Humidity-dependent structural transition of guanosine and disodium adenosine 5′-triphosphate crystals studied by low-frequency Raman spectroscopy

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The structural transitions of guanosine and disodium adenosine 5′-triphosphate (Na_2·ATP) crystals controlled by relative humidity are monitored by low-frequency Raman spectroscopy. Although the water absorption-desorption curves against relative humidity show large hysteresis, the transition is reversible in both cases. Five well-defined states of guanosine and four well-defined states of Na_2·ATP are distinguished, some of which are already known through x-ray analyses. In these states stacked bases form a long column. The transition schemes among well-defined states have the following characters. Microcrystals of guanosine undergo a phase transition, that is, the transition takes place cooperatively at a critical relative humidity. On the other hand, the transition of Na_2·ATP in the humidity range from 10 to 50% is characterized as a gradual addition of water molecules, resulting in the coexistence of two states without long correlation length.

INTRODUCTION

It is well known that the conformational transition of the sodium salt of DNA between the *A* form and the *B* form is controlled by the hydration number.[1] The transition is usually achieved by changing the relative humidity (RH). Similarly, the hydration number of nucleotide and nucleoside crystals is a function of relative humidity.[2] The crystal structure is expected to change according to the hydration numbers as in the case of DNA. However, structural analyses at various relative humidities have been carried out only on disodium inosine 5′-phosphate (Na_2·IMP) (Ref. 3) and disodium adenosine 5′-triphosphate (Na_2·ATP) (Ref. 4) as far as we know. Further, the water absorption-desorption curves of nucleotide crystals against RH show fine structure,[2] which suggests that there exist several stable states in each nucleotide crystal as indicated in ATP crystals.[4] Studying the method of hydration of nucleotide or nucleoside crystals will benefit in clarifying the hydration scheme of DNA. This system will also be a model case of hydration-induced structural transition of molecular crystals.

Through x-ray diffraction studies we found that nucleoside and nucleotide crystals undergo structural transitions in the course of water absorption and desorption processes. For guanosine crystals we distinguished three well-defined states.[5] In a water absorption process, a Na_2·ATP crystal takes different crystal structures, two of those being already determined.[4,6,7]

We reinvestigated gravimetric measurements on various nucleotide and guanosine crystals, changing the relative humidity at room temperature. The water absorption-desorption curves against RH show large hysteresis. Moreover, in some cases, these curves show a steplike shape, that is, the curves have plateau regions. It is suggested that the plateau regions generally correspond to different crystal states. In this paper, we report the results on guanosine and Na_2·ATP crystals. The results on disodium citidine 5′-phosphate (Na_2·CMP) and disodium adenosine 5′-phosphate (Na_2·AMP) will be reported elsewhere.

The change of crystal structure can also be monitored by low-frequency Raman spectra, since in the spectra there appear phonon modes which are sensitive to the structure. We report here the spectral changes of guanosine and Na_2·ATP crystals on varying the relative humidity. We confirm the existence of five different crystal states of guanosine, three of them being already known, and we distinguish four crystal states of Na_2·ATP.

In a previous paper,[8] we reported the existence of a Raman mode of nucleotide crystals, characteristic of the column structure of stacked bases. We called this mode the *S* mode; it lies around 20 cm^{-1} and is the lowest-frequency Raman-active mode in many cases. The *S* mode of these crystals has similar character to the *S* mode of the DNA double helix and polyriboguanylic acid [poly(rG)] quadruple helix.[9,10] Using the *S* mode we will discuss the manner of stacking of the bases in various crystal states.

The changing scheme of the transient spectra between two stable states of ATP is different from that of guanosine. The characteristics of the crystal transitions of ATP and guanosine are also discussed.

