Vibrational fluctuations of the hydrogen bonds around a nucleation defect of melting in poly(dG)-poly(dC)

Yup Kim
Department of Physics, Purdue University, West Lafayette, Indiana 47907
and Department of Physics and Research Institute for Basic Sciences, Kyung Hee University, Seoul 131, Korea

E. W. Prohofsky
Department of Physics, Purdue University, West Lafayette, Indiana 47907
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Vibrational fluctuations of the hydrogen bonds around a nucleation defect of strand-separation melting of a DNA polymer, poly(dG)-poly(dC) (dG denotes repeating guanine bases on one strand and dC the repeating cytosine bases on the other strand), are analyzed at 293 and 340 K using a modified self-consistent phonon theory with a complex Green's function. At 293 K the helix is very stable and the important frequency band for fluctuation is the band between 60 and 120 cm^{-1}. This is the same important band as that for the fluctuation of the hydrogen bonds which are far from the defect. At 340 K, near the temperature at which the hydrogen-bond instability in the neighboring cells to the defect occurs, there are two important frequency bands. One is the band between 60 and 120 cm^{-1} which is essential for the fluctuation of the bond near the major groove. A second band is the band under 20 cm^{-1} which is critical for the fluctuation of the bond near the minor groove. We also explain the directional effects around the defect when the instability of the bonds begins to occur based on the roles of the particular phonons.

I. INTRODUCTION

The DNA helix is a double helix in the sense that it is composed of two strands, each one made of covalently bonded atoms, which are held together by weaker hydrogen bonds (H bonds). On raising temperature the double strand separates into two single strands due to the instability of the weaker H bonds and this is generally referred to as a melting of the DNA. The thermal behavior is much like a melting. Recently we have developed a modified self-consistent phonon theory[1-3] based on phonon Green's functions[4,5] to study the strand-separation melting of a DNA polymer poly(dG)-poly(dC). Here dG and dC represent separate strands of repeating guanine and cytosine bases respectively. In the self- consistent phonon theory we calculate the vibrational fluctuation of the hydrogen bonds of the helix, the effective force constants of the hydrogen bond stretches and the resulting eigenfrequencies and the eigenfunctions of the DNA polymer. The melting is inferred from the development of an instability in the H bonds. Theoretically this instability comes from the anharmonic effects of the large thermal fluctuation.

The two strands of the DNA helix are held together by specific hydrogen bonds as well as a large number of nonbonded interactions[6] which give rise to a stacking energy. In our model for the melting of the DNA helix the vibrational modes of the double helix[3,6] have been calculated using the force constants for the hydrogen bond interactions as well as force constants for nonbonded interactions. Our eigenvectors of the vibrational modes represent the calculated displacements of the DNA atoms in the presence of all the atom-atom interactions in our model. In the following work we will focus on the stretch of H-bond coordinates as this is a convenient parameter which directly measures the local separation of the two DNA strands. We will treat the H-bond force constants self-consistently, but we will not correct the nonbonded interactions for temperature-dependent effects. The nonbonded interactions have a much weaker dependence on atom separation and should have much reduced temperature dependence. The growth in the strand separation displayed by our theory is a growth that takes place in the presence of both the H-bond force constants and nonbonded force constants. The instability is similarly an instability in the presence of all force constants.

Every calculation of our self-consistent phonon theory at each temperature is based on the temperature-independent Morse potential of the hydrogen bonds whose parameters are determined from the dynamical data at 293 K.[3,7] There are two reasons to believe that our parametrization for the Morse potential represents a reasonable approximation to the actual potential in the double helix. Firstly we predict the observed frequencies of a number of modes in DNA and in particular the 85 cm^{-1} cm^{-1} band[3,7] which is assigned as the principal H-bond stretch mode.[8] These modes are observed to be resonant rather than relaxational.[9] Secondly the predicted melting temperature is in reasonable agreement with observations.[3,4,6]

We have applied our model to the perfect infinite DNA polymer and we have predicted the melting of the perfect helix at 380 K.[3] Although this temperature is reasonably close to the experimental melting one,[10-12] this was a

