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VIBRATIONAL RELAXATION ON HYDROGEN BONDING IN DINUCLEOSIDE PHOSPHATE

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The specific interactions between bases, which depend on the dinucleoside phosphate conformations, were studied in terms of the vibrational dynamics of hydrogen-bonding. The hydrogen-bond stretching vibrations of the nucleotide complexes and dinucleoside phosphates were observed in the polycrystalline state by the Raman spectroscopy. The vibrational dynamics were investigated by measuring the line broadenings of hydrogen-bonding vibration observed in near $100cm^{-1}$.

The half band-widths of vibrational lines took their magnitudes from $4cm^{-1}$ to $10cm^{-1}$. The calculations for the relaxation processes revealed that the broadening of the vibrational lines was considered to originate in process, in which the vibrational energy was transferred among the lattice modes.

The doublet spectra observed in the GpC, ApT and GMP-CMP samples were rather symmetrical, in contrast to the asymmetric two peaks of the TpT, ApA, CpC, GpT and AMP-TMP. The relaxation time for the former group was 1.22×10^{-12} sec, whereas that for the latter group was 2.01×10^{-12} sec on the average. The difference between the relaxation times was attributed to that of the sugar-phosphate conformations in a crystal, on the basis of calculating coupled oscillator frequencies with respect to the sugar-phosphate conformations.

The relaxation time, 1.27×10^{-12}sec for the ApT crystal was faster than the one, 1.82×10^{-12}sec for the AMP-TMP crystal. This fact could be also explained by the relaxation processes depending on their crystal structures. The Watson-Crick A-T pairing in the ApT become highly stabilized dynamically, but is in low thermodynamic stability, since this type of pairing is found in the least interaction energy among the possible four base-pairings.

Key words : hydrogen bonding, dinucleotides, relaxation time

INTRODUCTION

It is well known that the four base-pairing schemes between adenine and either uracil or thymine have been observed in single crystal studies [6, 26]. However, Watson-Crick pairing between adenine and uracil has been exclusively observed in dinucleoside monophosphate crystals [19]. This suggests that the observed hydrogen-bonding

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scheme might depend on the sugar-phosphate conformation. We will consider the specific interaction between the bases, which is related to the dinucleoside phosphate conformations from the viewpoint of molecular dynamics.

The dynamic behaviors of base pairing of uracil and adenine derivatives in non-polar solutions were investigated by using the ultrasonic or dielectric relaxation methods [4, 8]. However, since these authors have based their evaluation of the kinetic measurements on the thermodynamic stability of the bonds, the data obtained from their relaxation studies do not yield information on the molecular dynamics of hydrogen-bondings. The associative behaviors of bases in a crystal formation can readily be understood, in part, in terms of the relaxation of the hydrogen-bonding vibrations. On associative collision, the encountered bases are for a moment bound by hydrogen-bonds, but the vibrational energy of hydrogen-bonding is left at the excited state. Unless the vibrational energy is quickly distributed among a number of lattice modes, dissociation into the originally encountered bases occurs within a period of a few vibrations before the bonding is stabilized [5]. Thus, vibrational relaxations might participate in a type of interactions which play an important role in selecting appropriate base-pairs toward certain hydrogen-bonds.

ROTHSCHILD et al. [17] discussed the presence of hydrogen-bonding in liquid $CHCl_3$ from the viewpoint of the dynamics of the vibrational relaxations. They studied vibrational relaxation by investigating the line broadening of Raman spectra in the carbon-hydrogen stretch, and they considered the vibrational relaxations of hydrogen-bonding as processes with the relaxation times from 10^{-11}sec to 10^{-12}sec. Similar time constants are thus expected for the vibrations of hydrogen-bonding in dinucleoside phosphate crystals.

In the present study, the normal modes of hydrogen-bond stretching vibration directly involved in hydrogen-bonding provide the basis for the dynamic process of relaxation. We have observed the line broadening of hydrogen-bond stretching vibrations for a number of dinucleoside phosphate and nucleotide crystals. All observations have been performed in the low frequency region of the Raman spectrum to hydrogen-bond vibration [3].

MATERIALS AND METHODS

Polycrystalline crystals were prepared for samples, since the single crystals of dinucleoside monophosphate complexes were not easy to obtain. Although the Raman spectrum in the polycrystalline state is observed as the sum of the intensities from the individual Raman tensor components, no variation in the Raman line is produced by variaion of the scattering angle or the relative orientation of the light beams and the crystal axis. In fact, the Raman lines of the nucleic acid bases and their complexes did not show essential differences between the single and polycrystalline states [3].

The nucleoside monophosphates of GpC, CpG, ApT, TpA, CpC, TpT, ApA and

GpT were purchased from P. L. Biochemicals and Boehringer Manheim. The samples, in the form of sodiums, were dissolved in dimethylsulfoxide at room temperature. The solutions were kept at 6°C and evacuated stepwise to a vacuum of 10^{-2}mmHg (finally). After a few days of standing, prismic, plate or needle-like polycrystalline crystals began to appear. The sodium salts of 5'-nucleotides were purchased from P. L. Biochemicals and Sigma Chemical Co. and were used without further purification. Equimolar quantities of AMP and TMP, or GMP and CMP were dissolved in dimethylsulfoxide at room temperature, respectively. The polycrystalline crystals were obtained by evacuating the solution at 6°C to a vacuum of 10^{-2}mmHg for a few days. The uv absorption spectra of solutions obtained from the washed crystals suggested a 1 : 1 complex.