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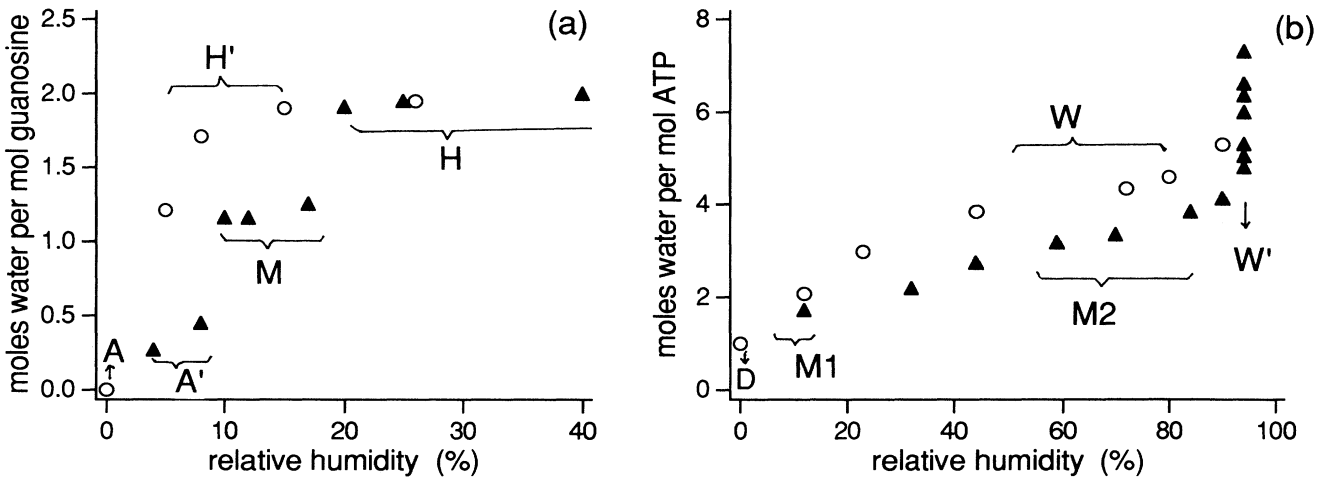

FIG. 1. Hydration numbers of guanosine (a) and Na_2·ATP (b) against relative humidity are plotted. Open circles correspond to the dehydration process and closed triangles correspond to the hydration process. The values of relative humidities include errors of ~2%. The ranges distinguished by capital letters are those where the Raman spectrum remained unchanged.

EXPERIMENT

Guanosine and Na_2·ATP were purchased from Yamasa Shoyu and Oriental Kobo, respectively. Guanosine was recrystallized from aqueous solution, and Na_2·ATP was recrystallized according to the method given in Ref. 4. The microcrystalline powder was mounted on a holder in a sealed humidity cell for Raman measurements. Relative humidity was controlled using saturated salt solutions.[11] The time development of the spectra was monitored to confirm the equilibrium state.

Raman spectra of low- and intermediate-frequency regions at room temperature were obtained using double-grating spectrometers Jobin-Yvon U-1000 and SPEX 1400, respectively, with photon-counting electronics. The excitation was made by the 488 and 514.5 nm lines of an Ar ion laser (NEC GLS 3200).

RESULTS

Hydration numbers of guanosine and Na_2·ATP crystals at typical relative humidity are plotted in Fig. 1. The water desorption and absorption processes show a large hysteresis. Capital letters represent the independent states of each crystal, which will be discussed in the following section.

Raman spectra of guanosine crystals at various RH's are shown in Fig. 2. The spectra marked by capital letters in Fig. 2 are observed at the RH range marked by the same letters in Fig. 1. Raman spectra *i*1 and *i*2 are the transient spectra. The series of spectra *A*-*A'*-*i*1-*M*-*i*2-*H* is observed in the course of a hydration process, while the series of spectra *H*-*H'*-*A'*-*A* is observed in the course of a dehydration process. The peak frequencies are listed in Table I together with those of Na_2·ATP.