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mean-field melting in which every set of the hydrogen bonds melts simultaneously. In contrast, the actual melting can be expected to involve nucleation sites. We have therefore applied the self-consistent theory to the hydrogen bonds around a simple but physically and biologically meaningful nucleation defect.[4] The nucleation defect for the melting we considered was the defect depicted in Fig. 1. As can be seen from Fig. 1, the three H bonds of the base pair of the cell (0) of the perfect helix has been cut. In our model it is assumed that no local change in conformation about the defect occurs. Long-lived defects might cause local deformation, but the details of the deformation are difficult to predict and incorporating such defects in our system greatly complicates the calculation. We then determine at what temperature the hydrogen bonds of the cell (1) and cell (-1) become unstable. The temperature of the instability should be lower than that of the mean-field melting, because the thermal fluctuation of the H bonds around the defect should be enhanced by the defect. We find the instability arises at 350 K (Ref. 4) which is very close to the experimental one.[10] We also find some directional effects when the H bonds begin to become unstable around defect. Around 350 K in the cell (1) the H bond adjacent to the major groove melts first followed by the other two bonds as in the case of the perfect helix. But in the cell (-1) the H bond near the minor groove first showed the instability followed by the other bonds.

On raising the temperature the double strand of the DNA helix separates into two single strands due to the thermal fluctuations breaking the weak hydrogen bonds which hold the two strands together. Since in this melting certain specific bonds are disrupted, there exists the possibility that certain vibrational excitations (or phonons) are more effective at enhancing the vibrational fluctuation which drives the melting. It is the purpose of this paper to analyze the role of the particular phonons of specific frequency in bringing about the melting of H bonds around the nucleation defect of Fig. 1. Specifically we want to see how phonons of specific frequency contribute to the vibrational fluctuations of the H bonds near the defect compared to those which lie far from the defect and how the directional effects arise near the temperature of the instability. First we calculate the frequency-dependent thermal fluctuation of the stretch of H bonds at 293 K. At this temperature the H bonds near the defect are quite stable. Next we calculate the fluctuations at 340 K near the temperature at which the H bonds around the defect become unstable. Although we have reported the result at 340 K briefly,[5] in this paper we want to explain the result at 340 K more specifically with comparison to the result at 293 K.

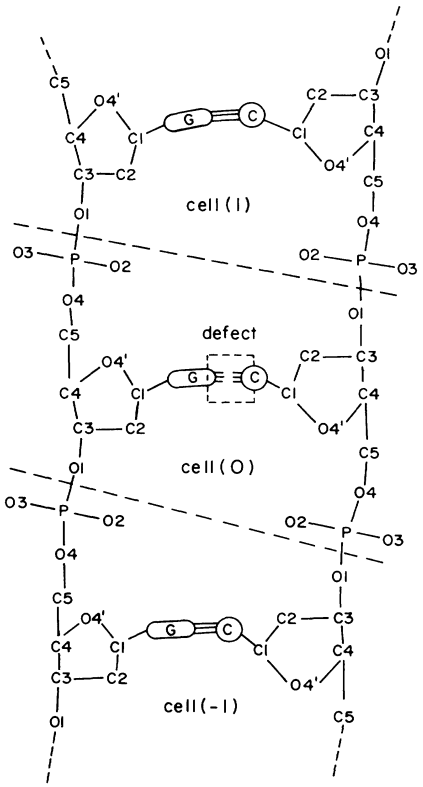

FIG. 1. Portion of three unit cells of poly(dG)-poly(dC) which shows the nucleation defect in hydrogen bonds of cell (0).

