A PROTON MAGNETIC RESONANCE STUDY OF THE INTERACTION OF ADENOSINE WITH POLYURIDYLIC ACID: EVIDENCE FOR BOTH ADENINE-URACIL BASE-STACKING AND BASE-PAIRING*

By Benedict W. Bangerter and Sunney I. Chan

NOYES LABORATORY OF CHEMICAL PHYSICS,†
CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA

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We report here a proton magnetic resonance (pmr) study of the interaction of adenosine with polyuridylic acid in aqueous solution. The results of this study indicate that the mode of interaction is adenosine intercalation and adenine-uracil base-stacking above 26°C, and verifies that a triple-stranded complex which is stabilized by both adenine-uracil hydrogen-bonding and adenine-adenine base-stacking is formed below this temperature. Some information regarding the dynamics of these processes has also been obtained.

Several studies of the interactions between biological bases and nucleosides in aqueous solution[1-3] have shown extensive association by vertical base-stacking, but no evidence for base-pairing by horizontal hydrogen-bonding has been found at the monomer level. In nonaqueous solvents such as dimethylsulfoxide (DMSO) and $CHCl_3$, however, interaction by horizontal hydrogen-bonding is apparently favored.[4-6] The incorporation of a particular base in a polynucleotide has been demonstrated to affect its mode of interaction with monomeric bases and nucleosides in aqueous solution, favoring hydrogen-bonding over base-stacking.[7-9]

Recently, we studied the binding of unsubstituted purine to polyuridylic acid by pmr spectroscopy,[10] and found that at 29°C, purine interacts with the polymer by base-stacking and intercalation only. The adenosine-polyuridylic acid system is of more direct biological interest because of the involvement of adenine-uracil base-pairs in the Watson-Crick bonding scheme. Studies of this system by infrared spectroscopy,[8] and by equilibrium dialysis, solubility, optical rotation, and hydrodynamic methods[9] have shown that adenosine binds to polyuridylic acid in a cooperative manner with a "melting temperature" near 20°C. The complex involves 1 adenosine per 2 uracil bases, and is presumably a rigid triple helix.

Materials and Methods.—Polyuridylic acid (poly U) of mol wt ~100,000 was obtained from Schwarz BioResearch, Inc. The poly U was converted from the original potassium salt to the sodium salt by passing the sample through a column of Dowex 50-W-X8 cation exchange resin and lyophilizing the resulting solution. Adenosine (A grade) was obtained from Calbiochem and was used without further purification. The poly U and adenosine were dried over P_2O_5 at ~25°C, and solution concentrations in D_2O were determined from the weight of the dry materials. A small amount of tetramethylammonium chloride (~0.01 *M*) was added to the solution to provide an internal reference for the chemical shift measurements. No other salt or buffer was added.

The 100 Mc/sec pmr spectra were obtained on a Varian HA-100 nuclear magnetic resonance (NMR) spectrometer operated in the frequency sweep mode. Tetramethylsilane (TMS) in a sealed capillary was used as an external reference and provided the field/frequency lock signal. Chemical shifts reported are referred to the TMS capillary

at 30°C, with the tetramethylammonium resonance used to correct for changes in bulk diamagnetic susceptibility differences between the TMS capillary and the D_2O solution with temperature. Spectra containing broad resonances were enhanced by use of a Varian C-1024 time-averaging computer.