The spectra *A* and *A'* are similar. The broadbands at 50–100 cm^{-1} become intense in the spectrum *A'*. The spectrum *H'* observed in the dehydration process is characterized by the absence of the 47 cm^{-1} mode of the spectrum *H*. The transient spectra *i*1 and *i*2 can be constructed by adding the neighboring two spectra, *A'*-*M* and *M*-*H*, respectively. Thus we obtain five independent spectra which correspond to five states. It is noted that the lowest-frequency mode is sharp and strong in every spectrum except in spectrum *M* which appears in the RH region between 10% and 20% of the hydration process.

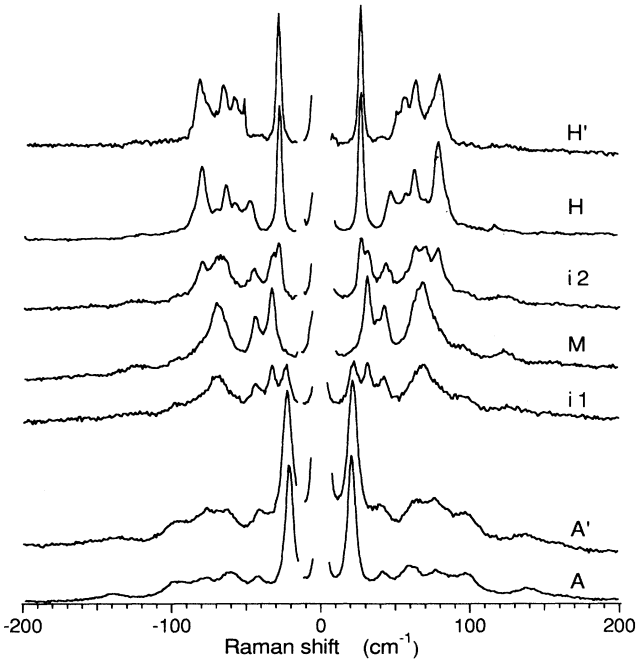

FIG. 2. Raman spectra of guanosine at various relative humidities. Capital letters correspond to the humidity ranges shown in Fig. 1(a). The spectra *i*1 and *i*2 were obtained at ~10% and ~20% RH, respectively. The order of spectral change corresponding to the hysteresis loop of hydration numbers is *H* (as grown) -*H'*-*A'*-*A*-*A'*-*M*-*H*.

TABLE I. Raman frequencies of low-lying modes of guanosine and ATP crystals. Note b: broad-band, w: weak band, and sh: shoulder.

States		Frequencies (cm^{-1})							
guanosine	*A*	21		41	60	77	96	138	
	A'	22		42	65b	75	95	135	
	M	32		43	66 sh	69	95b	125b	
	H	27		47	57	63	70w	80	
	H'	27		51	57	64		80	
Na_2·ATP	*D*	25	30	41		62	84		
	*M*1	23sh	30	44	56	69	84	95	121
	*M*2 17	23.5	35	44w	56b		80		
	W	23.5	28sh				80b		

Figure 3 shows the Raman spectra of Na_2·ATP at various relative humidities. The lower six spectra from *D* to *W'* show spectral changes with increasing relative humidity. The spectrum *W* is the one at 50% RH in the dehydration process from 95% RH. The spectra *D*, *M*1, *M*2, *W'*, and *W* are observed in the corresponding RH ranges indicated in Fig. 1. The spectra *i*1 and *i*2 are the transient spectra from the spectrum *M*1 to *M*2, and from the spectrum *M*2 to *W'*, respectively. The series of spectra *D*-*M*1-*i*1-*M*2-*i*2-*W'* is observed in a hydration process and the series of spectra *W'*-*W*-*M*1-*D* is observed in a dehydration process. The spectrum *W'* can be regarded to be the same as the spectrum *W*, except for some spectral activity in the 15–20 cm^{-1} region, since the peak frequencies in both spectra are the same. This point will be discussed in the next section. Then we can regard the four spectra *D*, *M*1, *M*2, and *W* (*W'*) in Fig. 3 to be independent. This means that four states are distinguished from the Raman spectroscopic point of view. In the spectra *M*1, *M*2, and *W* (*W'*), there exists a sharp and strong peak at ~25 cm^{-1}. The weak lowest-frequency mode lies at 15 cm^{-1} in the spectrum *M*2. The spectrum *D* has double peaks at ~25 cm^{-1}, instead of a single peak nearby.

