2.034, occurring when the magnetic field lies along the Z axis, arises from the promotion of one of the nonbonding electrons into the half-filled orbital of the unpaired electron. A small positive g-value change, $g_x = 2.007$, probably results from the promotion of an electron from the C–O bond into the half-filled orbital. The undeviated g-value, $g_y = 2.002$, corresponds to a magnetic field parallel to the orbital of the unpaired electron.

Conclusion

The results of the stability studies may be summarized briefly. Methyl radicals, which apparently diffuse through the matrix, disappear both by recombination and by hydrogen abstraction. If over-all radical concentrations are very large, the recombination reaction predominates; otherwise the hydrogen abstraction is more important. Small radicals, such as CHO and CH_2OH, are apparently immobilized to a considerable extent in the matrix by hydrogen bonding, and after the complete disappearance of the methyl radicals, decay by hydrogen abstraction from the matrix. The methoxy radical appears as an end product of this reaction.

The presence of methyl radicals in the photolysis of methanol with 2500 Å. radiation deserves comment since their presence is unexpected because of the low absorption in this spectral region. There may be some possibility of the CH_3 radicals being produced by residual 1849 Å. light but this appears to be unlikely. In view of the role that trace amounts of impurities have played in radiation chemistry it is reasonable to suspect such a cause. However, irradiation of alcohol samples to which small amounts of suspected impurities such as aldehydes, ketones, etc., have been added failed to produce sufficiently high concentrations of methyl radicals to account for the observations reported here. There is also the possibility that the methyl radicals arise from some secondary process. In fact in the approach used in this study it is not feasible to isolate the primary photolytic effect from secondary processes such as successive photolysis or reactions of hot primary radicals with other fragments or the matrix.

It may also be noted that the assignment of the resonance shown in Fig. 5 to the methoxy radical is not completely unambiguous since a similarly shaped resonance would be expected for an appropriate peroxy radical. Experiments with carefully deoxygenated samples, however, show that, if the radical is of the peroxy type, it does not result from reaction with dissolved oxygen.

[CONTRIBUTION FROM THE SPECTROSCOPY LABORATORY AND THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE, MASSACHUSETTS]

Hydration of Deoxyribonucleic Acid. II. An Infrared Study[1]

BY MICHAEL FALK,[2] KARL A. HARTMAN, JR., AND R. C. LORD

RECEIVED SEPTEMBER 24, 1962

The infrared spectrum of deoxyribonucleic acid (DNA) has been studied in the region of 4000 to 400 cm.$^{-1}$ as a function of relative humidity. From frequency and intensity changes of infrared bands it is concluded that the $PO_2^-Na^+$ groups of DNA become hydrated in the range of 0 to 65% relative humidity, while the hydration of the bases begins above this range. The strength of hydrogen bonding is greatest for the first water molecules to adsorb and decreases thereafter, approaching the strength of hydrogen bonding of liquid water.

Introduction

Deoxyribonucleic acid (DNA) is a large polymer molecule composed of many molecular subunits (purine and pyrimidine bases, deoxyribose, and diesterified phosphate groups) and gives rise to a complex infrared spectrum. A detailed assignment of every feature in the spectrum is not possible at present, but partial assignment of the more prominent bands is useful.

By comparison with the spectra of the constituent molecules many bands in the spectrum of DNA have been assigned to specific modes of vibration of particular groups of atoms. The first infrared studies of DNA were made by Blout and Fields[3] and by Blout and Lenormant,[4] who made many assignments. The infrared spectrum of DNA has been examined in detail by Sutherland and Tsuboi,[5] who were the first to report the changes in the spectrum of solid DNA films occurring as a function of relative humidity. Bradbury, Price and Wilkinson reported studies of DNA[6] as well as of nucleoprotamine[7] and nucleohistone[8] and were the first to present plots of the dichroic ratio of several infrared bands as a function of the water content of DNA. They also reported a plot of the frequency of the band due to the antisymmetric stretching vibration of the PO_2^- group of DNA against the water content of the specimen. Several new assignments were made by Tsuboi,[9] who also listed the changes in the spectrum of solid DNA upon dehydration and showed that some of the changes are similar to those which occur when DNA is denatured in solution by heating,[9] by treatment with deoxyribonuclease,[9] or by treatment with formamide.[10]