The Raman vibrational spectra revealed that the sample crystals did not contain the carbon-sulfur or sulfur-oxygen stretches of dimethylsulfoxide.

The Raman spectra in the low frequency region between 70cm^{-1} and 130^{-1} were observed with a Kawaguchi KR-5C double monochrometer. The Raman scattering system is shown in Fig. 1. The 514.5nm line from an argon laser at a power of 100mW served as the excitation source. 5mg of polycrystalline crystals were loaded into an openning of 2mm diameter and 0.5mm height which had been located in the center of an aluminum cell plate, and were sealed by thin cover glasses.

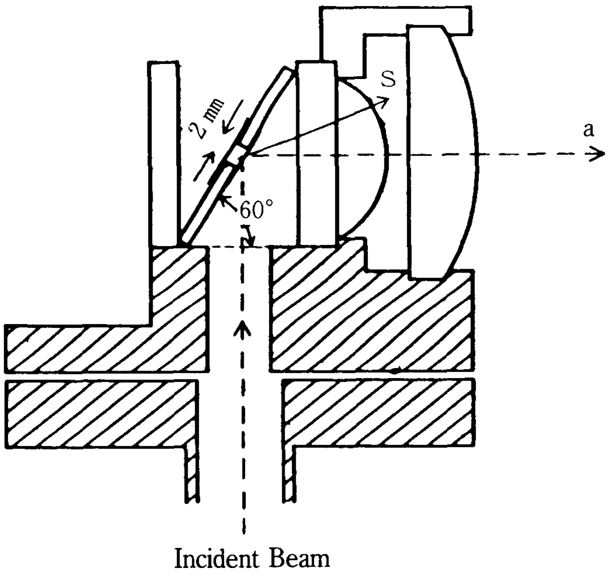

Fig. 1 Raman scattering system. S : scattered Raman, a : axis of scattered light. Scattered light, observed at 90° with respect to the transverse incident beam, was collected by a double lens system. The sample cell plate was placed at 60° to the axis of the scattered light. Both a spherical surface of cell lens and a condensing lens satisfy the aplanatic condition to the center of an inclined plate of 60°.

The spectral slit width was kept 1.0cm^{-1}. The spectrometer was scanned through the bands at a rate of 4cm^{-1}/min with a 5 sec integration time. Temperature control was obtained by placing a sample holder in a small box insulated from heat, through which temperature-controlled N_2 gas was passed. Temperature was automatically regulated by a copper-constantan thermocouple attached to the sample cell plate. The uncertainty in temperature was estimated to be ±1°C.

An instrumental profile was obtained by observing scattering from a solution of latex spheres (0.082 μm) diluted in water and was found to be approximately Gaussian with a typical half band-width of 1.0cm^{-1}. The measured spectrum was then considered to be a convolution of Gaussian instrumental function with the actual spectrum. In our analysis we obtained a least-squares fit of the measured spectrum to a Voigt function as shown in Fig. 2. It was found that the half band-width of the Gaussian component of the Voigt function was equal to the measured instrumental line-width indicating that the actual spectrum was Lorentzian. Since the order of convolution was not important, we simply adopted the measured spectrum as being Lorentzian.

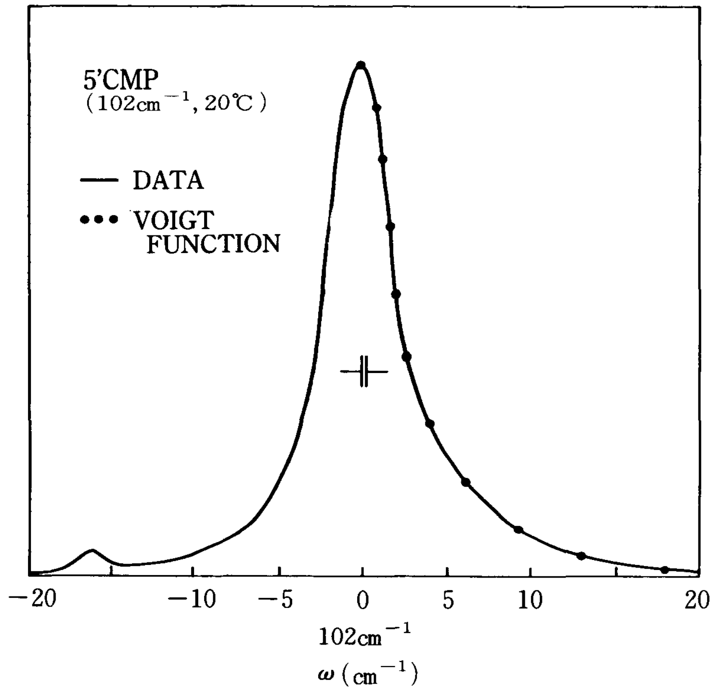

Fig. 2 Raman spectrum, 102cm^{-1} of 5'-CMP at 20°C. To save space only 82cm^{-1} to 122cm^{-1} frequency range is shown. Absissa in cm^{-1}, ordinate in scattering intensity (arbitrary). Voight function fits to a vibrationally broadened 102cm^{-1} Raman line of 5'-CMP at 20°C.
(instrumental width indicated as -||-)

TREATMENT OF DATA AND RESULTS

Studies of the low-frequency vibrations of nucleic acid bases in the infrared and Raman spectroscopies revealed that the pure hydrogen-bond stretching vibrations for these crystals appear in the region between 70cm^{-1} and 125cm^{-1}, and the out-of-plane librations appear strongly in the region below 100cm^{-1} [3, 22]. At present, librations are not available for investigation of the vibrational relaxation process. We thus tried to pick up the hydrogen-bond stretching vibrations among observed spectra in the range of 70cm^{-1} to 130cm^{-1}.

