FIGURE 1: The effect of concentration of **25**, where $R_1 = R_1 =$ Me, $R_3 =$ pentyl, and $n = 2$, on the ΔT_m of rA–rU (x—x) and rI–rC (●—●) helices in 0.1 M Tris-HCl, pH 7.25.

5.2° to destabilization of that of −1.0°, a $\Delta\Delta T_m = -6.2°$. For both rA–rU and rI–rC helices the effect of increasing R on the value of $\Delta\Delta T_m$ appears to depend on chain length, n. For example, for $n = 3$ and 4, there is a greater decrease in ΔT_m as R is varied from hydrogen to methyl and ethyl to propyl than from methyl to ethyl; whereas for $n = 5$ and 6, the reverse trend is observed (Tables III and IV).

The effect of concentration of **25** ($R_1 = R_2 =$ Me, $R_3 =$ pentyl, and $n = 2$) on the melting temperature of rA–rU and rI–rC helices is shown in Figure 1. At high concentration of **25**, a maximum lowering of the melting temperature of the rA–rU helix is reached ($\Delta T_m \cong -6.7°$). Similarly, the rI–rC helix also appears to reach a maximum lowering of the melting temperature ($\Delta T_m \cong -12.0°$). It is interesting to note that the rI–rC helix is considerably more destabilized than the rA–rU helix in the presence of **25** (see Discussion).

Finally, **26–30** ($R_1 = R_1 =$ Me and $R_3 =$ benzyl) were synthesized in order to determine the effect of a large hydrophobic substituent on T_m. The results which are recorded in Table II show that even at a low concentration, *i.e.*, 2×10^{-3} M, a lowering of T_m is observed. Studies at higher concentration of **26–30** are not possible due to the ultraviolet absorption of the phenyl rings in the 260-mμ region.

TABLE V: The Change, $\Delta\Delta T_m$, for rA–rU and rI–rC as a Result of a Change in R from Propyl to 2-Hydroxyethyl in $Me_2N^+(R)(CH_2)_nN^+(R)Me_2$ (c 2×10^{-2} M).

n	rA–rU[a]	rI–rC[b]
3	8.7	5.2
4	9.6	4.0
5	7.5	3.1
6	5.6	3.0

[a] Average deviation ±0.8°. [b] Average deviation ±1.0°.

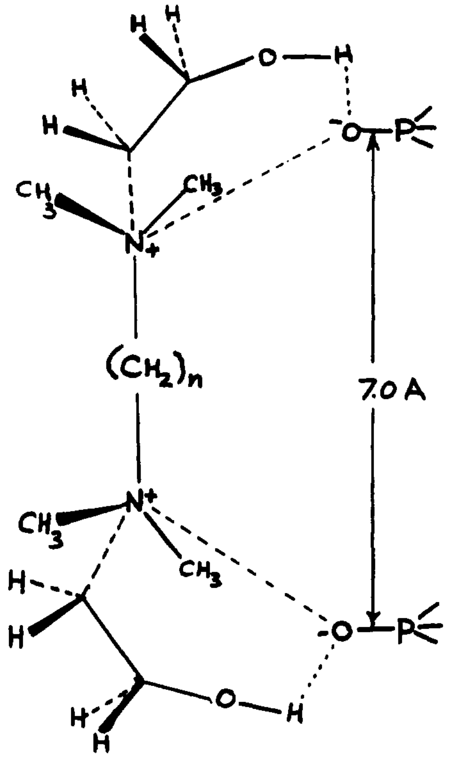

FIGURE 2: A possible scheme for the interactions of **35–38** with adjacent phosphate anions of a nucleic acid helix.

Effect of Polar Substituent. Substitution of a polar group on the diquaternary ammonium salts of the type $Me_2N^+(R)(CH_2)_nN^+(R)Me_2$ helps to stabilize the helices of both rA–rU and rI–rC. The effects of **35–38**, where R = 2-hydroxyethyl, on ΔT_m are recorded in Table II. The differences in ΔT_m between **35–38** and **20–23** where R = propyl are recorded in Table V. If it is assumed that the hydroxyethyl group is as bulky as a propyl group, the substantial increase in the stability of the helices, $\Delta\Delta T_m$, in going from propyl to 2-hydroxyethyl (Table V) is probably attributed to a hydrogen-bonding effect (Figure 2).