II. COMPLEX GREEN'S FUNCTION AND SELF-CONSISTENT PHONON THEORY

The vibrational equation of motion of the helix with the defect is

$$(F-\omega^2+C)q=0\ , \tag{1}$$

where F is the force-constant matrix of the perfect helix, C is the perturbation of finite size for the cut of the three hydrogen bonds of cell (0) (see Fig. 1) and ω^2 is the eigenfrequency. In the presence of a defect in an otherwise perfect helix, helical symmetry[13] is broken locally and a deviation from the perfect-helix phonon-dispersion relations[6] occurs. There are differences in the eigenvectors of the inband modes whose frequencies lie within the phonon dispersion curves of the perfect helix as well as the possible creation of localized modes within the branch gaps of dispersion curves of the perfect helix. To analyze the inband case we introduce the complex Green's function g of the perfect helix which is defined as

$$g(\omega^2)\equiv(\omega^2-F)^{-1}\ . \tag{2}$$

For ω^2 lying in the perfect helix dispersion curves $g(\omega^2)$ has first-order poles. Using the famous identity

$$\lim_{\epsilon\to 0}\frac{1}{x+i\epsilon}=\mathrm{P}(1/x)-i\pi\delta(x)\ , \tag{3}$$

the singularities of g appear as the imaginary part of complex g.[4,14] The imaginary part of the complex Green's function is very important in calculating the vibrational fluctuation of given H bonds. The defect Green's function $G(\omega^2)$ is then from Eqs. (1) and (2),

$$G(\omega^2)=g(\omega^2)+gT(\omega^2)g\ , \tag{4}$$

where

$$T(\omega^2)=C(1-g(\omega^2)C)^{-1}\ . \tag{5}$$

In numerical calculation we first calculate the perfect helix complex Green's function.[4] In this calculation at 293 K we have used the result of the phonon dispersion curves and eigenfunctions of the perfect helix.[6] In contrast at 340 K we have used the new dispersion curves and eigenfunctions calculated by the self-consistent phonon theory of the perfect helix in which the correction for the thermal vibration is included.[3] We then calculate the T matrix of Eq. (5) and then the defect Green's function of cell (1) and cell (-1). For the localized states which may occur within the branch gaps of the phonon dispersion curves of the perfect helix, the Green's function $g(\omega^2)$ never develops a singularity and g may be easily calculated. Then the new localized states are found by searching for zeroes in a determinant of finite size[3,4] and the corresponding eigenfunctions for the cells (1) and (-1) are evaluated by use of Eq. (1). Among 123 bands of the perfect helix, the frequency bands 28 to 123 are essentially dispersionless,[3,6] and we treat these bands in the Einstein approximation. The other bands whose frequencies are lower than 220 cm^{-1} are treated exactly.

Among all the force constants of DNA we assume that the six force constants for the hydrogen bond stretches of cells (1) and (-1) change significantly and the other force constants of the cells are assumed not to change significantly. Thus for the self-consistent calculation to examine the stability of the hydrogen bonds of the cells (1) and (-1) we do every calculation in the subspace of six hydrogen-bond stretch coordinates of cells (1) and (-1) of the DNA helix internal coordinates.[15] In each iteration of the modified self-consistent phonon theory there are three steps which include the anharmonic effects as was stated in the Introduction. First we calculate the correlation tensor D_i for each H bond which represents the thermal average of fluctuations of H-bond stretches. D_i can be written as[4]

$$D_i = \langle s^i s^i \rangle = \overline{\int} d\omega \, D_i^c(\omega) + \sum_\lambda D_i^\lambda(\omega_\lambda) , \tag{6}$$

where s^i means the stretch coordinate of ith H bond, $\langle\ \rangle$ means the thermal average, $\overline{\int}$ means the integration over all continuous frequency bands and $D_i^c(\omega)$ means the contribution of the inband modes to D_i and D_i^λ is the contribution of λth localized states to D_i. Here

$$D_i^c(\omega) = \frac{1}{\pi} \coth(\beta\omega/2) \mathrm{Im} G_i(\omega^2) \tag{7}$$

and

$$D_i^\lambda(\omega_\lambda) = \frac{1}{2\omega_\lambda} s_i^\lambda s_i^{\lambda *} \coth(\beta\omega_\lambda/2) , \tag{8}$$

