Calorimetric study of the complexes between polyuridylic acid and adenylic nucleotides

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ABSTRACT

Soluble complexes of poly(U) and adenylic nucleotides in NaCl solutions were studied by scanning microcalorimetry. The melting enthalpies, ΔH_m , of poly(U) complexes with adenosine, 2',3'-cAMP, 2'(3')-AMP, 5'-AMP, ADP, ATP in 1 M NaCl are 50.5; 45.0; 42.9; 28.6; 26.1 and 25.6 kJ/mole triplets, respectively. ΔH_m is independent of the complex melting temperature, T_m . The calorimetric enthalpies are considerably lower than the apparent $\Delta H_{v.H.}$ obtained from T_m dependence on free monomer concentration. The enthalpy of complex formation in 1 M NaCl depends neither on the number nor on the degree of ionization of the phosphate groups but is essentially determined by their 5'- or 2'(3')-position. In contrast to 2'(3')-AMP·2 poly(U), ΔH_m of 5'-AMP·2 poly(U) increases considerably at lowering Na^+ concentration. The enthalpy of poly(U) double helix melting in 1 M NaCl is 8.8 kJ/mole pairs which is 2.5 times lower than that in $MgCl_2$ solutions.

INTRODUCTION

Thermodynamics of interactions in nucleic acids was studied extensively by calorimetric methods at the level of oligo- and polynucleotides (review in (1)). Polynucleotide-monomer complexes proved to be useful models in the physical chemistry of nucleic acids (2-12), in studying the mechanisms of the template-directed synthesis (13,14) and prebiotic evolution (15). Direct calorimetric data on these complexes are however not numerous (7,10,11).

Among these systems, the complexes between polynucleotides and nucleotides are of special biological interest. Such complexes were observed in solutions of poly(C) with guanylic derivatives (3,6,14). For poly(U) with adenylic nucleotides, only insoluble complexes formed in the presence of Mg^{++} ions were found (16-18). The soluble complex was obtained in poly(U) mixture with cyclic 3',5'-AMP (10).

In the present paper the complex formation of poly(U) with adenylic nucleotides in NaCl solutions as well as the melting of poly(U) double helix was studied by adiabatic scanning micro-calorimetry. The following effects on the thermodynamics of A·U interaction were investigated: (i) addition of a phosphate group to the nucleoside, (ii) changing the position of the phosphate group in the nucleotide, (iii) changing the number of the phosphate groups, (iv) varying the degree of ionization of the terminal phosphate. This possibility to change easily the charge in the monomeric "strand" is one of the advantages of polynucleotide-monomer systems.

EXPERIMENTAL

Materials

Poly(U), adenosine, 2',3'-cAMP (Na-salt), 2'(3')-AMP, 5'-AMP, 5'-ADP and 5'-ATP (Na₂-salts) were obtained from Reanal (Hungary). 2'(3')-AMP was a mixture of 2'- and 3'-nucleotides with unindicated ratio of the isomers. Poly(U) was dialyzed in the cold against 10 mM EDTA and 0.1 M NaCl, then against doubly distilled water until the complete absence of UV-absorption in the dialysate, and finally lyophilized. The monomer preparations were used without additional purification. Paper chromatography showed that the mononucleotides were chromatographically pure, and ADP and ATP contained ca. 6 per cent of nucleotides with smaller numbers of the phosphate groups. This, however, was insignificant for the present study. Due to the dependence of the melting temperature (T_m) on monomer concentration such quantities of the admixtures could not form separate complexes with poly(U) which is evidenced by the presence of only one peak on the heat absorption curves (see Results).

The solutions were prepared in 0.1 M Na-cacodylate buffer with doubly distilled water. Most of the results were obtained for solutions with high ionic strength in the presence of 1 M NaCl. pH of the solutions was measured by a pH-121 potentiometer (± 0.04 pH units). The concentrations of the substances were determined spectrophotometrically using appropriate dilutions and the following extinction coefficients ϵ^{260} : adenosine, 14.9×10^3 ; the mononucleotides 15.3×10^3 ; ADP and ATP, 15.4×10^3 .

