PROTON DISPERSION FORCES

SECONDARY-STRUCTURE STABILIZING FORCES BETWEEN THE HYDROGEN BONDS OF THE POLYNUCLEOTIDES

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ABSTRACT In the double helix formed by the semiprotonated polycytidylic acid (poly C), both strands are linked via $NH^+ \cdots N$ hydrogen bonds. It is a known fact that such symmetrical hydrogen bonds with a double minimum potential well are extremely polarizable. This polarizability causes interaction effects, in particular the proton dispersion forces between such hydrogen bonds. These forces result in a shift of the energy levels and a continuum is observed in the infrared (IR) spectra of solutions in which such hydrogen bonds are present. The continuum occurs in the IR spectrum of the semiprotonated poly C, when the former is present in coiled state. If the double helix forms, an extremely broad band of the NH stretching vibration is observed instead of the continuum, since in the double helix all hydrogen bonds are oriented equally to one another and polarize each other mutually to a strong degree. The proton dispersion forces between the hydrogen bonds balance a considerable part of the electrostatic repulsion of the protons and hence enable the double helix to form. It is conceivable that an unsymmetrical double minimum potential well is present in the $NH \cdots N$ bonds in the DNA and RNA. Such bonds may likewise be considerably more polarizable than electron systems and thus, in this case too, proton dispersion forces would contribute to helix stabilization.

INTRODUCTION

The polarizability of symmetrical hydrogen bonds with a double minimum potential well (Fig. 1 *a*) is more than two orders of magnitude greater than customary degrees of polarizability (1, 2). This polarizability causes two interaction effects: firstly, the proton dispersion force between these hydrogen bonds (3, 4), and secondly, an ion-induced dipole interaction (5) between such hydrogen bonds and the fields of the anions, respectively, the dipoles of the solvate molecules. Both interaction effects lead to a considerable shift of the energy levels of the protons. Since in liquids the distances and orientations between the above hydrogen bonds and between the anions and these hydrogen bonds pass through a statistical variety of values, the strength of these interaction effects and thus the shift of the energy

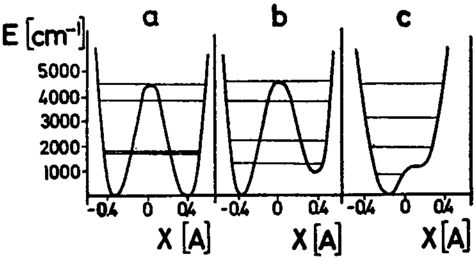

FIGURE 1 (*a*) Symmetrical double minimum potential well. (*b*), (*c*) Asymmetrical double minimum potential wells.

levels also pass through a variety of values. Thus a continuous absorption occurs in the IR spectrum of such solutions. This continuum is observed in many different systems in which $BH^+ \cdots B$ or $(BH \cdots B)^-$ hydrogen bonds are present e.g., see references 4–8.

Even in the case of unsymmetrical hydrogen bonds with a double minimum potential well, the polarizability may be considerably larger than electron polarizabilities (2). The polarizability is still approximately one order of magnitude greater than electron polarizabilities when the lowest energy level enables the proton to transfer to the second well (Fig. 1 *b*). Even if the proton can only migrate in the first excited state (Fig. 1 *c*), the polarizability may still be considerably larger than customary polarizabilities due to the shift of electrons (2).

THE SEMIPROTONATED POLY C DOUBLE HELIX

Numerous investigations (9–16) have shown that the salts of poly C in slightly acidic milieux form a double helix, whereby two base residues are each linked via a symmetrical $NH^+ \cdots N$ hydrogen bond forming between the N_1 atoms of the base residues.