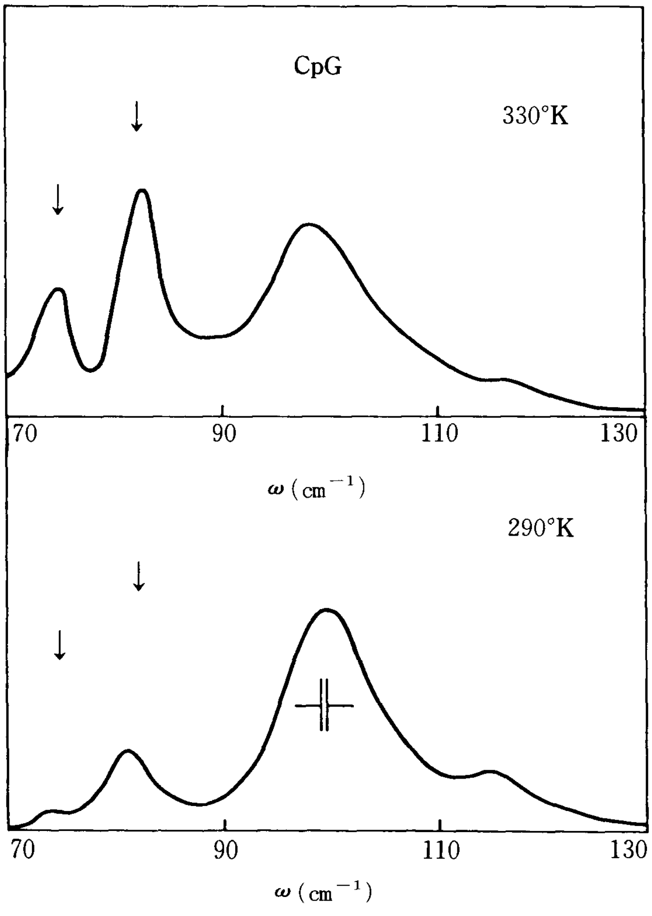

Fig. 3 Raman spectra of CpG at 290 K and 330 K. Base lines are not corrected. Vertical arrows show the change in the intensities with increasing temperature. Temperature control is given in the text. Temperature dependency of Raman spectra is as follows.

Temperature (K)	
290°	vibrations and librations
310°	vibrations and librations
330°	vibrations and strong librations
350°	broadened, weak vibrations and librations