The spectrum *i*1 in Fig. 3 is a typical one observed in the relative humidity range from 20% to 44%. We observed the RH-specific spectrum, which cannot be represented by the addition of the spectra *M*1 and *M*2. For example, the phonon peaks at 55 and 95 cm^{-1}, characteristic of the spectrum *M*1, disappear at ~20% RH, while traces of other peaks remain. Phonon peaks observed in the spectrum *M*2 gradually appear with increasing RH. The peaks at 24 and 30 cm^{-1}, which are characteristic of the spectra *M*1 and *M*2, respectively, both appear in the intermediate spectrum. The intensities of these two peaks interchange with increasing relative humidity.

The spectrum *i*2 in Fig. 3, which is observed after increasing RH up to 95%, differs from the spectrum *M*2 in the intensity of the central region below ~20 cm^{-1}. The intensity at 13–17 cm^{-1}, the dip region between the central component and the 23 cm^{-1} mode, increases before the spectrum changes to the spectrum *W'*.

Figure 4 shows the Raman spectra of the intramolecular vibrational region corresponding to the four independent states of ATP in the range from 600 to 1600 cm^{-1}. The spectra *D* and *M*1 are similar, and the difference between *M*2 and *W* is small, while the spectrum *M*1 clearly differs from the spectrum *M*2.

DISCUSSION

The peaks in the Raman spectra shown in Figs. 2 and 3 are phonon modes which are characteristic of the crystal structure. From the fact that in most cases there exist well-defined phonon modes, we conclude that the crystals undergo structural transitions preserving crystal states. In other words, no overall disordered state appears in the course of structural transitions. This coincides with x-ray results showing that these compounds continue crys-

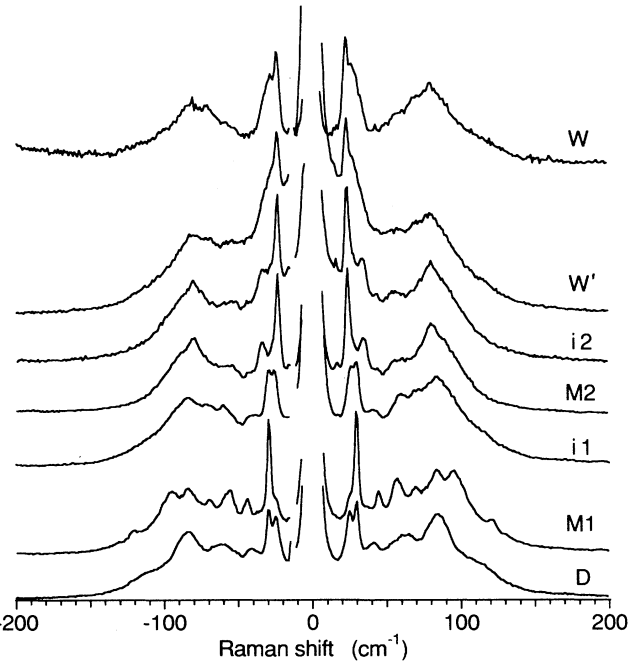

FIG. 3. Raman spectra of Na_2·ATP at various relative humidities. Capital letters correspond to the humidity ranges shown in Fig. 1(b). The spectra *i*1 and *i*2 were obtained at ~20% and ~95% RH, respectively. The order of spectral change corresponding to the hysteresis loop of hydration numbers is *W* (as grown) -*M*1-*D*-*M*1-*M*2-*W'*-*W*.