Poly(U) concentration (per nucleotide residue) was determined by the method of (19) which gave the value of $\xi^{260} = 9.4 \times 10^3$, in good agreement with the literature (20).

Calorimetry

The measurements were made in a DASM-1M scanning microcalorimeter (21). The poly(U) - monomer mixture was in the measuring calorimeter chamber and the reference chamber contained, as a rule, the monomer at the same concentration as in the mixture. This was done to compensate the heat effect of nucleotide unstacking (as known, nucleic monomers in aqueous solutions form stacks dissociating with increasing temperature (2)). A similar method was used in (7). In the 0.1M buffer the pH did not change on heating and its value after heating coincided with the initial one within ± 0.1 unit of pH. The high ionic strength made it possible, if necessary, to cool the solution down to -4°C and so to start calorimetric recording at temperatures below 0°C . The heating rate was usually 1 K min^{-1} . The accuracy of temperature determination was $\pm 0.1 \text{ K}$. Since the heat absorption curves were rather symmetrical (see Results) the temperature of the maximum heat capacity change was taken as the midpoint of the transition (the melting temperature). The calorimetric melting enthalpy ΔH_m was determined from the area under the heat absorption curve. The estimated uncertainty of ΔH_m values is about 3%, due mainly to the inaccuracy in determination of poly(U) concentration. The melting entropy was determined as
$$\Delta S_m = \frac{\Delta H_m}{T_m}.$$

Optical Measurements

UV melting curves were obtained in 0.1-cm thermostated cells on a Carl Zeiss Jena VSU-2P spectrophotometer. The reference cell contained the monomer solution of the same concentration as in the mixture with poly(U).

IR spectra were measured on a Perkin-Elmer M-180 spectrometer in thermostated CaF_2 cells of $80 \mu\text{m}$ pathlength. The thickness of the cells was estimated by the interference method with the error less than 1%. The compensation of D_2O absorption in the sample and reference cells was checked against the D_2O band at 3845 cm^{-1} . The compensation of the absorption of light

water admixture was checked against the OH-vibration band at 3420 cm^{-1} .

RESULTS

Poly(U) complexes with adenylic nucleotides.

Figure 1 shows examples of calorimetric recordings for the melting of poly(U) complexes with adenosine and adenylic nucleotides. The monomers were taken in high excess to ensure complete formation of the complexes and to increase their T_m values: the complex melting temperature is known to increase with the free monomer concentration (5,8).

In all cases melting of the complex resulted in only one, rather symmetrical, peak on the heat absorption curve. This indicates that only one complex is formed in a poly(U)-monomer

Figure 1. Microcalorimetric recordings of the melting of poly(U) complexes with adenosine and adenylic nucleotides. The curves are numbered according to Table I (a,b) and II (c,d).

mixture and that the complex dissociates directly to its components as the temperature increases. The heat capacity change upon melting, ΔC_p^d , was in most cases less than the error of its determination (Fig.1). The narrowness of the transitions (the half-widths do not exceed 7 K) provided the rather high accuracy of ΔH_m determination.

The complex formation in the systems studied is completely reversible. This was proved by the complete reproducibility of the recordings at repeated heating of one and the same sample and by the coincidence of the thermodynamic parameters obtained at different heating rates (0.5 or 1 K min⁻¹).

All the calorimetric data are presented in Tables I and II. The ΔH_m values were calculated proceeding from the assumption of a complete complex formation which was ensured by high excess of the monomer. The completeness of the reaction is proved by the constancy of ΔH_m values upon changing the free monomer concentration (Table I). The heat absorption curves for each complex were obtained at least for two values of free monomer concentration. The dependence of melting parameters on pH and on free monomer concentration was studied in more detail for poly(U) complex with 5'-AMP in 1 M NaCl.