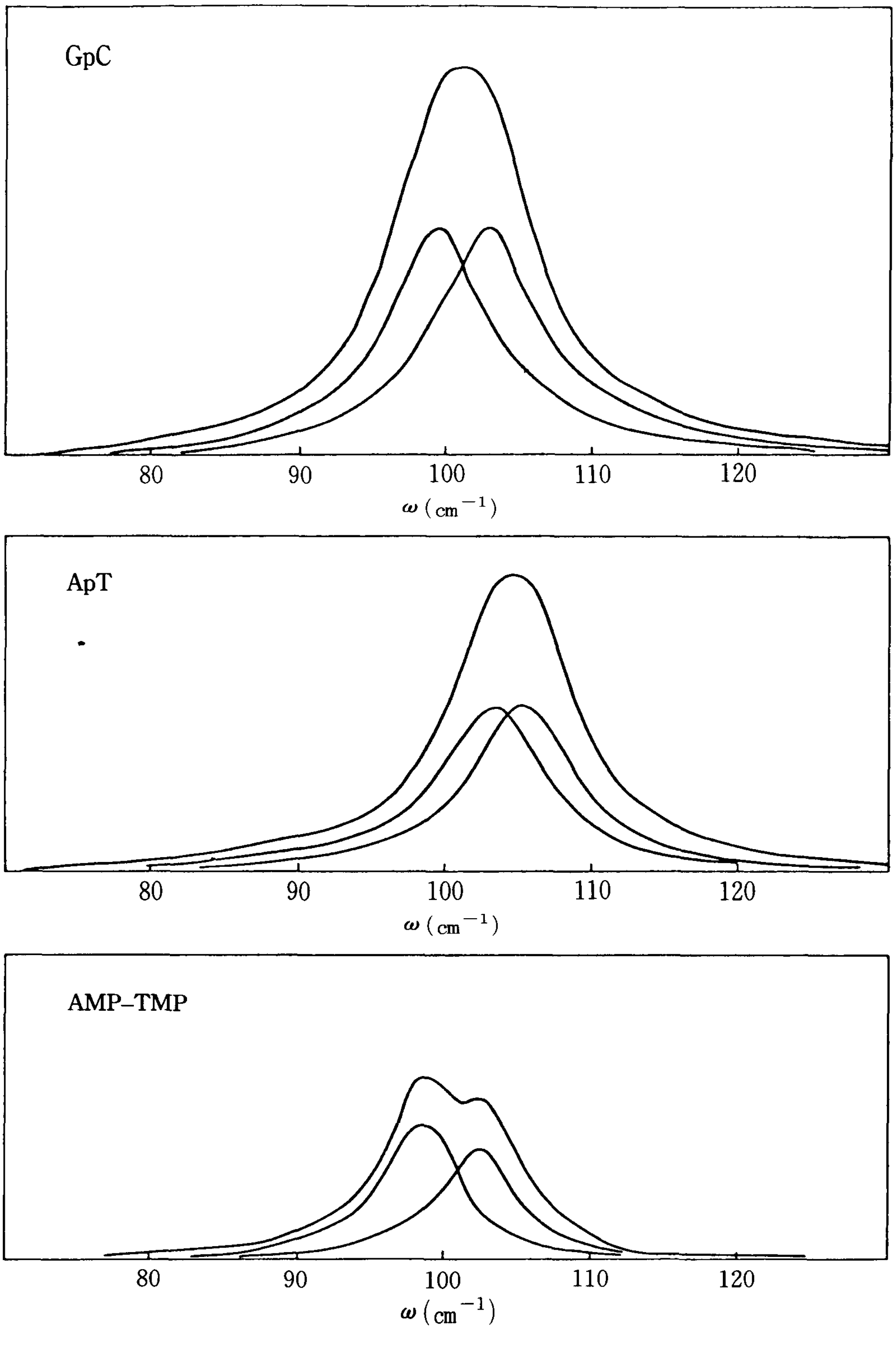

Fig. 4 True spectra of GpC, ApT and AMP–TMP drawn from computations. The observed spectra are fitted to Lorentz functions with a significance of fit less than 0.01 using non-linear least square approximations. The parameters ; peak position, peak height, and half band-width are chosen for a number of line components.

In order to get information about the vibrational modes of the observed frequencies, a normal coordinate treatment for the lattice vibrations was performed on the crystals, from which crystallographic data were available [20]. The calculations gave reasonably better results at the expense of introduction of an additional parameter ; however, these results are limited to the surveys of streching frequencies. In order to eliminate the librations directly from the observed spectra, the changes in the intensity of the Raman lines were checked for temperature increase. As an example, Raman spectra of the CpG at two temperatures, 290°K and 330°K, are shown in Fig. 3. As the temperature rises, the crystal lattice gradually expands, allowing the molecules to rotate freely through increasingly larger angles. The lines, which become stronger with temperature rises, were attributed to the out-of-plane librations (see vertical arrows, in Fig. 3). The lines arising from the librations were excluded from the members of the observed lines. Thus, the lines selected from the observed spectra were employed for the relaxation study, and were tentatively attributed to the hydrogen-bond stretching vibrations.

The spectral shapes are often overlapped with two or more bands. To obtain the true spectrum of the hydrogen-bond stretching vibrations, it was necessary to separate overlapping bands and to approximate the band envelopes by a Lorentzian function. After the raw data were numerically corrected for base line drift with linear and quadratic shape, non-linear least-square analysis served for fitting the envelopes of the singlet and multiplet Raman scattering band system [15]. Typical spectra of the ApT, GpC and AMP-TMP samples are shown in Fig. 4. The observed frequencies in a cm^{-1} unit, relative peak heights, and half band-widths in a cm^{-1} unit for all the observed crystals have been listed in Table 1. No significant difference was found in the Raman lines between ApT and TpA, or GpC and CpG ; therefore the CpG and TpA lines were omitted from the table.

The doublet of the GpC, ApT and GMP-CMP crystals were rather symmetrical, in contrast to the asymmetric peaks of the TpT, ApA, CpC, GpT and AMP-TMP samples. The bands near $100\mathrm{cm}^{-1}$ found in the former group (W) probably arose from the two vibrations of about the same frequency, and were then split into the two peaks with a few cm^{-1} separations by the vibrational coupling which occured between the stretching coordinates of nearest-neighbor molecules. One reason for this is that the double helical geometry would give a close doublet with the definite intensity [7, 24]. Also, the magnitudes of observed splitting can be directly related to the potential which perturbs the isolated molecule energies and thereby couples the motions of pairs of the molecules in a crystal, following the idea of Hexter [7]. The values in the splitting found in the former were in good agreement with those calculated ones from the dipole-dipole interaction energies as the potential between the two base-pairs, in which the interaction energies between the bases were given by Fujita, et al. [2].

On the other hand, the two peaks with several cm^{-1} separations in the latter group

TABLE 1 *Summany of the present results for observed frequencies, relative peak heights, half band-widths and relaxation times calculated from half band-widths*

MOLECULES	FREQUENCY (cm^{-1})	RELATIVE HEIGHT (arbitrary)	HALF BAND-WIDTH[a] (cm^{-1})	RELAXATION TIME[b] ($sec \times 10^{-12}$)
GpC (W)[c]	102	4.0	8.6	1.23
	99	4.0	8.8	1.20
ApT (W)	105	3.0	8.2	1.29
	103	3.1	8.4	1.26
GMP–CMP (W)	110	2.7	8.0	1.33
	107	2.8	9.4	1.13
CpC (N)	101	4.0	4.5	2.36
	95	4.3	4.0	2.65
TpT (N)	106	3.3	5.2	2.04
	100	4.0	6.0	1.77
ApA (N)	110	1.5	5.0	2.12
	104	2.3	5.3	2.00
GpT (N)	108	1.7	6.2	1.71
	100	2.9	5.8	1.83
AMP–TMP (N)	109	2.0	6.1	1.74
	104	2.5	5.6	1.90
(W) -Type			8.57±0.50	1.24±0.07 (SD)
(N) -Type			5.37±0.72	2.01±0.28 (SD)

(a) Half band-width is the width at half-peak height, $\Delta \nu$.