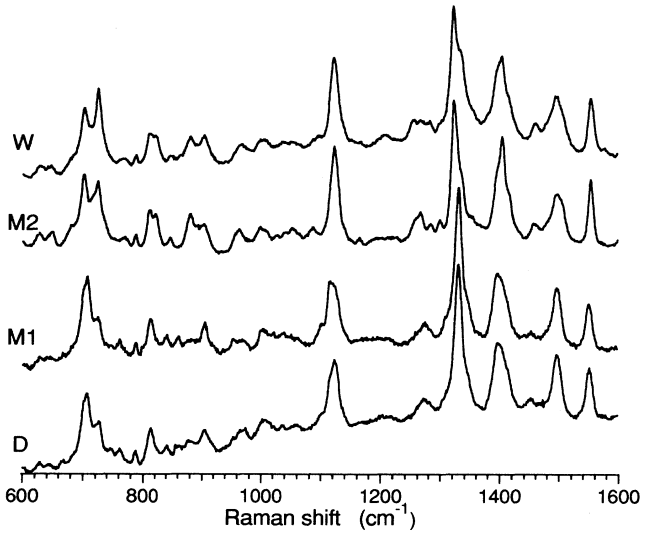

FIG. 4. Raman spectra of Na_2·ATP in the intramolecular vibrational region. Capital letters have the same meaning as those in Fig. 3.

talline during the transitions except for the *A* state of guanosine.[4,5]

The sharp low-frequency mode at 20–30 cm^{-1} appearing in most spectra is referred to as the *S* mode according to the abbreviation given in the previous paper.[8] The Raman intensity of the *S* mode becomes salient when the bases stack to form a columnar structure. When the stacking manner is better, the *S* mode becomes sharper and stronger. We conclude that this mode originates from the cooperative motion of bases stacked in a column. The frequency is influenced not only by the manner of base stacking but also by the surroundings of a column. The origin of other low-frequency bands has not yet been clarified.

Now we discuss the crystal transition monitored by Raman spectra.

A. Guanosine

The independent Raman spectra *H*, *H'*, *A'*, *A*, and *M* correspond to the different crystal states *H*, *H'*, *A'*, *A*, and *M*, respectively. From the gravimetric measurement and x-ray powder diffraction study, three crystal states, *H*, *A*, and *M*, are distinguished.[5] The Raman spectral change indicates that there exist two more states, the *H'* and *A'* states, in addition to these three states. Although the structure of guanosine dihydrate (the *H* state), which exists in the relative humidity range above 20%, is already known,[12] the other four crystal structures have not yet been determined.

A guanosine dihydrate crystal has the columnar structure of stacked bases, which are hydrogen bonded in the *b* direction forming a layer structure parallel to the *bc* plane, as shown in Fig. 5.[12] There exist two kinds of water molecules, i.e., interlayer water molecules and intralayer water molecules, in the *H* state. The intralayer water molecules bridge the stacked bases and the interlayer water molecules bridge the ribose moieties of the adjacent layers. In the *M* state, only the intralayer water molecules would exist, as illustrated in Fig. 5.[5] The stacked bases along the *c* direction are bridged by intralayer water molecules as in the *H* state. Further, direct hydrogen bonds between ribose moieties of the adjacent layers must be formed as in the case of Na_2·ATP·$2H_2O$.[4] In the *A* state, the intralayer water molecules which bridge the bases are lost, and the ribose moieties are disordered to be free from the hydrogen bonds.[5] The frequency of the *S* mode must be influenced by the hydrogen-bonding network mentioned above. In particular, the higher frequency of the *S* mode in the *M* state may be due to the tightly bound structure.