Results and Discussion.—The pmr spectrum of poly U at 30°C, shown in Figure 1*a*, exhibits resonances for the uracil H_6 and H_5 and ribose $H_{1'}$ protons with linewidths of ~2–3 cps, about three times the linewidths observed for the uridine monomer. The H_6 and H_5 resonances are spin-spin doublets with $|J_{H_5-H_6}| = 8.0$ cps, and $H_{1'}$ is a doublet from spin-spin coupling with $H_{2'}$ with $|J_{H_{1'}-H_{2'}}| = 5.4$ cps. At 4°C, the linewidths of the H_6, H_5, and $H_{1'}$ resonances are only about 50 per cent greater than at 30°C, as shown in Figure 1*b*. The chemical shifts change only slightly over this temperature range after correcting for the effect of temperature on bulk susceptibility differences between the TMS reference capillary and the D_2O solution. The coupling constants $|J_{H_5-H_6}|$ and $|J_{H_{1'}-H_{2'}}|$ are virtually independent of temperature over this temperature range. The relatively narrow poly U resonances observed indicate that the polymer assumes a relatively disordered, random coil structure over the temperature range 30°C to 4°C under the present conditions of ionic strength and pD (~7.0).

The effect of adding adenosine to the poly U solution at 30°C is depicted in Figure 1*c*, *d*, and *e*. The low solubility of adenosine in D_2O prevented increasing the adenosine concentration beyond ~0.08 *M*. At 30°C, the addition of adenosine can be seen to shift the three monitored uridine resonances to higher fields. The adenosine-induced shifts are summarized in Table 1, and are compared with the purine-induced shifts observed under comparable conditions in our purine-binding study.[10] These upfield shifts indicate that the adenosine interacts with the polymer by intercalation and stacking of the planar adenine and uracil bases. Such shifts have been shown to result from base-stacking in studies of the interaction of unsubstituted purine with bases and nucleosides,[2] dinucleoside monophosphates[11] and poly U,[10] and are a consequence of the ring-current magnetic anisotropy of the purine base. The relative order of the adenosine-induced upfield shifts of the poly U resonances ($H_5 > H_6 > H_{1'}$), and the magnitudes of these shifts, agree closely with the results of our earlier purine-poly U binding study. Thus purine and adenosine both stack with the uracil bases of poly U to about the same degree. As in the purine-poly U interaction, the rate of exchange of adenosine and uridine residues between bound and free environments is rapid on the NMR time scale ($>\sim 10^3$ sec^{-1}). This base-stacking interaction, which we estimate to involve ~5–10 per cent of the uracil and adenine bases in complex formation at these concentrations, was not evident in the previous studies of this system by other physical methods.[8, 9]

The additional resonances appearing in the spectrum after the addition of adenosine to the poly U solution are those of the H_8, H_2, and $H_{1'}$ protons of adenosine, with the assignments indicated. These adenosine resonances are readily distinguished from the poly U resonances on the basis of their spectral positions and their relative intensities. As in uridine, the $H_{1'}$ resonance in adenosine