(b) Relaxation time τ is given by $\tau = 1/(\pi c \Delta \nu)$ and c is the velocity of light.

(c) In Text, the former group corresponds to (W) in this table and the latter one (N).

(N) could be attributed to independent hydrogen-bond stretcthing vibrations belonging to the gerade species, where the hydrogen-bond vibrations between bases or other hydrogen-bond vibrations may exist in the lattice modes. After all possible treatments of the data were performed, the Raman lines listed in Table 1 were assigned to the hydrogen-bond stretching vibrations.

Relaxation times in the last column of Table 1 were determined, not by direct integration of the correlation function from the Lorentzian, but from the half band-widths, using

$$\tau = 1/(\pi c \Delta\nu)$$

where τ is the relaxation time and $\Delta\nu$ is the half band-width. As seen in Table 1, it is noticed that the average relaxation time for the former group is $1.24 \pm 0.07 \times 10^{-12}$ sec, while that for the latter group is $2.01 \pm 0.3 \times 10^{-12}$sec (SD). The statistical t-test showed that the value of t was significant under 1% level of significancy.

DISCUSSION AND CONCLUSIONS

Relaxation Mechanism

As seen in Table 1, the half band-widths of hydrogen-bond vibrational lines took their magnitudes approximately from 4cm^{-1} to 10cm^{-1}. The half band-widths were treated as being completely determined by the relaxation processes of the hydrogen-bond vibration. There are three plausible mechanisms which can account for the vibrational relaxation processes observed in crystal lattices : the electrical interaction of dipole molecules, perturbation of the vibrational energy levels (anharmonicity), and vibrational-rotational interaction.

As mentioned above, the dipole interaction between the two base-pairs has been veryfied by a few cm^{-1} splittings of the band for GpC, ApT and GMP-CMP by the idea of correlation field splitting. A calculation was made for the broadening induced by the dipole-dipole interactions between the base pairs, following the idea of Valiev [25, 17]. These broadenings were estimated to be no more than 2.0cm^{-1}. The dipole-dipole interaction does not account for the observed band-widths of the hydrogen-bond vibrations.

The broadening of vibrational lines is also related to the perturbation of the vibrational energy levels, such as anharmonicity. Quantum mechanically, the expression for the band-width due to the third order anharmonic forces is too complex to evaluate explicitly the broadening in relation to the relaxation process in our crystals [14]. Classically, the vibvational energy at the excited mode is distributed among a number of lattice modes through the mutual interactions occuring between modes (vibrational-vibrational energy transfer), and the mode behaves like a damped harmonic oscillator. The spectral broadening of such a random process can be given in terms of the velocity correlation function. In the one dimentional lattice, the correlation function was assumed to be an exponentially damped function of the time t,

$$\rho(t)=e^{-Wt/2N} \quad (1)$$

where W is the oscillator frequency and N is the number of lattice modes. The decay constant, or relaxation time

$$\tau=2N/W \quad (2)$$

is such that in the time τ, a wave front originating at the first excited mode and travelling with the velocity of very long waves in the lattice will pass over around a cyclic chain [18, 23]. In our system, the lattice points are given by a number of momer units, that is, bases, sugars or phosphates of the dinucleoside phosphate crystal. If we take $W=100cm^{-1}$ ($=1.9\times10^{13}sec^{-1}$ as angular frequency) and $N=10$ in a unit cell, the relaxation time is calculated to be $1.06\times10^{-12}sec$, corresponding to the half band-width of $10cm^{-1}$. Although this value seems to be larger than the observed ones, this type of relaxation process explains the observed broadening of the vibrational line.

Energy transfer between the oscillators in a crystal lattice is almost always accompained by internal rotational transfer. Vibrational-rotational energy transfer might be most efficient when the frequency of vibration is low and the moment of inertia of the rotation is small. The hydrogen-bond vibrational frequency, which is approximately $100cm^{-1}$, is rather low, but the moment of inertia, which is roughly $10^{-38}g\cdot cm^2$ for a momer unit, is fairly large. The vibrational-rotational relaxation time may be estimated to be several times slower than the vibrational-vibrational ones. Accordingly, we concluded that the vibrational-vibrational relaxation is a major contributing agency in explaining the observed line broadenings.

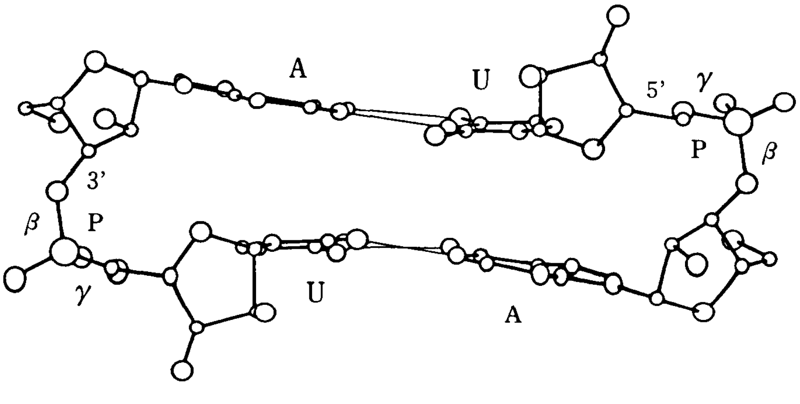

Fig. 5 Molecular structure of ApU double helix proposed by Seeman, et al. (1976). A view of the structure down the dyad axis in which the helical axis is vertical and the bases are viewed edge on. The two torsion angles about the phosphateester bonds are β and γ . For double-helical nucleic acids these will have values near 300°. γ must be in the vicinity of 300° in order to have a structure in which the ribose moieties are roughly parallel, that is a helical structure. When β is 90° or 180°, the relative positions of the base point away from the helix axis.