Let us first consider the range 1700–1600 cm^{-1} in Fig. 2. A band at 1655 cm^{-1} is observed with the solution of the unprotonated poly C. This band is the CO stretching vibration to which the ring itself also contributes, as shown by a ^{15}N shift of this band (17). In the case of the D_2O-hydrated sample this band is observed at 1653 cm^{-1}. When the HCl required for the semiprotonation is slowly added to a poly C solution, three bands are observed, one at 1660 cm^{-1}, one at 1683 cm^{-1}, and one at 1725 cm^{-1}. The band at 1725 cm^{-1} is to be ascribed to the base residues at which a proton is located. The corresponding band was already observed previously at 1725 cm^{-1} (in D_2O solution at 1709 cm^{-1}) on protonation of the cytidine by Shimanouchi et al. (18) and discussed. The bands at 1660 cm^{-1} and 1683 cm^{-1} are to be assigned on the one hand to the CO stretching vibration of the base residues where no proton is located, and on the other hand to the $—NH_2$ scissor vibration of these base residues. When the secondary structure has formed, the transition moments of these two vibrations occur in one plane and couple, which

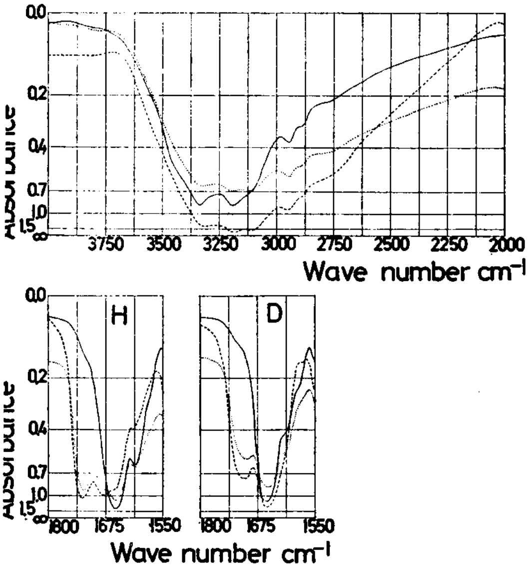

FIGURE 2

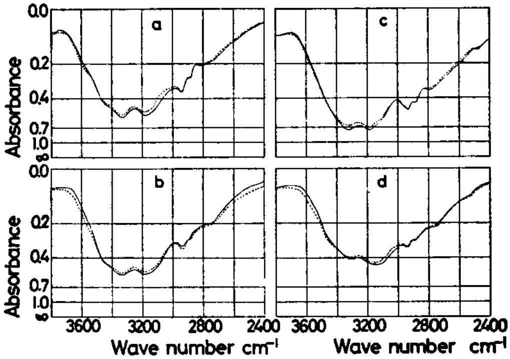

FIGURE 3

FIGURE 2 IR spectra of films of potassium salt of poly C ——, nonprotonated; ----, semiprotonated double helix; ····, semiprotonated, coiled.

FIGURE 3 IR spectra. ----, film at room temperature (293°K); ——, film at 85°K. (*a*) DNA, (*b*) RNA, (*c*) poly (A + U), (*d*) poly (G + C).

results in the splitting observed. The fact that the —NH_2 scissor vibration participates in this band pair is proved by the lack of a corresponding splitting in the presence of $-ND_2$ groups. Such coupling effects of vibrations of various groups indicating a secondary structure are also discussed in references 19–21. If the amount of HCl required for semiprotonation is added quickly to a very concentrated poly C solution, this band splitting is not observed (Fig. 2). This shows that the poly C has precipitated under these conditions in the coiled state, for in the coiled state the transition moment of the scissor vibration of the —NH_2 groups generally does not occur in the same plane as the stretching vibration of the CO groups. Hence these vibrations do not couple. No splitting is observed.

In contrast to the finding with unprotonated poly C in the case of the semiprotonated poly C double helix, an extremely broad band is observed extending from the bands of the —NH_2 vibrations in the range 3400–3100 cm^{-1} to smaller wave numbers up to 2100 cm^{-1}. This absorption is caused by the $NH^+ \cdots N$ hydrogen bonds. These $NH^+ \cdots N$ bonds were thoroughly investigated in conjunction with solutions of semiprotonated imidazole and pyrazole (8), as well as with numerous other N bases (22) and in particular with poly-L-histidine (8). Between these hydrogen bonds act proton dispersion forces which are an essential cause of the extreme band broadening. Further, these $NH^+ \cdots N$ bonds are polarized more or less strongly by the dipole fields of the hydration water molecules and by the fields of the $\rangle PO_2^-$ ions of the backbone. The strength depends on the extent to which the fields of these $\rangle PO_2^-$ ions are shielded by the neighboring cations. This induced dipole interaction also leads, of course, to a considerable broadening of the observed absorption.