The following points are implied from the Raman spectra of various hydration states. The anhydrous state (the *A* state) and the *A'* state are partially disordered states, because the phonon peaks are broad and weak except the *S* mode. The x-ray study also indicated the partial disorder of the *A* state.[5] The strong *S* mode in these states in-

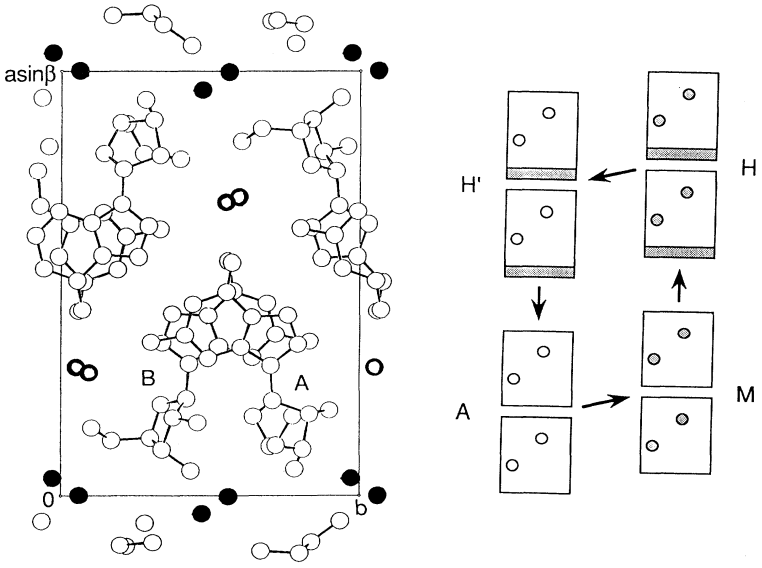

FIG. 5. Crystal structure of guanosine dihydrate and a scheme of the adsorption-desorption process. Guanosine molecules *A* and *B* are crystallographically independent. Water molecules between the layers (interlayer water molecules) are marked by closed circles and water molecules within the layer (intralayer water molecules) are marked by thick circles.

dicates that the bases stack with large overlapping area, that is, the base stacking structure is maintained in spite of partial disorder. The base stacking manner is similar between the A and A' states, judging from the S mode.

The M state is characterized by the shift and low intensity of the S mode. The intensity decrease of the S mode can be interpreted in the following three different ways. (1) The superlattice structure of the M state[5] induces the splitting of the S mode. (2) The mode coupling with a higher-frequency mode reduces the intensity of the S mode. (3) The distortion of base stacking caused by the packing correlates with the low intensity.[8] It needs further experiments to conclude which explanation is most reasonable.

The Raman spectrum H' is similar to the spectrum H, except for the 47 and 70 cm^{-1} modes. This suggests that the crystal structure of the H' state is similar to that of the H state, in spite of the water desorption. We consider that in the dehydration process holes of water molecules are formed at intralayer water sites. This hole formation causes the disappearance of the 47 and 70 cm^{-1} modes. In other words, these modes must strongly depend on the existence of intralayer water molecules.

We have observed the coexistence of two states at the transition point from the A' to M states and from the M to H states, that is, the transient spectra $i1$ and $i2$ are interpreted as the superposition of the two spectra. This suggests that microcrystals undergo a phase transition, which is rapid and cooperative. There must exist a critical relative humidity for the structural transition of microcrystals, though we could not determine its exact value because of the discontinuous RH control.

B. Disodium salt of ATP

In the hydration process of ATP there are four independent states which are distinguished by the Raman spectra D, $M1$, $M2$, and W. These states correspond to the monohydrate (D), dihydrate ($M1$), trihydrate ($M2$), and multihydrate (W) states, as marked in Fig. 1. The crystal structures of the $M1$ and $M2$ states were determined by single-crystal x-ray analysis.[4,6,7] For the following two reasons, the state represented by the spectrum W has the same crystal structure as that represented by the spectrum W', except for the number of water molecules. The peak positions of both Raman spectra are the same. A preliminary x-ray study indicated that the lattice constant a remains unchanged during the dehydration process from 95% to 50% RH. The lower intensity of the central region (below ~ 20 cm^{-1}) in the W spectrum indicates that the weakly bound water molecules are lost in the W state without any lattice shrinkage.[13]

The S mode exists throughout the structural transitions. This means that the base stacking is almost conserved. In fact, x-ray study revealed that the base stacking manners of the $M1$ and $M2$ states are similar, though the conformations of sugar and triphosphate moieties change drastically during the transition.[4] The frequency of the S mode of the $M1$ state is higher than that of the $M2$ state, because loss of the intercolumnar water molecules causes direct hydrogen-bond formation between ribose moieties in the $M1$ state[4] (see Fig. 6). The Raman spectra indicate that the bases stack well also in the D and W states. We should note that the S mode in the D state clearly appears as double peaks. This curious phenomenon arouses our interest in the crystal structure of the D state.