The obtained data show that the complex melting enthalpy does not depend on temperature (in the studied range of T_m).

The data of Table II were obtained for solutions without the supporting electrolyte where the high nucleotide concentrations made a significant contribution to the value of ionic strength. Therefore these data refer to solutions of variable ionic strength whereas the results presented in Table I correspond to conditions of nearly constant ionic strength.

As mentioned above, for poly(U) with adenosine mono- and triphosphates only insoluble complexes in Mg^{++} solutions have been found and studied in detail (17,18). The authors of these studies came to a conclusion that the energy of the phase transition (precipitation) is the necessary driving force for complex formation between polynucleotides and nucleotides. In this connection we studied the physical state of the complexes formed in Na^+ solutions.

To analyse the physical state of the solutions, we, as in

Table I. Melting parameters of poly(U) complexes with adenosine and adenylic nucleotides in 1.0 M NaCl, 0.1 M Na-cacodylate^{a)}

No	Mixture composition, (mM)		pH	T _m (K)	ΔH _m (kJ mol ⁻¹)	ΔS _m (J K ⁻¹ mol ⁻¹)
<u>poly(U)+adenosine^{b)}</u>						
1.	1.40	6.82	7.6	297.1	50.9	171
2.	2.41	10.6	7.6	299.8	50.1	167
<u>poly(U)+2',3'- cAMP</u>						
3.	2.67	7.17	7.2	292.7	44.5	152
4.	2.67	14.7	7.2	299.5	45.7	153
5.	2.53	28.0	7.2	304.2	44.9	148
<u>poly(U)+2'(3')-AMP</u>						
6.	1.70	31	5.7	301.4	42.3	140
7. ^{c)}	2.74	32	7.2	300.3	42.7	142
8.	2.84	52	7.2	302.1	42.7	141
9.	2.84	68	7.2	304.3	43.2	142
<u>poly(U)+5'-AMP</u>						
10.	2.71	20.5	7.2	280.3	-	-
11.	2.67	42	7.2	286.1	28.6	100
12.	3.08	61	7.2	288.8	29.0	100
13. ^{c)}	1.44	69	7.2	291.6	28.6	98
14.	2.66	69	8.0	288.6	28.2	98
15.	2.64	68	5.7	290.3	28.2	97
16.	2.61	125	7.2	294.3	28.6	97
<u>poly(U)+5'-ADP</u>						
17. ^{c)}	2.78	30	7.2	284.5	-	-
18.	2.63	33	6.6	287.6	26.1	91
19. ^{c)}	2.78	64	7.2	289.5	26.1	90
20.	2.63	67	6.6	291.2	26.5	91
21. ^{c)}	1.79	88	5.7	293.2	25.7	88
<u>poly(U)+5'-ATP</u>						
22.	2.63	75	7.2	281.5	25.2	90
23.	2.79	113	7.2	284.1	26.0	92

a) The values of ΔH_m and ΔS_m are given per mole of base triplets.

b) In 1.0 M NaCl, 0.05 M Na-cacodylate.

c) In the presence of 0.1 M EDTA.

Table II. Melting parameters of poly(U) complexes with adenosine 2'(3')- and 5'-monophosphates in 0.1 M Na-cacodylate^{a)}

No	Mixture composition		pH	T _m (K)	ΔH _m (kJ mol ⁻¹)	ΔS _m (J K ⁻¹ mol ⁻¹)
<u>poly(U)+2'(3')-AMP</u>						
1.	1.96	60	5.7	290.7	43.1	148
2.	1.96	60	7.2	283.3	42.7	151
<u>poly(U)+5'-AMP</u>						
3.	2.73	139	5.7	283.5	41.0	145
4.	2.73	139	7.2	284.9	41.8	147
5.	2.64	217	7.2	286.3	38.8	135
6.	1.63	305	5.7	295.8	30.1	102
7.	1.63	305	7.2	299.4	31.0	104

a) The values of ΔH_m and ΔS_m are given per mole of base triplets.