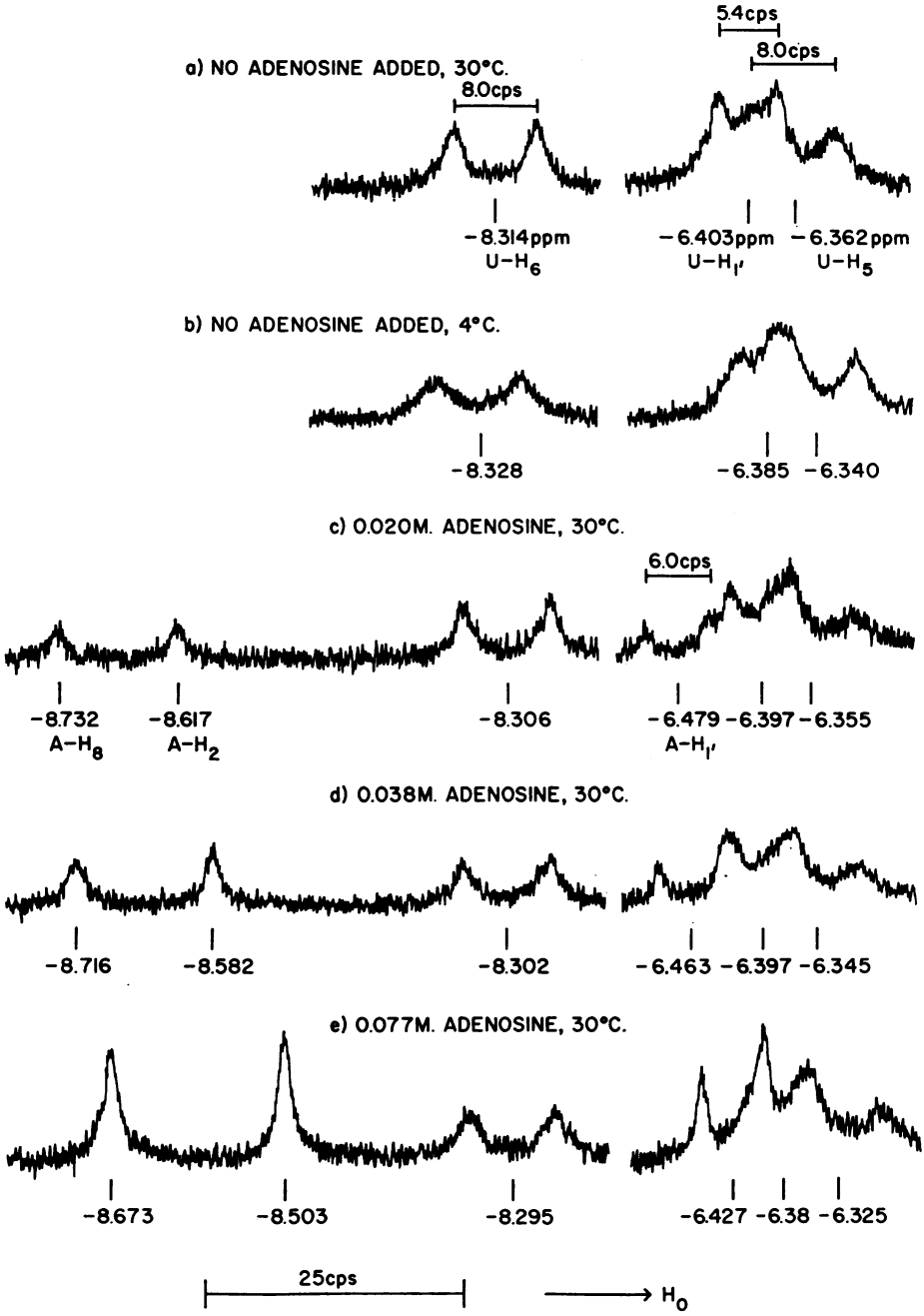

FIG. 1.—Polyuridylic acid (sodium salt), 0.078 *M* in uridine, and adenosine proton resonances at 100 Mc/sec; (*a*) no adenosine added, 30°C; (*b*) no adenosine added, 4°C; (*c*) 0.020 *M* adenosine, 30°C; (*d*) 0.038 *M* adenosine, 30°C; (*e*) 0.077 *M* adenosine, 30°C.

TABLE 1. *Comparison of adenosine-induced shifts and purine-induced shifts for poly U protons.**

		H_6	H_5	$H_{1'}$
Adenosine	Poly U			
0.020 *M*	0.078 *M*	0.8	0.7	0.6
0.038	0.078	1.2	1.7	0.6
0.077	0.078	1.9	3.7	~2.
Purine	Poly U			
0.027 *M*	0.100 *M*	0.6	0.9	0.8
0.054	0.100	1.7	2.2	1.8
0.102	0.100	3.6	5.0	3.5

* Cps at 100 Mc/sec.

also appears as a doublet due to spin-spin coupling with the $H_{2'}$ proton of the ribose ring. The $H_{1'}$-$H_{2'}$ coupling constant is 6.0 cps.