Some Raman bands in the intramolecular vibrational region are sensitive to the molecular conformation, such as the ca. 720 cm^{-1} bands of the ring stretching mode and the ca. 810 cm^{-1} bands of the O-P-O symmetric stretching mode. The Raman spectra shown in Fig. 4 indicate that conformation-sensitive Raman bands are characteristic of each state. We consider that the molecular conformation in the D state is similar to that in the $M1$ state and that the conformation in the $M2$ and W states is similar. The crystal structure of the D state must differ from that of the $M1$ state, judging from the low-frequency spectra, although the molecular conformation of these two states is similar. On the other hand, the crystal structure and molecular conformation of the $M2$ and W states are both similar. These facts are useful to determine the crystal structures of the D state and the W state.

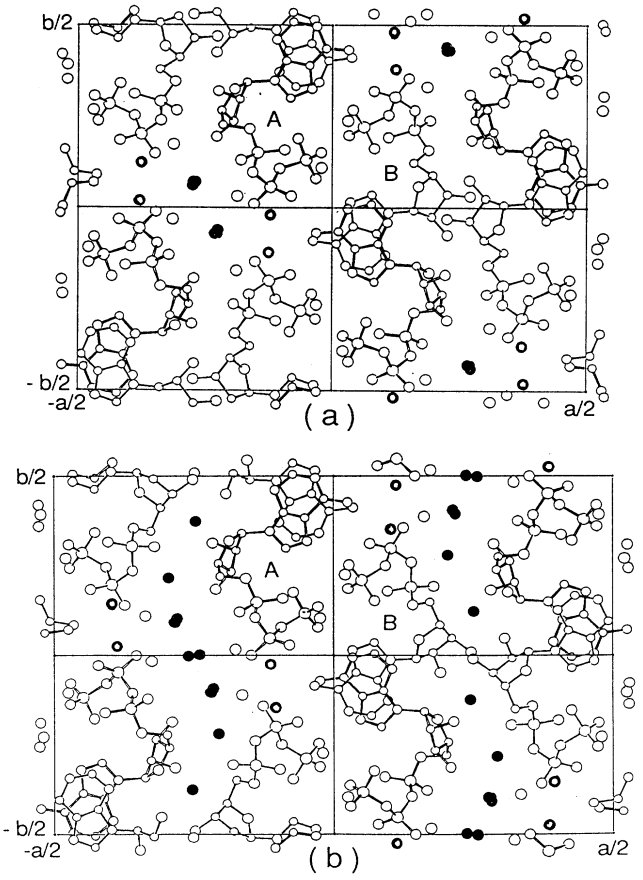

FIG. 6. Crystal structure of $Na_2 \cdot ATP \cdot 2H_2O$ (a) and $Na_2 \cdot ATP \cdot 3H_2O$ (b). ATP molecules A and B are crystallographically independent. Water molecules which construct water layers are marked by closed circles and other water molecules are marked by thick circles. Sodium ions are marked by thin circles.

From now on, we discuss the transition scheme from the $M1$ to $M2$ states. The factor discriminating the structure of the $M2$ state from that of the $M1$ state is the existence of water molecules located between the column of ATP molecules as shown in Fig. 6.[4]

The intermediate Raman spectrum $i1$ between the $M1$ and $M2$ states in Fig. 3 cannot be represented by an addition of the spectra $M1$ and $M2$, except for the S mode. The intermediate spectrum changes depending on the relative humidity, which suggests that the crystal structure of the intermediate state continuously changes.