Conformational Dependence of Relaxation

Since the vibrational mode in a lattice of coupled molecules depends strongly on the lattice geometry, the relaxation process should be quite sensitive to the geometry. A crystal lattice structure of the dinucleoside phosphate dimers for the ApU, which was proposed by SEEMAN, et al. [19] is shown in Fig. 5. KIM et al. [9] showed that the torsion angles, γ and β, about the phosphate bonds are sensitive to the overall structure of the dinucleoside phosphate crystals (Fig. 5). γ must be in the vicinity of 300° in order to have a structure in which the ribose moieties are roughly parallel, that is, a helical structure. It is only when β is also near 300° that the backbone is in the double helical conformation. The backbone torsion angles of the related crystals are listed in Table 2 together with the helical features and hydrogen-bonding aspects.

For calculating the vibrational relaxations of the coupled vibrations in crystals, the W in Eqn. 1 should be replaced by the matrix $[W_{ij}]$ made by the coupled oscillator frequencies. We calculated the distribution of oscillator frequency element $[W_{ij}]$'s for the two sets, one consisted of torsion angles, $\gamma/\beta = 300°/300°$ pairs, and the other of $\gamma/\beta = 300°/180°$ pairs. Detailed computations on this model are in Appendix.

The ratio of the relaxation time of 300°/300° angles to the relaxation time of 300°/180° pairs was calculated to be 0.82. On the other hand, the observed relaxation times for the 300°/300° angle pairs in their structures, were approximately 1.24×10^{-12}sec, whereas the ones for the 300°/180° angle pairs was 2.01×10^{-12}sec on the average (Table 1). The ratio of the relaxation time of the former group (W) to the average ones of the latter group (N) was about 0.61. Hence, for the two possible helical conformations, the ratio between the calculated relaxation times was close to that between the observed relaxation time. The results indicated that the vibrational relaxations of hydrogen-bonding are related to the backbone conformations.

Dynamic Stability of Hydrogen Bonding on Relaxation.

The relaxation process is of crucial importance in forming the hydrogen-bonded complexes. As mentioned in Treatment of Data and Results, the doublets observed in the GpC, ApT and GMP-CMP crystals arose from the two vibrations of about the same frequency. This apparently indicates that these crystals, having 300°/300° torsion angles about the phosphates, should be ordered with respect to the 2_1 helical axis. These crystals must be in favarable conformation to dissipate the vibration energy by travelling along to helix axis. In going from monomers to dimers, the associates of the GpC or ApT become highly stabilized dynamically, since their vibrational energies of hydrogen-bondings are quickly dissipated among a number of lattice modes.

The 4_1 helix conformation has been found for pdTdT [1]. Although an extensive hydrogen-bonding exists, but no base-base bonding occurs. The molecule, in which β angles are near 180°, is less favored to relax the vibrational energy. Although crystallographic data are not yet available for the ApA, some results of an X-ray

TABLE 2 *Backbone torsion angles, their helical conformations and hydrogen-bond aspects for dinucleoside phosphate molecules*

NUCLEOTIDES	TORSION ANGLES[a]		HELICAL FEATURES[b]	HYDROGEN-BOND ASPECTS
	05'–P : γ	P–03' : β	Kim's normenclature	
ApU	285	285	P 3	WC pairing
GpC	285	285	P 3	WC pairing
pdTdT	288	163	P 2	no base pairing
ApA^+	297	181	P 2	base pairing between adenines
A^+pA^+	92	76	A 1	
pdGdC[c]				WC pairing
pdGdT[c]				no base pairing

(a) We adopted the alphabetical nomenclature for dihedral angles in the polynucleotide backbone followed by SEEMAN, et al. (1976)

(b) The nomenclatures for the helical conformations are proposed by KIM, et al. (1973)

(c) NMR studies in H_2O solutions (T. R. KRUGH, M. A. YOUNG, 1975) diffraction study of $Ap^{-}A^{+}p^{-}A^{+}$ have been reported [21]. On the ApA duplex, rotamers about 05'-P and P-03' might have the torsion angles of 300°/180° on the average [10]. Therefore, the vibrational energy between adenines does not become distributed rapidly among the oscillators. The CpC conformation, which is proposed by the NMR study [13], also does not seem to differ essentially from the ApA dimer. The NMR study revealed that the pdGdT does not give evidence of a hydrogen-bonded dimer conformation such as that found in the pdGdC [11]. The close similarities between the solution backbone conformations and those found in the crystalline state for several dinucleosides indicated that the pdGdT crystal was not in a favorable conformation for energy relaxation. Thus, the bands near $100\mathrm{cm}^{-1}$ observed in these crystals have in common the asymmetric two peaks with several cm^{-1} separations, which might be arisen from the hydrogen-bond vibrations between the bases or other hydrogen-bond vibrations. These crystals may be in unfavarable conformation to relax the vibrational energy. We concluded that dinucleoside phosphate dimers of the non-Watson-Crick pairings are less dynamically stable than those of the Watson-Crick base pairings.