The proton resonances of unsubstituted purine, under similar conditions, experience extreme line broadening in the presence of poly U.[10] This has been shown to result from intercalation of purine between adjacent uracil bases of the polymer. In the present system, at low adenosine concentrations, the H_8 and H_2 resonances are only slightly broadened in the presence of poly U and become narrower with increasing adenosine concentration. The bulky ribose group in adenosine apparently hinders the adenine base from assuming the close proximity to the ribose-phosphate backbone of poly U required for the line broadening to occur. The adenosine resonances are also shifted to higher fields with increasing concentration, a result of the extensive self-association of adenosine which has been demonstrated previously.[3]

Since previous studies of this system[8, 9] indicated an adenosine-poly U interaction only below ~25°C, the effect of temperature on several adenosine-poly U solutions was studied. The results for a solution 0.078 *M* in poly U and 0.038 *M* in adenosine (base ratio A:U = 1:2) are shown in Figure 2. Only the spectral regions involving the adenosine H_2, H_8, and uridine H_6 protons are presented here. At temperatures from 37° to 26°, the linewidths of the monitored resonances are constant and the chemical shifts change only slightly. Below 26°, however, all the resonances broaden markedly with decreasing temperature. This line broadening is of approximately the same magnitude for all the adenosine and uridine resonances. In addition, the adenosine resonances shift abruptly to higher fields with decreasing temperature below 26°, paralleling the linewidth behavior. The resonances of the uridine protons shift little with temperature between 37° and 20°. All the monitored resonances are broadened beyond detection at 4°C.

The narrow temperature range over which the pmr spectral behavior of this system changes, particularly the onset of the extreme line broadening of the proton resonances observed, suggests the formation of a rigid, ordered complex through a cooperative interaction of adenosine with poly U. The absence of detectable resonances at 4° suggests that either all of the reactants present are involved in complex formation or there is rapid exchange of the adenosine and poly U molecules between the free and complexed environments. The upfield