where $G_i(\omega^2)$ is the diagonal part of the ith H bond of the defect Green's function from Eq. (4), and s_i^λ is the complex eigenfunction of the ith H bond of localized states with the eigenfrequency ω_λ. In the first iteration D_i is calculated from the defect Green's function of inband modes and localized states for H bonds of the cells (1) and (-1) using the method of the first part of this section. After the first iteration D_i can be calculated by the result of the previous iteration. Next using a Morse potential we calculate the force constants for the hydrogen-bond stretches of the cell (1) and (-1). The Morse potential parameters[3] used here are determined by fitting the experimentally observed dynamic behavior at 293 K (Ref. 3). In the subsequent calculations we use the parameters without modification even though we vary temperatures. Final step in the iteration is the calculation of a new $G_i(\omega)$ and new localized states in terms of the calculated force constants as the new force constants. In this step the method of the first part of this section is taken to calculate the new $G_i(\omega)$ of Eq. (4) and new localized states.

Our calculations of the force constants differs from the standard self-consistent phonon approach. We allow for thermal expansion in a self-consistent manner.[1] The centroid of the fluctuations in bond length is not at the minimum of the potential function. If it were the symmetric distribution of fluctuations about the minimum would guarantee that all odd power terms in an expansion of the potential about the minimum would cancel. In our asymmetric calculation odd power contributions do occur about the thermal expansion. The location of the centroid of the fluctuations is the self-consistent midpoint between the classical turnaround points of the oscillator. This definition of equilibrium position is consistent with standard theories of thermal expansion.

III. RESULTS AND DISCUSSIONS

The three steps are iterated and we get self-consistency both at 293 and at 340 K. As was stated in the Introduction, it is our purpose to see the detailed frequency dependence of $D_i^c(\omega)$ for each H bond of the two neighboring cells to the cell with the defect at both room temperature 293 and 340 K which is near the temperature 350 K at which the H bonds are unstable.[4] As already explained, the critical bonds among the six H bonds at the defect are the bond adjacent to the major groove of the cell (1) and the bond adjacent to the minor groove of the cell (-1) at 350 K.[4] Here the $+z$ direction is the $3' \rightarrow 5'$ direction to the G backbone. The results of our calculation are seen in Figs. 2 and 3. The result for the bond which is located at the center of the three H bonds is not displayed, since this bond does not initiate melting in the temperature range between 293 and 340 K. Each bar in Figs. 2 and 3 is the average density $\overline{D}_i^c(\omega)$ of Eq. (7) in the corresponding cell which is defined as

$$\overline{D}_i(\omega_i) = \frac{1}{\Delta\omega_i} \int_{\omega_i}^{\omega_i + \Delta\omega_i} D_i^c(\omega) d\omega , \tag{9}$$

for the given frequency band with the range between ω_i and $\omega_i + \Delta\omega_i$. The area under the given bar from the very bottom of the graph is the contribution of the given band to the total fluctuation D_i of Eq. (6). The cell (∞) means a cell lying far away from the defect. The $D_i(\omega)$'s for cell (∞) are the same as those for the perfect infinite helix. We display the average fluctuation densities of the bond near the major groove in Fig. 2(a) for 293 K and Fig. 2(b) for 340 K and those of the bond near the minor groove in Fig. 3(a) for 293 and Fig. 3(b) for 340 K.

The frequency band structure of the perfect DNA polymer is as follows. Below ≈ 66 cm^{-1} the bands are over-

lapped and look like a single continuous band with no branch gaps.[3,6] From ≈ 66 to 1656 cm^{-1} there are 110 discrete bands with relatively small bandwidths. We do not display the fluctuation densities for the frequency band above 130 cm^{-1} because the densities of the neighboring cells are essentially the same as those far from the defect. At 293 K the fluctuation densities of the neighboring cells to the defect are slightly increased around 10 cm^{-1} and between 60 and 120 cm^{-1}. Compared to the perfect helix this includes an effect from the localized states in this frequency range. At 293 K the H bonds near the major groove of both cell (1) and (−1) have more thermal fluctuation than the other two bonds. This situation is similar to those of H bonds far from the defect. There are no critical fluctuations or critical directional dependences at 293 K. There is a slight increase in the thermal fluctuation of the bond of cell (1) adjacent to the major groove from the band between 80 and 110 cm^{-1} [Fig. 2(a)] and a slight increase in that of the bond near the minor groove of the cell (−1) from the band around 10 cm^{-1} [Fig. 3(a)]. At 293 K the helix is very stable around the defect.[2] In contrast, at 340 K near the critical temperature the fluctuation densities around the defect are increased critically for the band below 20 cm^{-1} and that between 60 and 120 cm^{-1} [Figs. 2(b) and 3(b)].