In the present study we have investigated the infrared spectra of solid films of lithium and sodium salts of DNA (NaDNA and LiDNA) in both deuterated and undeuterated form, in the range 4000 to 400 cm.$^{-1}$. Both polarized and non-polarized spectra were recorded as a function of relative humidity (r.h.) with special care taken to attain equilibrium. As we are largely in agreement with the frequencies and assignments reported by previous investigators, only the *changes* in the infrared spectrum of DNA brought about by hydration and dehydration will be described, particularly those changes which provide information about the ranges of relative humidity in which various molecular subgroups

(1) This work was supported by Grant No. A-2262(C3) from the National Institutes of Health, Public Health Service.

(2) Atlantic Regional Laboratory, National Research Council (Canada), Halifax, Canada.

(3) E. R. Blout and M. Fields, *Science*, **107**, 252 (1948); *J. Biol. Chem.*, **178**, 335 (1949); *J. Am. Chem. Soc.*, **72**, 479 (1950).

(4) E. R. Blout and H. Lenormant, *Biochim. et Biophys. Acta*, **17**, 325 (1955).

(5) G. B. B. M. Sutherland and M. Tsuboi, *Proc. Roy. Soc.* (London), **A239**, 446 (1957).

(6) E. M. Bradbury, W. C. Price and G. R. Wilkinson, *J. Mol. Biol.*, **3**, 301 (1961).

(7) E. M. Bradbury, W. C. Price and G. R. Wilkinson, *ibid.*, **4**, 39 (1962).

(8) E. M. Bradbury, W. C. Price, G. R. Wilkinson and G. Zubay, *ibid.*, **4**, 50 (1962).

(9) M. Tsuboi, *Prog. Theoret. Phys.* (Kyoto), Supp. 17, 99 (1961).

(10) Y. Kyogoku, M. Tsuboi, T. Shimanouchi and I. Watanabe, *J. Mol. Biol.*, **3**, 741 (1961).

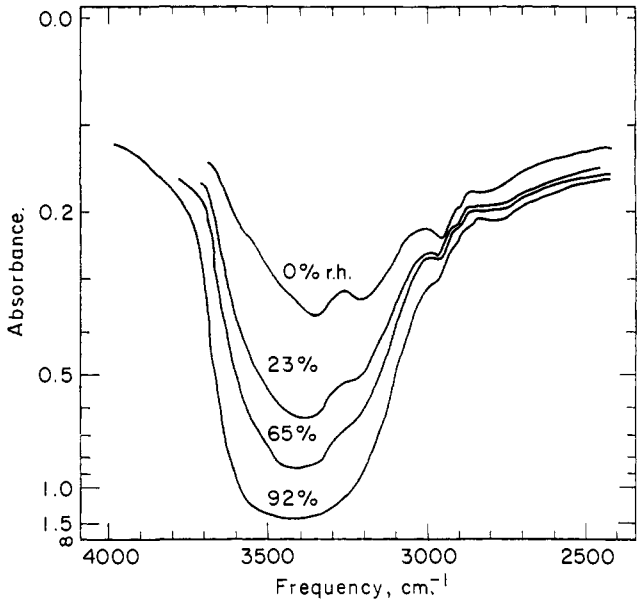

Fig. 1.—Spectra of NaDNA in the hydrogen stretching region at several relative humidities (Perkin–Elmer 237).

in DNA become hydrated and about the strength of the hydrogen bonding between water and the DNA substrate. The closely connected question of the nature of the changes in the DNA structure which accompany hydration and dehydration will be discussed in the following paper[11] along with the results of a study of oriented DNA using polarized infrared and ultraviolet radiation.

Experimental

Samples of NaDNA and LiDNA used in this study were the same as in the gravimetric work.[12] An additional sample of calf-thymus NaDNA prepared by Dr. P. F. Davison was used to check the results. The specimens for infrared study were prepared by spreading and evaporating the correct amount of an aqueous solution of DNA to form a film 1 to 5 microns thick on a rectangular plate of silver chloride or fluorite. The plate then was cemented as one window of a hygrostatic cell, similar to that described by Sutherland and Tsuboi,[5] except that for convenience the reservoir holding the appropriate saturated salt solution and forming the bottom of the cell could be detached by means of a ground glass joint. This made it possible to change reservoirs rapidly. The salts used in maintaining constant r.h. were the same as in ref. 12 and the films were deuterated by placing in the reservoir a saturated solution of one of the salts in D_2O. Anhydrone or phosphorus pentoxide was placed in the reservoir to obtain 0% r.h.