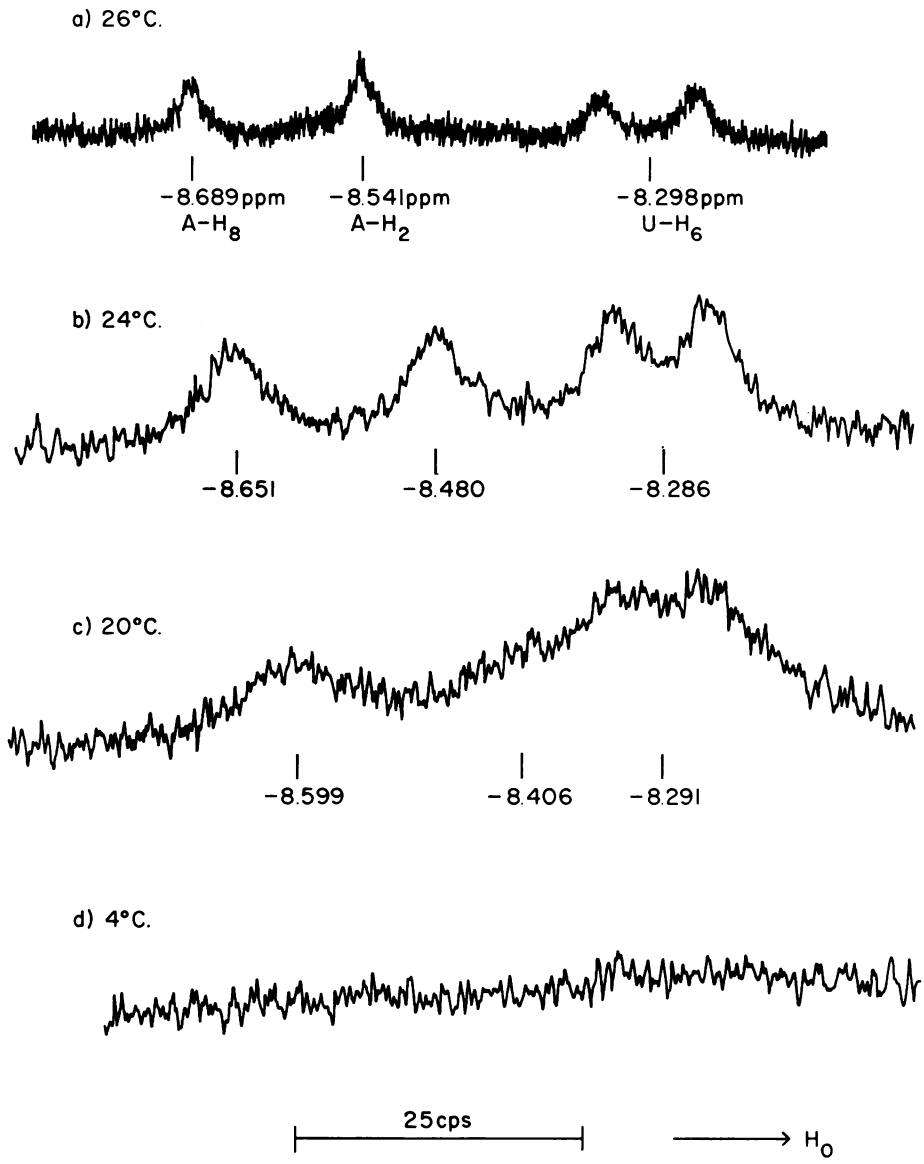

FIG. 2.—Poly U H_6, 0.078 *M* in uridine, and 0.038 *M* adenosine H_8 and H_2 resonances at 100 Mc/sec. Base ratio A:U = 1:2. (*a*) 26°C, single scan; (*b*) 24°C, 17 scans; (*c*) 20°C, 26 scans; (*d*) 4°C, 20 scans.

shifts of the adenine proton resonances observed as the adenosine-poly U complex is formed indicate a high degree of adenine-adenine base-stacking in the complex. The absence of any appreciable shifts in the uracil base proton resonances with complex formation shows that adenine-uracil base-stacking is not involved. Uracil-uracil base-stacking cannot be detected by pmr, since the uracil base does not exhibit measurable ring-current magnetic anisotropy.

The nature of the poly U-adenosine complex below 26°C was further investigated by examining the proton magnetic resonance spectral behavior of a 0.078 *M* poly U solution containing 0.077 *M* adenosine (base ratio A:U = 1:1) over the same temperature range. Above 20°C, the behavior of the base proton resonances in this solution is similar to that for the A:U = 1:2 solution. The adenine H_8 and H_2 and uracil H_6 resonances broaden, and the adenine resonances shift upfield as the temperature is lowered. Below 20°C, however, the adenine resonances begin to narrow with decreasing temperature as shown in Figure 3. The uracil H_6 doublet, however, remains broad beyond detection. From the intensities of the adenine resonances at −3°C and +30°C, it is estimated that the low temperature absorptions of the adenosine proton resonances account for approximately half the total adenosine in solution. These results clearly indicate that the chemical exchange of adenosine between the free and complexed environments is slow on the NMR time scale below ~20°C, and that the stoichiometry of the adenosine-poly U complex formed below 26°C involves 2 uracil bases per adenosine. Thus, the adenosine-poly U complex formed below 26°C is a triple-stranded structure, with stacked adenosine molecules forming horizontal adenine-uracil hydrogen-bonds with two poly U strands. Presumably both Watson-Crick and Hoogsteen hydrogen-bonding schemes are involved. However, in view of the strong tendency for monomeric adenosine to associate in aqueous solution,[3] and the high degree of adenine-adenine base-stacking in the 1A:2U adenosine-poly U complex demonstrated here, it is felt that the vertical interactions between adjacent bound adenosine molecules provide an important part of the driving force toward the formation of the complex.