Discussion

Effect of Charge Separation. The stabilization of nucleic acid helices by diamines $NH_2(CH_2)_nNH_2$ has been studied by Tabor (1962) and Mahler and Mehrotra (1963). It was shown by Tabor that the melting temperature of calf thymus deoxyribonucleic acid (DNA) ($T_m = 77.4°$), in the presence of 10^{-3} M diamines, increased by 4.5, 6.4, 6.0, 6.8, and 6.0° as n was varied from 2 to 6, respectively. Similarly, Mahler and Mehrotra (1963), in an extensive study of the effect of diamines on a number of nucleic acids derived from biological sources, concluded that maximum stabilization occurs at $n = 5$. Since the nucleic acids rich in adenine and thymine exhibited a greater stabilization in the presence of diamines, Mahler postulated a bridging role for diamines, *i.e.*, specific binding between phosphate residues of one strand and the adenine and/or thymine residues of the other.

Our results do not agree with the above interpretation, since stabilization of rI–rC helix by diquaternary ammonium salts I does occur, albeit it is not as pronounced as with rA–rU helix. Furthermore, our results unequivocally show that maximum stabilization of both helices occurs at $n = 3$ for the simple diamines,[4] 1–4, in accord with what would be expected for simple electrostatic and hydrogen-bonding interactions of adjacent phosphate anions.

Molecular framework models of nucleic acid helices were constructed according to the specification of the stereochemical detail of the X-ray structure of DNA outlined by Wilkins (1963). Assuming that the bases are spaced 3.4 A apart along the axis of the molecule and there is one turn of the helix every 34 A, it is found that adjacent phosphate anions are separated by approximately 7.0 A. In order to measure directly from models the distance between adjacent negatively charged oxygen atoms in the presence of $N^+H_3(CH_2)_nN^+H_3$, II, the following assumptions were made. (1) The distance between the positively charged nitrogen and the negatively charged oxygen of the phosphate group is approximately 2.8 A. (2) The $N^+H \ldots\ldots O^-$ angle is 180°. The assumptions are reasonable and what might be expected for normal hydrogen bonding (Pauling, 1960). Under these conditions it is found that adjacent negatively charged oxygen atoms must be brought closer together for $n = 2$ than for $n = 3$; *e.g.*, actual measured distances are 6.3 and 6.9 A, respectively (Figure 3 and Table VI).

TABLE VI: Measured Distance, d, between Adjacent Negatively Charged Oxygen Atoms as a Result of Varying the Distance, r, between the Positively Charged Nitrogen of I and Negatively Charged Oxygen of the Phosphate Residue (See Text).

r^a	d^a for $n = 2$	d^a for $n = 3$
2.8	6.3	6.9
3.2	6.4	7.0
3.6	6.6	7.0
4.0	6.9	7.0

[a] Values are in angstroms.

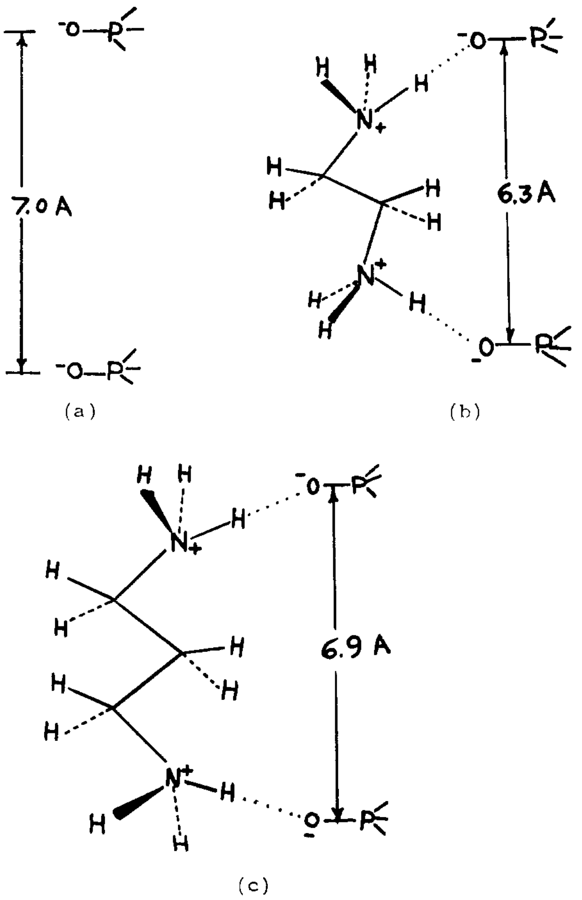

FIGURE 3: Measured distances in the molecular framework. (a) Distance between adjacent phosphate anions as measured from a molecular framework model of the Watson–Crick helix. (b and c) Distances between negative charges obtained by measurement of molecular framework models of $N^+H_3(CH_2)_nN^+H_3$, where $n = 2$ and 3. At $n = 4$ the natural distance of 7 A is easily accommodated.