The spectra were recorded on several instruments: a Perkin–Elmer 112 single-beam double-pass spectrophotometer with NaCl and LiF prisms, a Perkin–Elmer 237 grating double-beam spectrophotometer, a Perkin–Elmer 137 double-beam spectrophotometer with a NaCl prism and a Baird AB-2 double-beam spectrophotometer with NaCl and KBr prisms.

In these and most other present-day infrared spectrophotometers, the sample is placed between the source and the monochromator and is thus subjected to the radiant power of all wave lengths emitted by the source. In the case of DNA this is a crucial problem, as the radiation drives water rapidly from the sample. In a typical continuous operation the water content decreased by 30% in 5 minutes (as determined from the intensity of the water bands), even though the temperature of the silver chloride plate (as measured by a thermocouple imbedded in it) rose only by about 3.5°.

This problem was avoided by recording the spectrum over wave length intervals of the order of one micron at rapid scanning rates. Scanning times of 1 to 2 minutes were used with at least 30 minutes allowed for return to equilibrium. These short bursts of radiation were found not to affect the water content of the sample appreciably, as indicated by the undiminished intensity of the water bands. An alternate and equally effective method, applicable only below 2000 cm.$^{-1}$, was to place an infrared filter (Kodak No. 230) in front of the sample.

(11) M. Falk, K. A. Hartman, Jr., and R. C. Lord, *J. Am. Chem. Soc.*, **85**, 391 (1963).

(12) M. Falk, K. A. Hartman, Jr., and R. C. Lord, *ibid.*, **84**, 3843 (1962).

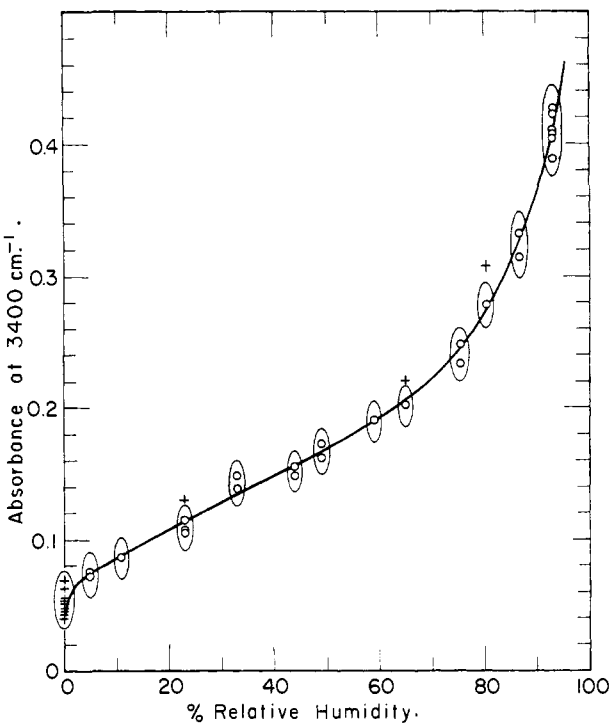

Fig. 2.—Plot of the absorbance at 3400 cm.$^{-1}$ in NaDNA as a function of relative humidity. Circles and crosses indicate values reached by increasing and decreasing relative humidity, respectively.

The attainment of equilibrium between the water content of the DNA films and the r.h. of the ambient atmosphere was followed spectroscopically and was found to require several days, the length of time increasing with sample thickness. Equilibrium was approached several times with each sample from both high and low r.h. to detect possible hysteresis.

Results

As the extent of hydration of DNA is varied, frequency and intensity changes of water bands and DNA bands are observed.