With the semiprotonated poly C double helix merely an extreme band broadening is observed instead of the continuum. This is due to two reasons: firstly, the distances and the orientations of the $NH^+ \cdots N$ bonds do not pass through a variety of values, on account of the secondary structure, and secondly, the $NH^+ \cdots N$ bonds neighbor each other to such an extent that these bonds polarize each other so strongly that the tunneling frequency decreases considerably.

Fig. 2 likewise shows an IR spectrum of semiprotonated poly C in the coiled state. In this case the extremely broad intensive band is not observed, instead a continous absorption towards small wave numbers extends, as anticipated, for example with the solutions of semiprotonated N bases (8, 22).

Besides the usual intermolecular interaction effects which stabilize double helical structures, the interaction effects discussed, in particular the proton dispersion forces, are of great significance for the stability of the double helix of the semiprotonated poly C, for the strong electrostatic repulsion of the excess protons would hinder the stability of the double helix. This electrostatic repulsion of the excess protons is compensated for to such an extent by the energy gain connected with the mutual polarization of the bonds that the double helix can form. This mutual polarization leads to an energy gain, for the lower energy levels occupied preferably by the protons in the hydrogen bonds were lowered by this interaction. This is shown by Fig. 110 in reference 4, p. 204.

DNA, rRNA, POLY (A + U), AND POLY (G + C)[1]

Fig. 3 shows spectra of DNA, rRNA, poly (A + U), and poly (G + C), not only for samples at room temperature but also at 85°K. The intensive band doublet is to be assigned to the stretching vibration of the hydrogen-bonded NH group of the $—NH_2$ group and the harmonic vibration of the $—NH_2$ group coupled by Fermi resonance (20). The maximum of a weak band is observed at about 2750 cm^{-1}. This has an extremely large half-width. Although this band is scarcely intensive, it extends towards small wave numbers to approximately 2400 cm^{-1}, and towards large wave numbers it merges with the broad band complex. The assignment of this band was discussed in detail in reference 21. With poly (A + U) Shulman (23) assigns this band to the NH stretching vibration in the hydrogen bond linking the N_1 of the adenine with the N_3 of the thymine. The band in the case of poly (G + C) can be assigned analogously. As shown in Fig. 3, the half-width of the band at 2750 cm^{-1} remains extremely large even at low temperatures.

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[1] poly (A + U), polyadenylic acid + polyuridylic acid; poly (G + C), polyguanylic acid + polycytidylic acid.

As discussed in detail by Fritsche (24), certainly no symmetrical double minimum potential well occurs in the NH ··· N hydrogen bonds. Shulman (23) discusses an unsymmetrical double minimum potential well in the NH ··· N bond in the poly (A + U)[2].

Since we know that even unsymmetrical hydrogen bonds of the above-mentioned type may be considerably more polarizable than electron systems (2), an important reason for the extremely large half-width of this band could be the interaction effects resulting from this polarizability between the hydrogen bonds in the double helix. It is in any case conceivable that even with the DNA and RNA the polarizability of the hydrogen bonds makes a contribution to the polarizability of these polynucleotides orthogonal to the double helix axis.

Even if the proton dispersion forces between unsymmetrical hydrogen bonds were not by far as large as in the case of symmetrical bonds with a double minimum potential well, they may be much larger than dispersion forces between the base residues determined by the polarizability of the electrons, for the polarizability of unsymmetrical bonds with a double minimum potential well may be considerably larger than customary electron polarizabilities (2). That is, when dispersion forces contribute essentially apart from the hydrophobic interaction to the stacking energy and thus to the stabilization of the double helix, the proton dispersion forces between the NH ··· N bonds presumably contribute decisively, too.

EXPERIMENTAL PROCEDURES

The experimental procedures are discussed in detail in reference 20. The spectra of the samples at 85°K were plotted with the IR low temperature cell described in reference 30 (see also reference 4, p. 265 ff.).

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[2] Numerous SCF calculations (26–29) provide other and contradictory results with respect to this potential well. The problem with these calculations, however, is the fact that base pairs are treated in isolation from their environment. The hydration of the DNA and the stacking of the base pairs certainly influence the potential well in these hydrogen bonds decisively.

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