As the size of the cations in $R_1R_2R_3N^+(CH_2)_nN^+R_1R_2R_3$, I, is increased it would be expected that the distance, r, between the positively charged nitrogen and the negatively charged oxygen would increase as well. Table VI shows the actual measured distances, d, between adjacent negatively charged oxygen atoms of the helix as a result of binding I for $n = 2$ and 3, as r is varied from 2.8 to 4.0 A. In order to bridge I for $n = 2$ at low values of r, it is necessary to distort the regular dimensions of the helix by bringing adjacent negatively charged oxygen atoms closer. However, as r increases, the natural distance of 7 A separating adjacent anions on the helix begins to be accommodated by I where $n = 2$. These stereochemical consequences are in line with our results; *e.g.*, in the series of compounds $Me_2N^+(R)(CH_2)_nN^+(R)Me_2$, as R is

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[4] Using an entirely different approach, *i.e.*, by studying the kinetics of binding a dye, acridine orange, to rI–rC in the presence of $N^+H_3(CH_2)_nN^+H_3$, II, $n = 2$–5, at 25°, we have been able to show that the second-order rate constant, k_2, varies inversely with the relative ability of II to stabilize the rI–rC helix; *e.g.*, k_2 is a minimum at $n = 3$ (E. J. Gabbay, in preparation).

made larger, maximum stabilization of the helices shifts from $n = 3$ to $n = 2$ (see Table II).

It is particularly interesting to note that the shift in maximum stabilization, $n = 3$ to $n = 2$, for rI–rC occurs by going from $N^+H_3(CH_2)_nN^+H_3$, II, to $Me_2N^+(H)(CH_2)_nN^+(H)Me_2$, V, whereas the same shift for rA–rU takes place in going from $Me_2N^+(Et)(CH_2)_nN^+(Et)Me_2$, VI, to $Me_2N^+(Pr)(CH_2)_nN^+(Pr)Me_2$, VII. This difference is significant and suggests, since r increases in going from II to V to VI to VII, that in the rI–rC helix, adjacent negatively charged oxygen atoms are at a closer distance and/or can become closer than in the rA–rU helix in the presence of I. If this is the case, it follows that in order to minimize the bond-angle strain, either the distance between bases in rI–rC is smaller or the number of bases per turn of helix is greater than in rA–rU. These conclusions can easily be demonstrated by molecular framework models of the Watson–Crick helix. We prefer the first alternative, *i.e.*, closer packing of bases, since it is supported by the following observations. (1) Stabilization of rI–rC for a given diquaternary ammonium salt, I, is not as pronounced as that of rA–rU. It suggests that maximum electrostatic interactions are not attained because of the inability of I to bind to the denser and more closely packed rI–rC helix at the same close distance as it does with rA–rU. (2) A net destabilization of the rI–rC helix as compared with rA–rU helix is always observed at a smaller R group for all values of n of IV. If it is assumed that rI–rC is denser than rA–rU, then the greater sensitivity to alkyl substituents exhibited by rI–rC may arise as a consequence of a greater percentage of the helix surface being in contact with a nonpolar medium. (3) The rI–rC helix is more stable than the rA–rU helix, as evidenced by a higher T_m.

The symmetrical salts **31–34**, $R_1 = R_2 = R_3 =$ ethyl, present a special problem. Table II shows that maximum stabilization or, as in the case of rI–rC, minimum destabilization occurs at $n = 3$ and not at $n = 2$ as might be expected since r is large. This serious anomaly may be rationalized by assuming that in the case of **31**, where $n = 2$, special steric and/or solvation effects[5] are operating which cause a net destabilization of both rA–rU and rI–rC helices, *i.e.*, −0.9 and −1.8°, respectively.

Effect of the Size of Substituents. Our results indicate that the effect of increasing the size and number of alkyl substituents of I decreases the stabilization of both rI–rC and rA–rU helices. Levine *et al.* (1963) find that an increase in the number and size of alkyl substituents on carbamates, alcohols, amides, and ureas increases the denaturing effectiveness toward DNA. The effect has been interpreted as being due to a decrease in the ion-solvating power and an increase in the hydrophobic character of the solvent medium resulting in a relative stabilization of denatured DNA. At the low concentrations of I used in this study, changes in solvents are expected to be small or negligible. Nevertheless, Jencks' conclusion (Levine *et al.*, 1963) is still very much applicable, since by electrostatically binding the diquaternary ammonium salts, I, the effective medium at the surface of the helix depends on the nature of the substituent; *i.e.*, increasing the size of the groups on I effectively lowers the dielectric constant of the surrounding medium and hence lowers the stability of the highly polar helical structure. Similarly, it may be argued that the effect of increasing the size of the substituent in I causes a stabilization of the random coils. The latter alternative is unlikely, since the interaction between I and a polynucleotide coil still remains electrostatic in nature. Therefore the same behavior as with the helix may be expected for such interactions; *i.e.*, increasing the size of the substituent should result in lowered affinity of I for the helix as well as the random coil.