The variation of the spectral positions of the adenosine H_2 and H_8 resonances with temperature for the A:U = 1:1 adenosine-poly U system sheds additional light on the dynamics of the system. At 21°C, where the spectrum for these protons consists of one broad envelope centered at ~−8.36 ppm, the resonances for these protons are at appreciably higher fields than their normal spectral positions in the absence of poly U. Since the adenosine concentration is in excess of the stoichiometric concentration required for the formation of the poly U-adenosine complex, it is clearly evident that the chemical exchange of the adenosine molecules between the bound and uncomplexed environments is fairly rapid, approaching conditions where the resonances would collapse into an averaged resonance whose position is determined by the weighted mean of the chemical shifts for the two adenosine environments. The resonances of the adenosine protons are at appreciably higher fields in the complex than in bulk solution because of the enhanced adenine-adenine base-stacking in the triple-stranded complex. At 13°C, the onset of slow chemical exchange between the two adenosine environments is apparent. Not only are the H_2 and H_8 adenosine resonances noticeably narrower, but they also appear at considerably lower fields, more characteristic of their positions in the absence of poly U at this temperature. Below 13°C, the spectrum thus consists of relatively narrow resonances of uncomplexed adenosine superimposed on the very broad resonances of the complex. Note that as the adenosine H_2 and H_8 res-