Association of Bases

It is widely known that there are the two types of interactions between the bases, hydrogen-bonding and base stacking. Quantum mechanical calculations have been performed to evaluate the relative contributions of the types of interactions to the overall conformation of nucleic acids [2]. Observing the values in Table 3, it is clear that the hydrogen-bonding energies for the G-C pairing are higher than the vertical stacking energies, while the vertical energies for the A-T pairing are higher than the hydrogen-bonding energies. If the hydrogen-bonding terms are predominant over the interactions between the bases, the bases associate preferentially by way of hydrogen-bonding energies. These conditions seem to be applicable to nucleoside and nucleotides. Also, as seen in Table 1, the relaxation time of the GMP-CMP crystals is almost equal to

TABLE 3 *Average stacking ($\overline{S}$) and hydrogen bonding ($\overline{H}$) energies per base pair of double-stranded DNA–like polymers from CNDO methods* (FUJITA *et al.*, 1974)

POLYMER	$\overline{S}$ (eV)	$\overline{H}$ (eV)
(G−C) . (G−C)(a)	−0.201	−0.484
(G) . (C)	−0.134	−0.484
(A) . (T)	−0.131	−0.076
(A−T) . (A−T)	−0.121	−0.076
(A−U) . (A−U)	−0.127	−0.056
(A) . (U)	−0.141	−0.065

(a) (x−y) . (x'−y') indicated poly (x−y) . poly (x'−y')

TABLE 4 *Interaction energies and dipole moments for the four A–T base-pairings*

BASE PAIRING TYPE	INTERACTION ENERGY BETWEEN BASES (eV)[a]				DIPOLE MOMENT[b] (Debye)
	charge charges	charge induced-dipoles	dispersions	total	
Watson–Crick	−0. 20	−0. 01	−0. 03	−0. 24	1. 5
Reversed Watson–Crick					3. 8
Hoogsteen	−0. 26	−0. 01	−0. 04	−0. 30	7. 1
Reversed Hoogsteen	−0. 24	−0. 01	−0. 04	−0. 29	5. 3

(a) These data were obtained from quantum chemecal computations by PULLMAN, et al. (1969)

(b) These data were given by R. F. W. HOPMANN (1973) that of the GpC or ApT. Although no crystal structure have been available for the GMP-CMP as yet, the GMP-CMP might be in the same double helical conformation as the GpC, in which the vibrational energy can be quickly distributed among the lattices. In view of the above discussions, we concluded that the G-C pairing are formed with both highly thermodynamic and dynamic stability.

On the other hand, the association of A-T pairs, in which the vertical stacking energies are higher than the hydrogen-bonding energies, results primarily in vertical stacking of the bases. At this stage, a further optimization is required for the base pairing between adenine and thymine. In going from monomers to dimers, it is possible to select a favorable pairings on its way among the four base pairing schemes between adenine and thymine. The interaction energies between adenine and thymine have been calculated for four types of base pairings on the assumption that interaction energies are originated from electrostatic and dipole derivative forces [8, 16] (Table 4). Comparison of the pairing schemes showed that the Hoogsteen arrangement is the most stable one in the case of the A-T pairing. This type of hydrogen-bonding has been generally found for associations between adenine and thymine.

However, pairing of thymine and adenine in the nucleic acids is largely due to the conformation of the polynucleotide backbone, since the pairing alone gives rise to a unit which is able to fit into a regular double-strand helix. The conformational dependence of the pairing can be understood, in part, in terms of the relaxation process. The relaxation time, 1.29×10^{-12}sec or 1.26×10^{-12}sec, found for the ApT crystal, is faster than the relaxation time, 1.74×10^{-12}sec or 1.90×10^{-12}sec, for the AMP-TMP. The ApT forms a small segment of a right-handed anti-parallel helix in the crystal, which is able to dissipate quickly the hydrogen-bonding energy into lattices. On the contrary, in light of our present knowledge it is unlikely that the Watson-Crick type A-T pair will be formed when AMP is paired with TMP.

As seen in Table 4, the Watson-Crick A-T pairing is found in the least interaction energy among the four pairings. Not only the hydrogen-bonds in their own are required to fit the pairing into the double strand helix with a highly dynamic stability. The hydrogen-bonds are also required to separate easily the complementary stands with low thermodynamic stability. The Watson-Crick A-T base pairing meets the two requirements best : high dynamic and low thermodynamic stability.

Appendix : Coupled Vibration of a Cyclic Chain

A simplified model of the normal mode vibration was chosen to represent to a cyclic chain of particles, which consists of four base-sugar units having the same mass m, respectively (Fig. 5). The nearest neighboring particles are connected by elastic springs whose force constants are expressed as K_B, K_X, K_B, and K_Y, in which K_B is one force between the bases, K_X or K_Y are the other forces between two base-sugar units. The phosphates are only treated as being of a swiveling nature. K_X is given by the vector sum of the two forces, which are derived from the two torsion angles of β and γ, as is K_Y. The vector confined by the two torsion angles of the backbone was estimated from the CPK-atomic model (Ealing Scientific, London). When the torsion angles of γ and β have the values of 300° and 300°, the force vectors acting to the phosphates from the base-sugar units are nearly parallel, whereas when their angles are 300° and 180° the vectors point in the direction of right angles each other. The solution of such a coupled oscillations on this model can be handled best using the matrix method.