In contrast, the S mode exists as an addition of the two peaks corresponding to those of the $M1$ and $M2$ states throughout the transition. The S mode exists when the bases stack in a column. Even in a short DNA fragment in a nucleosome core, the well-defined S mode exists.[14] Three-dimensional (3D) crystallinity seems not to be necessary for the S mode, that is, the S mode is a one-dimensional collective mode. Further, it should be noted that the S mode appears as a single peak during the A-B transition of DNA,[15,16] where the correlation length is roughly estimated to be of the order of one pitch of a double helix.[17] In other words, the S mode reflects the average structure of a column of stacked bases. Then the double peaks whose frequencies are just the same as those of the $M1$ and $M2$ states indicate that there exists intercolumnar disorder instead of intracolumnar disorder. If the two frequencies reflect the two different environments where the intercolumnar water layer is formed or not, it is possible that the intermediate state is a completely random mixture of the $M1$ layer and the $M2$ layer. However, the random interchange of the two layers might produce structural stress. So it is more likely that the two domains having the $M1$ and $M2$ structures coexist and the frequencies correspond to each of them.

X-ray analysis[4] revealed that the transition from the $M1$ to $M2$ states takes place without any change of the crystal state, the lattice constant a linearly increases with increasing relative humidity from 5% to 50%, and the diffraction peaks do not split at intermediate states. The single diffraction peak indicates that the domain size or the correlation length of the $M1$ or $M2$ states along the a axis is less than $\sim 10^2$ nm. The domains of each structure locate without a long correlation length along the a axis. The region of the $M2$ domain expands with increasing RH.

Thus we conclude that the intermediate state is a random mixture of domains of the $M1$ and $M2$ states with rather short correlation length ($< 10^2$ nm). The two frequencies of the S mode correspond to each domain. The transition from the $M1$ to $M2$ structure within one layer may occur cooperatively, that is, no structural disorder exists within the layer. The relatively broad peaks of the spectrum $i1$ also suggest that the 3D crystallinity is partly lost during the transition.

Next the transition from the $M2$ to W' states is discussed. This transition is characterized by the steep increase of the hydration number at $\sim 90\%$ RH, that is, there exists a critical relative humidity or water vapor pressure for this transition. A slight conformational change of ATP molecules occurs, judging from the higher-frequency Raman spectra (Fig. 4). The most remarkable change during this transition is the frequency decrease and line broadening of the 34 cm^{-1} mode. This behavior reminds us of the 30 cm^{-1} mode of A-DNA which disappears in the course of the A-B transition.[15,16] This mode must be directly influenced by the increasing intercolumnar water layer. The Raman spectrum $i2$ shown in Fig. 3 differs from those of the $M2$ and W' states. The 34 cm^{-1} mode still exists, but a considerable intensity increase is observed at the dip region (13–17 cm^{-1}). This increase must be due to the response of the additional water molecules which are weakly bound by ATP molecules.[13] The excess water molecules induce a slight change of the molecular structure, and immediately the crystal reaches the stable W' state. Since the difference between the spectra $M2$ and W' is less than the difference between the spectra $M1$ and $M2$, the coexistence of the $M2$ and W' states cannot be proved.

CONCLUSIONS

The structural transitions of guanosine and Na_2·ATP controlled by relative humidity is monitored by low-frequency Raman spectroscopy. Well-defined crystal states are distinguished, some of which are already known through x-ray diffraction studies. Since the intensity of the S mode is strong, the bases are considered to be well stacked, forming a long column in every state except the M state of guanosine. The transition schemes among well-defined states are different between the two species. (1) Microcrystals of guanosine undergo a phase transition, that is, microcrystals change their structure cooperatively. (2) The $M1$ to $M2$ transition of Na_2·ATP is characterized as a gradual addition of water layers, and only a small domain has 3D crystallinity.

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