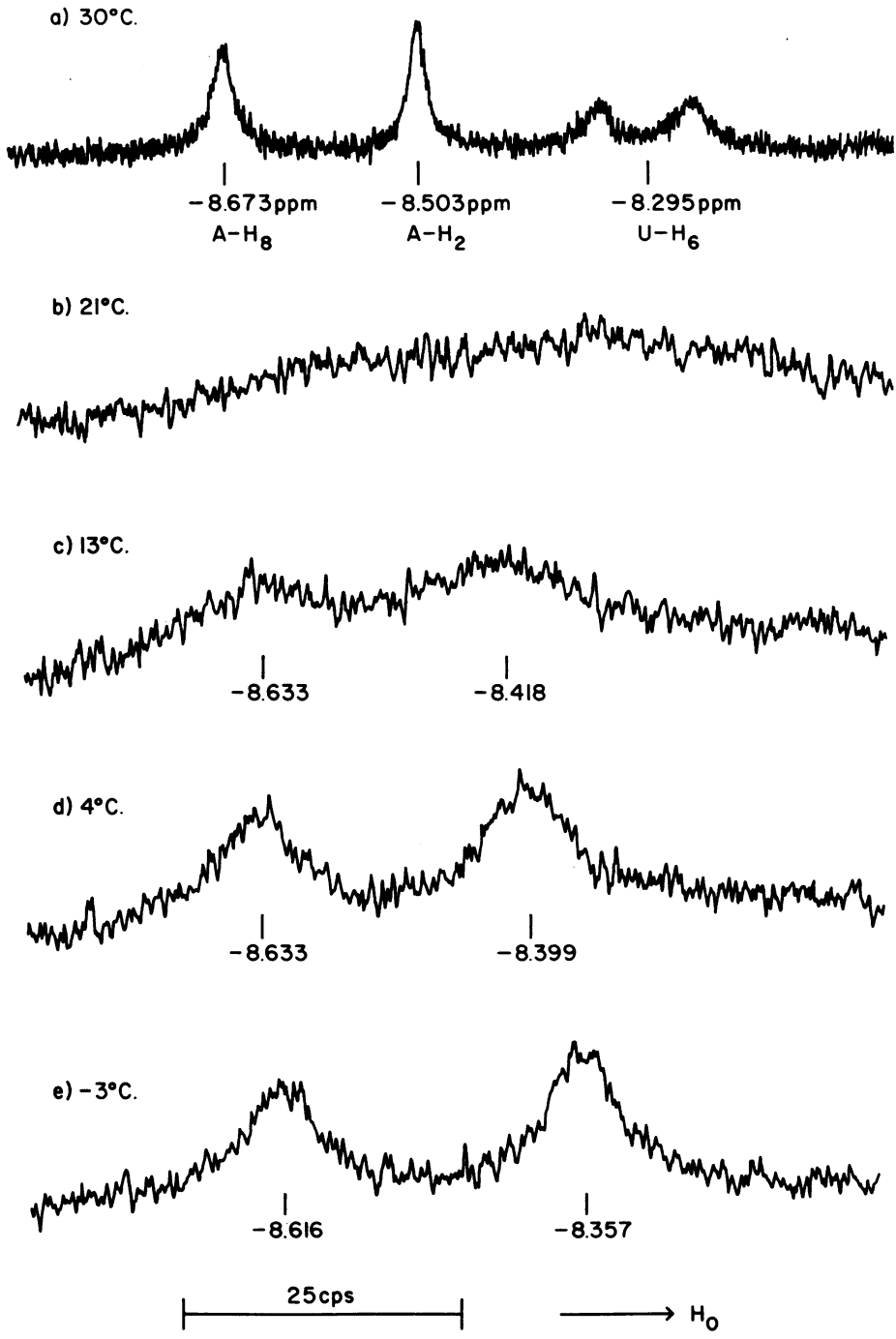

FIG. 3.—Poly U H_6, 0.078 *M* in uridine, and 0.077 *M* adenosine H_8 and H_2 resonances at 100 Mc/sec. Base ratio A:U = 1:1. (*a*) 30°C, single scan; (*b*) 21°C, 20 scans; (*c*) 13°C, 20 scans; (*d*) 4°C, 15 scans; (*e*) −3°C, 15 scans.

onances narrow with decreasing temperature their spectral positions are also shifted to higher fields. These upfield shifts, we feel, arise from the increasing self-association of unbound adenosine in bulk solution with decreasing temperature.

Finally, we note that, even at $-3°C$, the linewidths of the resonances due to the excess adenosine in the poly U solution are significantly broader than those for free adenosine in the absence of the adenosine-poly U complex. Since the HOD resonance and that of the tetramethylammonium standard are still extremely narrow ($\sim$1 cps), these widths cannot be interpreted on the basis of viscosity effects, but instead reflect the rate of chemical exchange of adenosine molecules between the two environments. In the limit of slow exchange, this leads to an additional broadening of the individual resonances by $(1/\pi)(1/\tau)$ (cps), where τ is the mean lifetime of an adenosine molecule in the uncomplexed environment. At $-3°C$, the linewidths of the adenosine H_8 and H_2 resonances (Fig. 3*e*) are $\sim$7.5 cps. The intrinsic linewidths for the free adenosine in the absence of chemical exchange are $\sim$1 cps. From the additional broadening, we thus obtain a lifetime of 5×10^{-2} sec for the free adenosine molecule in solution in the presence of the triple-stranded complex. A similar analysis can be carried out for the adenosine resonances observed at 4°C and 13°C, although the linewidths are less reliable because of weak intensities and significant overlap between the H_2 and H_8 resonances. Lifetimes of $\sim 4 \times 10^{-2}$ sec and $\sim 2 \times 10^{-2}$ sec can be obtained for these temperatures. From the variation of the lifetimes with temperature, we obtain an apparent activation energy of 8 kcal/mole for the exchange process.

Conclusions.—This study reveals two modes of interaction between adenosine and polyuridylic acid, depending upon the temperature. At temperatures above 26°C or so, monomeric adenosine binds to the polymer by noncooperative A-U base-stacking. Below this temperature, a rigid triple-stranded 1A:2U complex is formed, presumably via cooperative hydrogen-bonding, as has previously been reported. These results not only clearly illustrate the importance of base-stacking in nonspecific interactions between bases, nucleosides, and nucleotides, they also reveal the important role of the base-stacking interactions in cooperatively formed structures involving specific base-pairing where such behavior is possible.

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