The statistical evaluation of the autocorrelation function of Eqn. 1 is identical that for magnetic relaxation which starts with a Chapman-Kolmogorov equation [12]. The autocorrelation functions are made up of the superpositions of the exponential by the damping function with eigen values of $[W_{ij}]$.

$$\rho(t) = \frac{1}{2}\left(e^{-1.1t} - e^{-3.63t} - e^{-7.0t}\right) \quad \text{for } 300°/300° \text{ pairs}$$

$$\rho(t) = \frac{1}{2}\left(e^{-0.77t} - e^{-2.57t} - e^{-4.94t}\right) \quad \text{for } 300°/180° \text{ pairs}$$

The overall relaxation time of Eqn. 2 is the negative reciprocal of the sum of [W]'s.

REFERENCES

1) CAMERAMAN, N. & FAECETT, I. K. (1973) : Structure of a dinucleid *Science* **182**, 1142–1143

2) FUJITA, H., IMAMURA, A. & NAGATA, C. (1974) : A molecular orbital study of a stability and the conformation of double-stranded DNA-like polymers *J. Theor. Biol.*, **45**, 411–433

3) HARADA, T. & LOAD, D. C. (1970) : Low-frequency IR and Raman spectra of some adenine and uracil crystals *Spectrochimica Acta*, **26A**, 2305–2318

4) HAMMES, G. G. & PARK, A. E. (1968) : Kinetic studies of hydrogen bonding *J. Amer. Chem. Soc.* **90**, 4151–4157

5) HAMMES, G. G. (1978) : Principle of chemical kinetics ; simple kinetic theory of collision dynamics, p.31, New York, Academic Press

6) HOOGSTEEN, K. (1963) : The crystal and molecular structure of a hydrogen-bonded complex *Acta Cryst.*, **16**, 907–916

7) HEXTER, R. M. (1960) : Intermolecular coupling of vibrations in molecular crystals *J. Chem. Phys.*, **33**, 1833–1841

8) HOPMAN, R. F. W. (1973) : A chemical relaxation study of base pairing *Berichte der Busen-Geselschaft.*,**77**, 52–60

9) KIM, S. H., BERMAN, H. M., SEEMAN, N. D. & NEWTONE, M. D. (1973) : Seven basic conformations of nucleic acid structure units *Acta Cryst.*, **29**, 703–709

10) KONDO, N. & DANYLUK, S. S. (1976) : Conformational properties of ApA in aqueous solution *Biochemistry*, **15**, 756–768

11) KRUGH, T. R. & YOUNG, N. A. (1975) : NMR studies of hydrogen bonded complexes of oligonucleotides in aqueous solution *Biochem. Biophys. Res. Comm.*, **62**,

1025–1031

12) Kubo, R. (1962) : The fluctuation-dissipation theorem in stochastic theory of line shape relaxation (D. Ter Haas ed.) p.24–62, London, Oliver and Boyd

13) Lee, C. H., Ezra, F. S., Kondo, N. S., Sarm, R. H. & Danyluk, S. S. (1976) : Conformational properties of dinucleoside monophosphate in solution *Biochemistry,* **15**, 3627–3639

14) Maradudin, A. A. & Fein, A. E. (1961) : Scattering of neutrons by an anharmonic crystal *Phys. Rev.,* **128**, 2589–2608

15) Pitha, J. & Jones, V. (1966) : A comparison of optimization methods for fitting curves to infrared band envelopes *Canad. J. Chem.,* **44**, 3031–3056

16) Pullman, B. & Pullman, A. (1969) : A molecular oribital study *Progr. Nucl. Acid Mol. Biol.,* **9**, 327–402

17) Rothschild, W. C., Rasaco, R. C. & Livingston, R. C. (1975) : Dynamics of molecular reorientational motion and vibrational relaxation in liquid *J. Chem. Phys.,* **62**, 1253–1268

18) Rubin, R. J. (1960) : Statistical dynamics of simple cubic lattices *J. Math. Phys.,* **1**, 309–318

19) Seeman, N. C., Rosenberg, J. M., Suddath, F. L., Kim, J. J. P. & Rich, A (1976) : RNA double-helical fragments at atomic resolution *J. Mol. Biol.,* **104**, 109–144

20) Shimanouchi, T. & Harada, I. (1964) : Far-infrared spectra of cyanuric acid and uracil *J. Chem. Phys.,* **41**, 2651–2655

21) Suck, D., Manor, D. C., Germain, G., Schwalbe, C. H., Weiman, G. & Saenger, W. (1973) : X-rays study of helix loop and base pair stacking *Nature New Biology,* **246**, 161–165

22) Susi, H., Ard, J. S. & Purcell, J. M. (1973) : Vibrational spectra of nucleic acid constituents *Spectrochimica Acta,* **29A**, 725–733

23) Toda, M. (1962) : Damping behaviors and space dimmentions *Prog. Theor. Phys. Suppl.,* **23**, 172–176

24) Turrell, G. (1972) : Infrared and Raman spectra of crystal ; factor group analysis of molecular crystals p.113, p.193, New York, Academic Press

25) Valiev, R. A. (1951) : On the theory of line widths in vibrational and Raman spectra of molecules *Opt. Spectrosc.,* **11**, 253–255

26) Voet, D. & Rich, A. (1970) : Hydrogen bonding between uracil and adenosine *Prog. Nucleic Acid Res. Mol. Biol.,* **10**, 183–265