From Fig. 2(b) we see that a band at ≈ 70 cm^{-1} is very

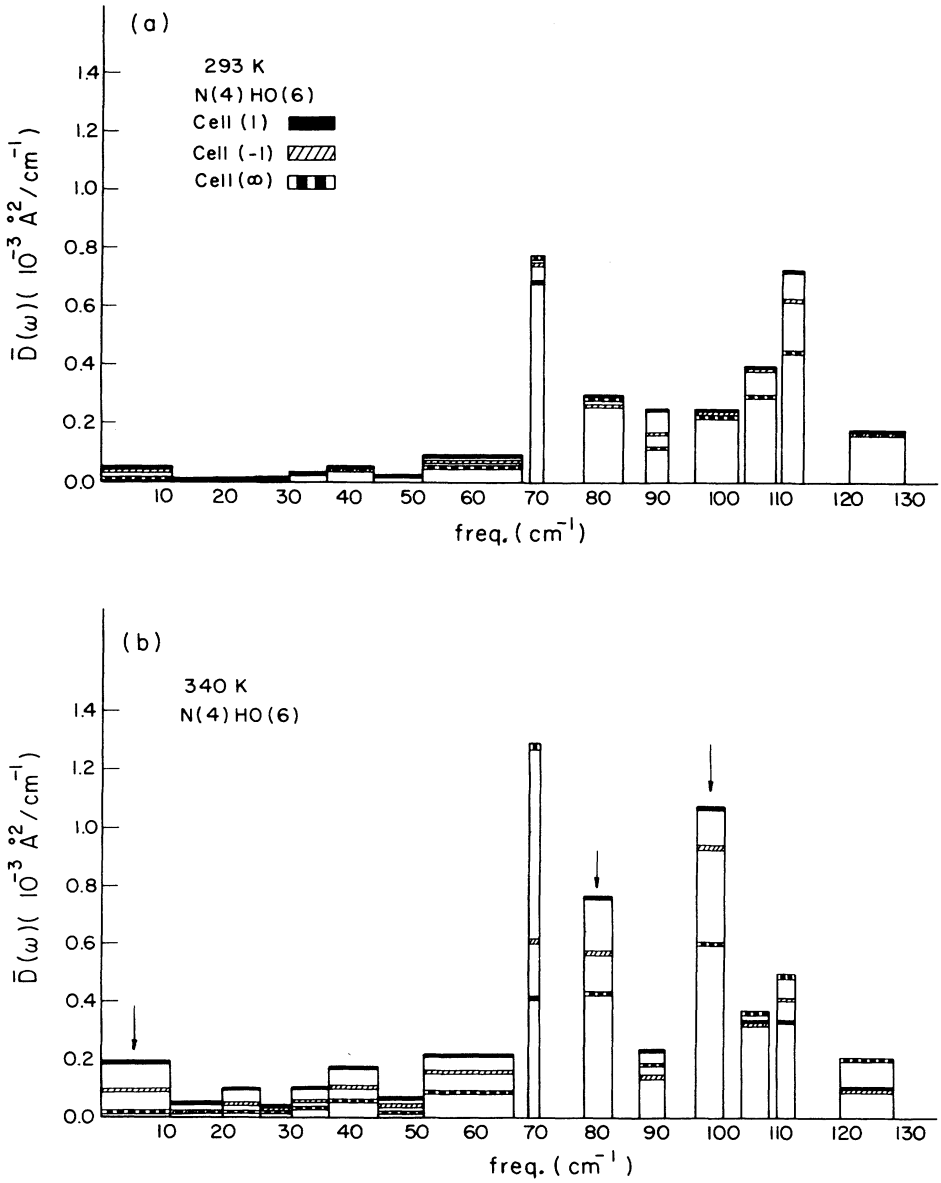

FIG. 2. The average thermal fluctuation density $\bar{D}(\omega)$ of Eq. (9) of the bond near the major groove [N(4)HO(6)] for the given frequency bands. The bold bar denotes that of cell (1), the cross-hatched bar denotes that of cell (−1) and the bold-dotted bar denotes that of cell (∞). (a) graph for 293 K; (b) graph for 340 K. The area under the given bar from the very bottom of the graph is the contribution of the given frequency band to the total fluctuation of the H bond of Eq. (6).

effective at inducing thermal fluctuations in the H bond near the major groove in cells far from the defect. Another band above 120 cm^{-1} also contributes to this motion. This closely mimics the bands most effective at inducing melting in a perfect helix as found in our calculation of mean-field melting.[3]

The bond near the major groove of cell (1) is the most critical bond in cell (1). The total fluctuation D_i of Eq. (6) of this bond is increased by 1.3 $Å^2$ compared to that of the corresponding bond of the perfect helix. About 20% of this increase comes from the band under 20 cm^{-1}. About 60% of this increase comes from the band between 60 and 120 cm^{-1} including the contribution from the pure localized states whose frequencies are 67.2, 68.75, 72.83, 84.45, 86.47, 102.79, 108.50, and 116.14 cm^{-1} [see Fig. 2(b)]. The contribution from the two bands whose frequencies are between 78.16 and 82.7 cm^{-1} and between 95.65 and 100.51 cm^{-1} is about 30%. These are thought to be the critical bands for the fluctuation of the H bond near the major groove around the defect. Thus bands at 80—100 cm^{-1} are very effective at inducing the thermal fluctuations of the H bond in the cell (1) which in our model is the $3' \rightarrow 5'$ direction for the guanine strand.

In the cell (-1) the most critical bond for the instability is the bond near the minor groove. The total fluctuation D_i of this bond is increased by 1.25 $Å^2$ comparing that of the perfect infinite helix. About 70% of this increase comes from the bands under 20 cm^{-1} [see Fig. 3(b)]. This band is the critical band for the fluctuation of the H bond near the minor groove.

We may state our conclusion as follows. There are two critical frequency bands around the nucleation defect.