1. **Water Bands.**—At values of r.h. near 92%, when the molar ratio of adsorbed water to DNA is of the order of twenty water molecules per nucleotide,[12] infrared bands characteristic of liquid water are observed in the spectrum of the DNA film. The band due to the bending vibration of the water molecule is centered at 1660 ± 15 cm.$^{-1}$ for H_2O in DNA (1640 ± 5 in liquid H_2O)[13] and at 1230 ± 10 cm.$^{-1}$ for D_2O in DNA (1205 ± 5 cm.$^{-1}$ in liquid D_2O). The librational modes of water molecules give rise to a band centered at 710 ± 25 cm.$^{-1}$ for H_2O in DNA (710 ± 25 cm.$^{-1}$ in liquid H_2O) and at 515 ± 15 cm.$^{-1}$ for D_2O in DNA (530 ± 25 cm.$^{-1}$ in liquid D_2O). The absorption usually assigned to the combination of the bending vibration with the librational modes occurs at 2210 ± 50 cm.$^{-1}$ for H_2O in DNA (2120 ± 15 cm.$^{-1}$ in liquid H_2O). The corresponding D_2O band (at 1620 cm.$^{-1}$ in liquid D_2O) could not be observed in DNA because of strong absorption in this region.

By far the most intense infrared band of water is that due mainly to the antisymmetric OH stretching vibration. In Fig. 1 the spectrum of DNA in the OH stretching region is shown in the range 0–92% r.h. At 0% r.h. all absorption due to water disappears and the underlying absorption due to DNA remains. It consists of bands arising from the NH stretching modes at 2800 ± 50, 3206 ± 10, and 3353 ± 10 cm.$^{-1}$ and the aliphatic CH stretching modes at 2892 ± 10 and 2951 ± 10 cm.$^{-1}$. The band corresponding to the stretching

(13) The frequencies of liquid H_2O and D_2O are from M. Falk and P. A. Giguère, *Can. J. Chem.*, **35**, 1195 (1957).

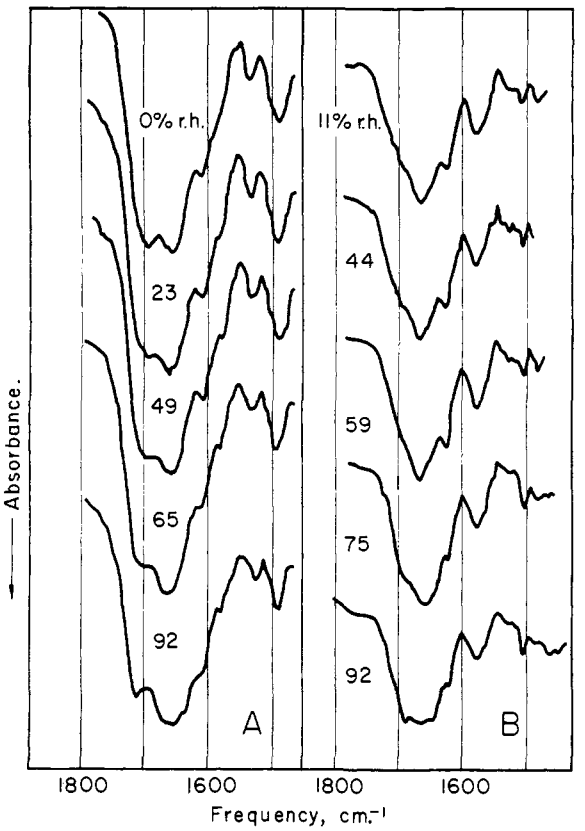

Fig. 3.—Spectra in the carbonyl stretching region of NaDNA (A) and of deuterated NaDNA (B) at several relative humidities (Perkin-Elmer 237).

modes of the aromatic CH groups at 3103 ± 10 cm.$^{-1}$ shows up more clearly in the spectrum of deuterated DNA. This underlying absorption can be approximately accounted for by subtracting the spectrum of 0% r.h. from the spectra at higher r.h. It is then found that the frequency of the water band increases from 3390 ± 10 cm.$^{-1}$ at low r.h. to 3420 ± 10 cm.$^{-1}$ at high r.h. In liquid water this band centers at 3400 ± 10 cm.$^{-1}$. A similar increase occurs in the frequency of the corresponding D_2O band near 2500 cm.$^{-1}$.

In Fig. 2 the absorbance at 3400 cm.$^{-1}$ has been plotted against r.h. The data were obtained for several DNA films of different thicknesses and the absorbance values were normalized by dividing by the absorbance of the band at 1220–1240 cm.$^{-1}$. The film thickness could be estimated roughly from the results of Sutherland and Tsuboi[5] and the effective value for Fig. 2 is approximately 1 micron.