(17), followed first of all the appearance of turbidity and the formation of precipitate during cooling poly(U) mixtures with the nucleotides down to 0°C. Under all studied salt conditions the solutions of poly(U) and the nucleotides taken separately remained transparent on storage at 1°C up to 5 days. The mixtures of poly(U) with 2'(3')- and 5'-AMP in 0.1 M Na-cacodylate buffer and poly(U) with 2'(3')-AMP in the presence of 1 M NaCl at the component concentrations given in Tables I and II also remained transparent during 60 h of observation at 1°C. Poly(U) mixtures with 5'-AMP in 1 M NaCl became turbid after 40 to 60 h (depending on AMP concentration). At poly(U) complex formation with ADP and ATP in 1 M NaCl a white precipitate also appeared eventually. The addition of 0.1 M EDTA prolonged the existence of the complexes in the soluble form. The detailed results of our study on the physical state of poly(U) complexes with the adenylic nucleotides and the comparison of the thermodynamic parameters of complex formation in Na⁺ and Mg⁺⁺ solutions will be presented elsewhere.

Even in the mixtures of poly(U) with 5'-nucleotides in 1 M NaCl where precipitation occurs sooner or later, the complexes are soluble for shorter time intervals which is indicated by

their UV-melting curves (Fig.2). The Figure shows that UV-melting is completely reversible and that keeping the complex at 0°C for 3 h results in no increase of light diffusion at 320 nm. (Noteworthy, the complex formation in Mg^{++} solutions could not be detected by UV or ORD spectroscopy because of the precipitation (17)). Prior to melting in the calorimeter the solutions are kept at temperatures near 0°C for 1-2 h, hence our calorimetric data (Tables I and II) refer to the soluble complexes.

Thus, under certain salt conditions (1 M NaCl) the soluble form may be a metastable state of poly(U) complexes with adenylic nucleotides which gradually aggregate to form a precipitate. At low Na^+ concentrations (Table II) the precipitation of the complexes was never observed. Franck and Ackermann (10) noted that poly(U) complexes with AMP and ATP became soluble if Mg^{++} was replaced by sufficiently high concentrations of Na^+ ions. However, the evidence for the existence of these complexes and thermodynamic parameters of the complex formation were not obtained. Our results, in accordance with these authors' statement, indicate that the transition into another phase, i.e. precipitation (17,18), is not a necessary precondition for complex formation between polynucleotides and nucleotides at neut-

Figure 2. Absorbance vs. temperature profiles for the complex between poly(U) (2.92 mM) and 5'-AMP (43 mM) in 1.0 M NaCl, 0.1 M Na-cacodylate, pH 7.2. The complex was formed (○, Δ), then kept at 0°C for 3 h and then melted (●, ▲).

ral pH. It is rather a secondary effect which accompanies the complex formation under certain conditions.

To calculate the values of thermodynamic parameters, one should know the stoichiometry of complex formation. The 2 poly-nucleotide: 1 monomer stoichiometry was found by equilibrium dialysis for poly(U) complexes with adenosine (5,8), and also with 3',5'-cAMP (10). It is difficult to use this direct method for poly(U) complexes with non-cyclic adenylic nucleotides because of the necessity of high monomer excess. Therefore, to characterize the stoichiometry of these complexes, we used IR spectroscopy. As shown by Miles et al. (22,23), characteristic differences are observed between the IR spectra of poly(U)·poly(A) and 2 poly(U)·poly(A) in the 1500-1800 cm^{-1} region. At the same time the IR spectra of triple-helical polynucleotide-polynucleotide (2 poly(U)·poly(A)) and polynucleotide-monomer (Ado·2 poly(U)) complexes are quite similar (4). Figure 3 presents IR spectra of poly(U) mixtures with 5'- and 2'(3')-AMP at different temperatures. Both in the position and intensity of the bands the IR spectra of the complexes between poly(U) and the nucleotides resemble that of the triple-helical 2 poly(U)·poly(A). In particular, in the IR spectra of the complexes the 1657 cm^{-1} band is observed (Fig.3) which is characteristic of the triple-helical complexes and absent in IR spectra of the double-helical ones (23). These data strongly suggest the 2 poly(U): 1 nucleotide stoichiometry of the complexes studied. It is worth to mention that the insoluble poly(U) complexes in Mg^{++} solutions are also of the 2:1 stoichiometry (17,18).