The fact that a limiting value of ΔT_m for rA–rU is reached with increasing concentration of 25 ($R_1 = R_2 =$ Me, $R_3 =$ pentyl, and $n = 2$) suggests that relative stabilization of the coils with respect to the helix is not likely. Saturating all available sites on the helix with **25** results in a complex that has a finite T_m which does not change with further increase in concentration of **25** (see Figure 1). However, if we were dealing with a relative stabilization of the coils with respect to the helix, a concentration-dependent decrease of T_m might be expected.[6] Such is the case for the effect of increasing concentrations of urea on the T_m of rA–rU helix, *e.g.*, T_m is found to be 52.4, 48.0, and 43.8° at 1, 2, and 3 M concentrations, respectively (E. J. Gabbay, 1966, unpublished results).

The results obtained with $Me_2N^+(R)(CH_2)N^+(R)Me_2$, IV, suggest that the extent of stabilization for a given change in R depends on the chain length. For example, in going from ethyl to propyl there is a greater decrease in ΔT_m for rA–rU helix than from methyl to ethyl for $n = 3$ and 4, whereas for $n = 5$ and 6 the reverse trend is observed (see Table III). A possible interpretation would be that for $n = 3$ and 4, binding of IV to the helix leads to a new complex as R is varied from ethyl to propyl which drastically interferes with solvation (*e.g.*, causing the liberation of a bound water molecule); in contrast, the same effect is noted as R is varied from methyl to ethyl when $n = 5$ and 6.

A net destabilization of the rI–rC helix as compared with the rA–rU helix is always observed at a smaller R group for all values of n of IV. Closer packing of the bases in rI–rC is one possible interpretation, since

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[5] Destabilization of the helices by **31** might be a consequence of having two bulky ethyl substituents sticking into the highly polar groove between adjacent negatively charged oxygen atoms, hence lowering the dielectric constant of the surrounding media. In the case of **32**, where $n = 3$, the distance between the positively charged nitrogen atoms is larger and therefore it might be reasonable to assume that it would be relatively more difficult to force the two ethyl substituents into the same groove and maintain maximum electrostatic interactions.

[6] It has been pointed out by a referee that the results are also consistent with relative stabilization of the random coils. Admittedly, the results may be interpreted in terms of relative stabilization of the random coils, or destabilization of the helical structure, and/or both.

it would predict that the denser helix would be more sensitive to alkyl substituents; *i.e.*, a greater percentage of the helix surface will be in contact with the nonpolar substituent. Other explanations are possible; however, the more tightly packed model is preferred since it explains other behavior of the rI–rC helix.

Effect of Polar Substituent. The change of R substituent in IV from propyl to 2-hydroxyethyl results in a marked stabilization of both helices (Table V). The maximum change in $\Delta\Delta T_m$ occurs at $n = 4$ and $n = 3$ for rA–rU and rI–rC, respectively, which may again indicate a closer distance between anions in the rI–rC helix. Figure 2 depicts a possible scheme for the interactions of **35–38** with nucleic acid helices.

We conclude that through the use of diquaternary ammonium salts, I, the study of the topography of nucleic acids *in solution* is possible. It has been shown above that the rA–rU and rI–rC helices conform with the requirements of the Watson–Crick model, although the helices are not identical in dimensions. The conclusion that the bases in rI–rC are more closely packed than the rA–rU suggests that a similar situation may exist with native DNA. For example, the distances between adjacent bases in a double-stranded DNA helix are expected to differ according to the structure of the bases.

It might be interesting to extend this work to the hybrid homopolymer pairs. For instance, the results of Chamberlin (1965) indicate that the T_m increases in the following series of homopolymer pairs: dI–rC < dI–dC < rI–dC < rI–rC. In the light of our study of the effects of diquaternary ammonium salts, I, on the stability of rA–rU and rI–rC helices, it would be reasonable to expect that the above series of homopolymer pairs also reflects the order of more densely packed helices.

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