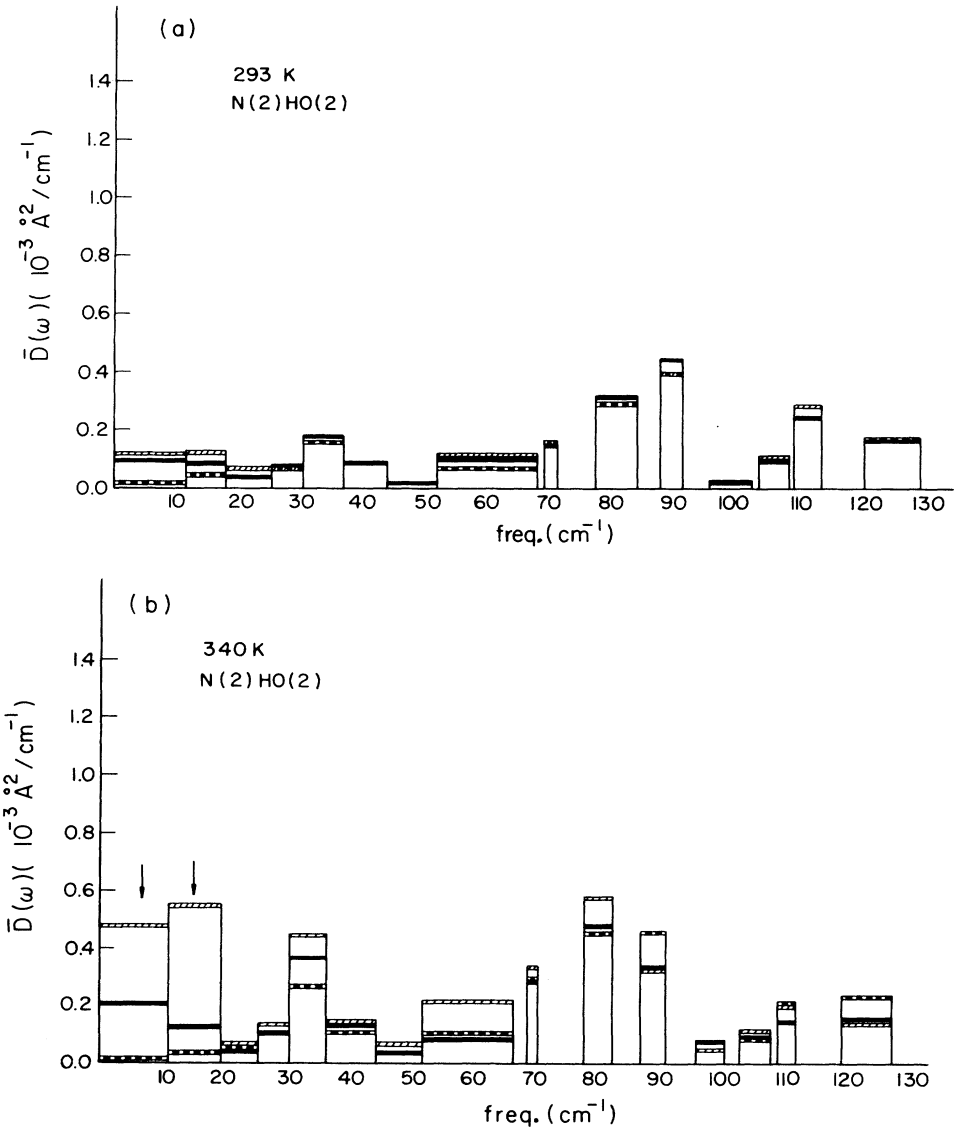

FIG. 3. The diagram for the H bond near the minor groove [N(2)HO(2)]. The notation is the same as that of Fig. 2. (a) graph for 293 K; (b) graph for 340 K.

One is the band between 60 and 120 cm^{-1} and the other is the band under 20 cm^{-1}. The first band is important for the melting proceeding in $+z$ direction, and the second band is important for the melting proceeding in $-z$ direction. The fluctuation at 10 cm^{-1} is a virtual resonance[16] similar to others found in microwave region located around the terminus.[14]

In all our calculations the integration over bands was carried out by summation over a mesh of frequencies for that band. Sharp resonant modes like that found in a previous DNA Green's-function calculation[14] may not show up explicitly in these calculations. Further calculations on a finer mesh are needed particularly in the broad bands at the lowest end of the spectrum.

This paper calculates the onset of an instability in a set of bands holding the double helix together. We have associated this instability with helix melting but this is not a detailed theory of helix melting. Broken bands can reform. The actual melting occurs when the free energy of the melting system is lower than that of the unmelted system. The instability calculated here is related to melting as the onset of the instability raises the free energy of the unmelted system substantially. The free energy of the unmelted system is related to the fluctuation parameter D. As shown in a previous work this parameter increases without bound at the temperature of the onset of the instability.[2] The rapid increase in free energy of the unmelted phase when no particular increase should occur in the melted phase should result in the melted state being at the lower free energy. In a previous work we showed that changes in salt concentration, etc., did alter the temperature[3] at which the instability occurs. We believe that effects such as salt, water structure, etc., alter melting by altering the onset of the instability. With the onset of the instability with the associated rise in unmelted free energy the melting occurs.

The fine points of melting and the applicability of this approach to a theory of melting which properly handles the cooperativity within a large system are still being explored. The principle point of this paper is to develop a calculation which can suggest local details of critical behavior in a very complex system. The result of this calculation does indicate that very specific local effects can occur as a result of details of the excitation spectra. The DNA helix is seen to be a system capable of complex responses to the introduction of excitations. The actual biologically significant structures are more complex than in the system considered here and should be capable of far more complex responses.

ACKNOWLEDGMENT

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