The apparent melting enthalpies for the polynucleotide-monomer complexes were obtained from the dependence of T_m on the free monomer concentration in the midpoint of the transition (8,24):

$$\frac{d \ln C_m}{d \left(\frac{1}{T_m} \right)} = - \frac{\Delta H_{app}}{R} \quad (1)$$

The dependence of $\frac{1}{T_m}$ on $\ln C_m$ obtained from our calorimetric data is well described by a straight line (Fig.4, the UV melting data also included). For the complex between poly(U) and 5'-AMP

Figure 3. Infrared spectra observed during melting of poly(U) complexes with 5'-AMP (a) and 2'(3')-AMP (b) in D₂O. Conditions: 1 M NaCl, 25 mM phosphate buffer, pH 7.2. Concentrations: 7.4 mM poly(U), 25.5 mM 2'(3')-AMP, 42 mM 5'-AMP.

Figure 4. Reciprocal T_m of 5'-AMP-2 poly(U) as a function of C_m . Conditions: 1.0 M NaCl, 0.1 M Na-cacodylate, pH 7.2. Open symbols: calorimetric data, closed symbols: optical data.

this gives $\Delta H_{app} = 92$ kJ/mole of triplets which is three times (!) as high as the direct calorimetric ΔH_m . Large differences between ΔH_m and ΔH_{app} values were found also for poly(U) complexes with adenosine and 2-aminoadenosine (7,8). To correlate the values of ΔH_m and ΔH_{app} , an empirical factor α was introduced (10) the physical meaning of which, as the authors themselves note, is not clear. Our data show that the values of α may differ considerably for poly(U) complexes with different nucleotides: $\alpha = \frac{\Delta H_m}{\Delta H_{app}} \approx 0.3$ for 5'-AMP·2 poly(U) while for 3',5'-cAMP·2 poly(U) $\alpha \approx 0.7$ (10). Moreover ΔH_m of 5'-AMP·2 poly(U) is considerably less than that of Ado·2 poly(U) (Table I) whereas the inverse is true for the ΔH_{app} values of these complexes ((8) and this study). Since we obtained ΔH_m and ΔH_{app} from the same calorimetric data these results demonstrate that Equation 1 does not give true values of the melting enthalpy for polynucleotide-monomer complexes. The reason of this discrepancy remains, however, unclear (cf. (24)).

The mean values of the thermodynamic parameters for poly(U) complexes with adenylic nucleotides in 1 M NaCl are listed in Table III. Since $\Delta C_p \approx 0$, the Gibbs energy of complex stabilization, $\Delta G(T)$, was calculated as $\Delta G(T) = \Delta H_m - T \cdot \frac{\Delta H_m}{T_m}$. The ΔG values are given not only for the standard temperature of 25°C (298.2 K) but also for 0°C. For comparison with the earlier data the values of ΔH_m are given in calories too.

Table III. Thermodynamic parameters of poly(U) complexes with adenylic nucleotides in 1.0 M NaCl, pH 7.2^a)

Nucleotide	2',3'-cAMP	2'(3')-AMP	5'-AMP	5'-ATP ^b)	5'-ATP
$\Delta H_m, \text{kJ mol}^{-1}$	45.0 (10.8)	42.9 (10.3)	28.6 (6.8)	26.1 (6.2)	25.6 (6.1)
$\Delta S_m, \text{JK}^{-1} \text{mol}^{-1}$	151 (36.1)	141 (33.7)	99 (23.7)	90 (21.5)	91 (21.7)
$\Delta G(273.2), \text{kJ mol}^{-1}$	3.7	4.4	1.6	1.5	0.7
$\Delta G(298.2), \text{kJ mol}^{-1}$	-0.03	0.8	-0.9	-0.7	-1.5

a) The values in parentheses are in kcal mol^{-1} (ΔH_m) and $\text{cal K}^{-1} \text{mol}^{-1}$ (ΔS_m).

b) In the presence of 0.1 M EDTA.