The absorbance at 3400 cm.$^{-1}$ (2500 cm.$^{-1}$ for D_2O) is a smooth function of the H_2O (D_2O) content of the DNA films, which increases with increasing r.h. This band provides a convenient measure of film hydration and may be used to confirm whether equilibrium is attained and reproduced. As in the gravimetric study[12] a significant hysteresis has been observed between the adsorption curve (circles in Fig. 2) and the desorption curves (crosses in Fig. 2).

2. **DNA Bands**: (a) **The 1550–1720 cm.$^{-1}$ Region.**—In Fig. 3 the spectra of DNA and of deuterated DNA are shown in this region at various r.h. values.

As r.h. is increased the spectrum of non-deuterated DNA shows a continuous increase in the intensity of absorption due to the bending vibration of H_2O at 1660 cm.$^{-1}$. The frequency of the band at 1690 ± 2 cm.$^{-1}$ in dry DNA remains constant from 0 to at least 50% r.h. and then rises to 1701 ± 2 cm.$^{-1}$ at 65% r.h. and to 1712 ± 2 cm.$^{-1}$ at 92% r.h.

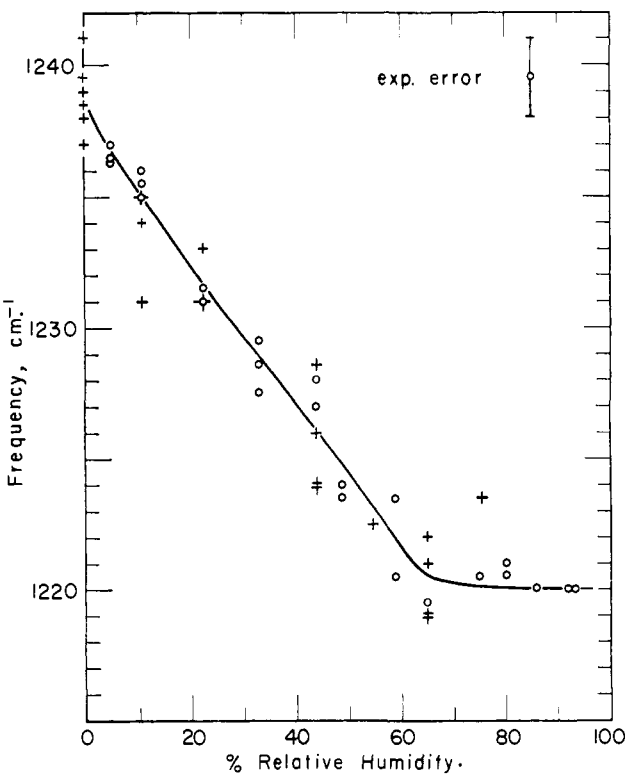

Fig. 4.—Plot of the frequency of the 1220–1240 cm.$^{-1}$ band as a function of relative humidity (Baird AB2 and Perkin-Elmer 112). Circles and crosses are as in Fig. 2.

The spectrum of deuterated DNA in this region shows no major change until above 65% r.h. Instead of one strong peak at 1664 ± 2 cm.$^{-1}$ below 65% r.h. three strong overlapping peaks appear at 92% r.h. These occur at 1690 ± 2, 1668 ± 2, and 1644 ± 3 cm.$^{-1}$. The weaker bands at 1620 ± 2 and 1574 ± 3 cm.$^{-1}$ do not appear to shift upon hydration.

These results are in general agreement with the observations of Tsuboi[5,9] and Bradbury,[8] who did not however investigate the r.h. range where the above changes occur.

(b) **The 1240 cm.$^{-1}$ Band.**—The strong band at 1240 ± 1.5 cm.$^{-1}$ in DNA at 0% r.h. shifts to 1220 cm.$^{-1}$ at high r.h. The frequency of this band has been studied very carefully as a function of relative humidity, and the results for several samples of NaDNA are shown in Fig. 4. The results for LiDNA are the same within the uncertainty of the data, which is about ±1.5 cm.$^{-1}$. The entire lowering of the frequency occurs in the humidity range of 0 to 65% r.h., and above 65% r.h. a single plateau is observed. These results differ from those of Bradbury, Price and Wilkinson,[8] obtained with polarized radiation and oriented samples. They reported one plateau near 90% r.h. and another near 70% r.h.