Melting of Poly(U) Double Helix

At temperatures near 0°C, poly(U) is known to form a hair-pin-like structure in solutions with high concentration of monovalent cations (25,26) the stability of which increases in the presence of Mg^{++} and diamines (27-29). Earlier the melting of poly(U) double helix was studied by calorimetric methods in solutions of Mg^{++} and diamines (29,30). We made an attempt to estimate the thermodynamic parameters of poly(U) double helix in Na^+ solutions.

Cooling to -4°C and heating at a low rate permitted us to obtain a complete heat absorption curve for poly(U) melting in 1 M NaCl (Fig.5). As seen from Fig.5, the value of T_m is 4.8°C and above 15°C poly(U) is devoid of a secondary structure, which is in accordance with the optical data (25). The same melting parameters were obtained at two concentrations of poly(U): 4.13 mM and 8.19 mM. The thermodynamic parameters of poly(U) double helix in 1 M NaCl are presented in Table IV. The value of ΔH_m obtained in 1 M NaCl is 2.5 times lower than that for Mg^{++} solutions (29,30). For poly(U) melting in 0.04 M MgCl_2 , pH 7.3 we obtained $T_m=6.3^\circ\text{C}$, $\Delta H_m=21.3$ kJ/mole of pairs, in good agreement with the values obtained by reaction calorimetry (29). The great difference in ΔH_m values for Na^+ and Mg^{++} solutions may point to a considerable difference in the poly(U) ordered

Figure 5. Temperature dependence of excess heat capacity of poly(U) in 1.0M NaCl, 0.1M Na-cacodylate, pH 7.6.

Table IV. Melting parameters of poly(U) double helix in 1.0 M NaCl, pH 7.6^{a)}

T_m (K)	ΔH_m (kJ/mole pairs)	ΔS_m (J/K mole pairs)	$\Delta G(273.2)$ (kJ/mole pairs)	$\Delta G(298.2)$ (kJ/mole pairs)
278.0	8.8 (2.1)	32 (7.6)	0.1	-0.7

a) The values in parentheses are in kcal mol⁻¹ (ΔH_m) and cal K⁻¹ mol⁻¹ (ΔS_m)

structures formed in the presence of mono- and divalent cations.

The formation of the poly(U) double helix in 1 M NaCl is accompanied by characteristic changes in its IR spectrum (Fig.6) which are similar to those observed in Mg⁺⁺ solutions (28) and in 0.2 M NaCl (31). As follows from (32), these changes may be caused not only by the formation of base pairs but also by base stacking in the double-helical structure. Therefore the interpretation of the IR spectra of poly(U) double helix taking into account base pairing only (28) must be considered with caution.

DISCUSSION

As indicated above, the melting temperature of polynucleotide-monomer complexes, in contrast to polynucleotide-polynucleotide ones, depends on the free monomer concentration. There-

Figure 6. Infrared spectra of poly(U) double helix (0°C) and coil (30°C) in D₂O. Conditions: 1 M NaCl, 25 mM phosphate buffer, pH 7.2. Poly(U) concentration is 14.8 mM.

fore to establish the possibility of complex formation between a polynucleotide and a monomer under given conditions it is necessary first of all to increase the monomer concentration up to the limit of its solubility. Obviously this was not done in (17), which led the authors to the conclusion that complex formation between poly(U) and adenylic nucleotides in Na^+ solutions is impossible. On the other hand the thermostability of different polynucleotide-monomer complexes can be compared only at equal C_m values (naturally, under the condition of complete complex formation). The analysis of the data of Table I in terms of the relationship between T_m and C_m (see Fig.4) shows that the thermostability of poly(U) complexes with adenylic nucleotides in 1 M NaCl at neutral pH, that is the value of T_m at $C_m = \text{const}$, changes in the order:

$\text{Ado} > 2',3'\text{-cAMP} > 2'(3')\text{-AMP} > 5'\text{-AMP} \geq 5'\text{-ADP} > 5'\text{-ATP}$

The values of ΔH_m for poly(U) complexes with adenine, adenosine and deoxyadenosine were found to be equal (7), hence the substitution of ribose of deoxyribose on the adenine ring has no effect on the enthalpy of the complex formation. Our data on ΔH_m and ΔS_m of $\text{Ado} \cdot 2 \text{ poly(U)}$ in 1 M NaCl (Table I) are close to those obtained in (7) for 0.6 M NaCl.

The results of the present study indicate that the thermodynamic parameters of complex formation between poly(U) and the adenylics, when changing from nucleoside to nucleotide, depend primarily on the position of the phosphate group and not on the value of its charge. The melting enthalpy depends neither on the presence or absence of the terminal phosphate (the data for poly(U) complexes with cyclic 2',3'-AMP and the non-cyclic 2'(3')-AMP) nor on the number of the phosphate groups (the data for poly(U) complexes with 5'-AMP, ADP and ATP). Either, ΔH_m does not depend on the degree of ionization of the phosphate group in the pH region from 5.7 to 8.0. In all these cases changing of the charge on the nucleotide results only in changes of the complex melting temperature.

On the other hand ΔH_m of 2',3'-cAMP $\cdot 2 \text{ poly(U)}$ (Table III) is less than that of 3',5'-cAMP $\cdot 2 \text{ poly(U)}$ (10) (note, however, that the data for the latter complex were obtained by mixing calorimetry). Especially great difference is observed between

ΔH_m and ΔS_m of poly(U) complexes with 2'(3')- and 5'-AMP in 1 M NaCl. The difference in the parameters is not associated with possible admixtures of divalent cations, which is shown by the experiments with the addition of EDTA (Table I).

Unexpectedly, ΔH_m of 5'-AMP·2 poly(U) was found to increase greatly at lowering the ionic strength whereas that of 2'(3')-AMP·2 poly(U) did not depend on Na^+ concentration (Table II). This led to the levelling of the difference in ΔH_m of poly(U) complexes with 2'(3')- and 5'-AMP at low Na^+ concentrations. However the melting temperature of 5'-AMP·2 poly(U) is considerably lower than that of 2'(3')-AMP·2 poly(U) both at high and low ionic strength. The data show that the difference in the values of T_m (at $C_m = \text{const}$) for these complexes reaches 15 K.

It is interesting that the difference between poly(U) complexes with 2'(3')-AMP and 5'-AMP manifests itself also in the pH-dependence of T_m (Tables I and II). Neutralization of the charge on the phosphate group at decreasing pH from 7.2 to 5.7 results in an increase of T_m for 2'(3')-AMP·2 poly(U) both at high and low ionic strength. As for poly(U) complex with 5'-AMP, its T_m increases in 1 M NaCl but decreases considerably at low Na^+ concentrations, when the pH is decreased from 7.2 to 5.7.

At present it is difficult to give a clear explanation for the differences revealed in poly(U) complex formation with 2'(3')- and 5'-AMP. Unfortunately, the similarity between the IR spectra in the $1500\text{--}1800\text{ cm}^{-1}$ region (Fig.3) does not permit an unambiguous conclusion about the similarity of base pairing in these complexes since not only the hydrogen bonding but also the base stacking (32) and other factors contribute to the IR spectra. Thus, a close resemblance is observed between the IR spectra of poly(U) complexes with adenosine and 2-aminoadenosine (4) while the latter complex has a considerably higher ΔH_m and probably one hydrogen bond more than the former one (4,7,8).

The differences in poly(U) complex formation with 2'(3')-AMP and 5'-AMP may be caused by the differences in the properties of the mononucleotides themselves. Let us consider first the differences in stacking of different adenosine monophosphates (33). These differences should manifest themselves in the

heat effect arising from re-distribution of the stacks due to monomer dissociation from the complex through the course of melting. The effect itself, however, must be quite insignificant since not more than 3 per cent of the total amount of the monomer is involved in the complex at the concentrations used (see also (7,10)). As a matter of fact our control experiments (corresponding to nrs. 1,15,20 of Table I) showed that the melting parameters are independent of whether the monomer or the buffer solution is on the tare side of the calorimeter. So, the differences in the degree of association of the mononucleotides cannot account for the large differences in ΔH_m and ΔS_m of poly(U) complexes with 2'(3')-AMP and 5'-AMP.

Secondly, it is known (34,35) that adenosine 2'- and 3'-monophosphates are predominantly *syn* whereas the 5'-phosphates are in the *anti* conformation in which the phosphate group is in an immediate proximity to the H8 of the imidazole ring. Hence there must be a repulsion between the phosphate group of 5'-AMP, on the one hand, and the uracil carbonyl group as well as the phosphates of poly(U), on the other hand. This may hinder the hydrogen bonding at the N7 of the 5'-AMP imidazole. Besides, the conformations of 5'-nucleotides in solution and in the complex with poly(U) may differ considerably, which also hinders complex formation. Obviously, both factors can affect the formation of only triple-helical complexes where the adenine monomers interact with the bases of the second poly(U) strand via the Hoogsteen type of pairing. An increase of the above-described repulsion with decreasing ionic strength may result in a change of the 5'-AMP conformation, the structure of 5'-AMP·2 poly(U) becoming close to that of 2'(3')-AMP·2 poly(U). Naturally this is only a possible explanation which needs further experimental verification.

Finally the differences in hydration of the AMP isomers may play a role in different poly(U) complex formation with 2'(3')-AMP and 5'-AMP. Because of the mutual sterical hindrance between the ribose and the phosphate group 2'(3')-AMP is hydrated to a less extent than 5'-AMP, as it was shown by the recent ultrasonic study (36). Thus, the less hydrated mononucleotide is more capable of complex formation with poly(U).

In view of the found differences in poly(U) complex formation with adenosine 5'- and 2'(3')-monophosphates it is interesting to note that the triple-helical complex between poly(C) and 5'-GMP is also formed with more difficulty (in a narrower pH region, at a lower temperature and a higher Na^+ concentration) than that with 2'(3')-GMP (6). Possibly, this is a manifestation of some common properties of nucleoside 5'-phosphates.

The main result of the present study is the finding that the enthalpy of complex formation between poly(U) and adenylic nucleotides is independent of the charge on the nucleotide molecule. This demonstrates directly that the destabilizing electrostatic interactions of the phosphate groups contribute little to the enthalpy of formation of the ordered structures in polynucleotides and their complexes. In a sense, this follows already from the coincidence (1) of the melting enthalpies for the triple-helical complexes between poly(U) and poly(A), and between poly(U) and adenosine. This conclusion is consistent with the results of the study on stabilization of the native structure of DNA by ionic strength (37) and with the existing theories (38,39) which show that the ion binding effects in polynucleotides and their complexes are primarily of an entropic nature. In connection with the mentioned theories it would be rather interesting to study the mechanism of the found ionic strength-dependence of the enthalpy of poly(U) complex formation with 5'-AMP. The observed peculiarities in complex formation with nucleoside 5'-phosphates may be also of some interest in studying regulation of the process of transcription where ion effects play an important role (39).

After this paper had been prepared we came across a recent IR spectroscopic study on the complexes between poly(U) and adenosine mono- and oligonucleotides (40). The results of this study are consistent with our data. The complex between poly(U) and 5'-AMP was not detected by these authors obviously because of low nucleotide concentrations used.

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