(c) **The 800 to 1100 cm.$^{-1}$ Region.**—The effect of r.h. on the spectrum of DNA in this region is shown in Fig. 5. Apart from a broadening of many bands, several changes in frequency and intensity occur upon dehydration.

The band at 1089 ± 2 cm.$^{-1}$ in dry DNA moves upon hydration to 1086 ± 1 cm.$^{-1}$. Since the frequency shift is small and the band broadens considerably on drying, it is not possible to determine the r.h. range in which the shift occurs. The band at 1066 ± 2 cm.$^{-1}$ moves to 1052 ± 1 cm.$^{-1}$ upon hydration. It appears that the entire shift occurs near 50% r.h. The band at 962 ± 2 cm.$^{-1}$ moves up to 970 ± 1 cm.$^{-1}$ between 0 and 60% r.h. Previous workers observed com-

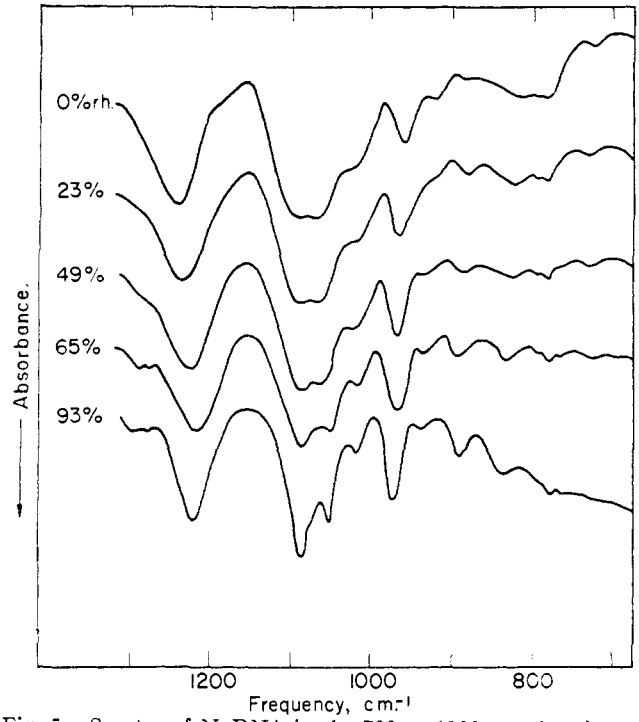

Fig. 5.—Spectra of NaDNA in the 700 to 1300 cm.$^{-1}$ region at several relative humidities (Baird AB2).

parable shifts in these bands but did not report the r.h. range in which the shifts occur.[5,6]

All the spectral changes observed in this work were reversible over many cycles of raising and lowering r.h. except for a small hysteresis, which will be neglected in the discussion that follows. The differences between NaDNA and LiDNA were often within the experimental uncertainty and will also be neglected in the discussion.

Discussion

1. The State of Adsorbed Water.—The plot in Fig. 2 agrees in shape with the hydration curve of DNA fibers obtained gravimetrically.[12] This agreement strengthens our belief that the hydration of DNA films adhering to rigid substrates does not essentially differ from the hydration of fibers, at least below about 75% r.h. At higher humidities the hydration of DNA proceeds with considerable swelling[12] and since the extent of swelling may be different for fibers and for rigidly mounted films, the hydration may also differ.

The OH stretching frequency of water in DNA is found to increase with increasing r.h. This indicates that the average strength of hydrogen bonding decreases[14] as subsequent water molecules adsorb. Since at 92% r.h. all the frequencies of adsorbed water are within experimental error of those observed for films of pure liquid water, and are quite different from those of ice,[15] we conclude that at high r.h. the bulk of the water molecules hydrating DNA form a structure similar to that of liquid water and that there is no infrared evidence for the existence of "icebergs."[16,17] The above result also justifies the assumption made by us previously in order to apply the Brunauer-Emmett-Teller treatment,[12] that all but the first layer of adsorbed water molecules have properties identical with those of liquid water.

(14) G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond," Reinhold Publ. Co., New York, N. Y., 1960.

(15) P. A. Giguère and K. B. Harvey, *Can. J. Chem.*, **34**, 798 (1956).

(16) B. Jakobson, *J. Am. Chem. Soc.*, **77**, 2919 (1955).

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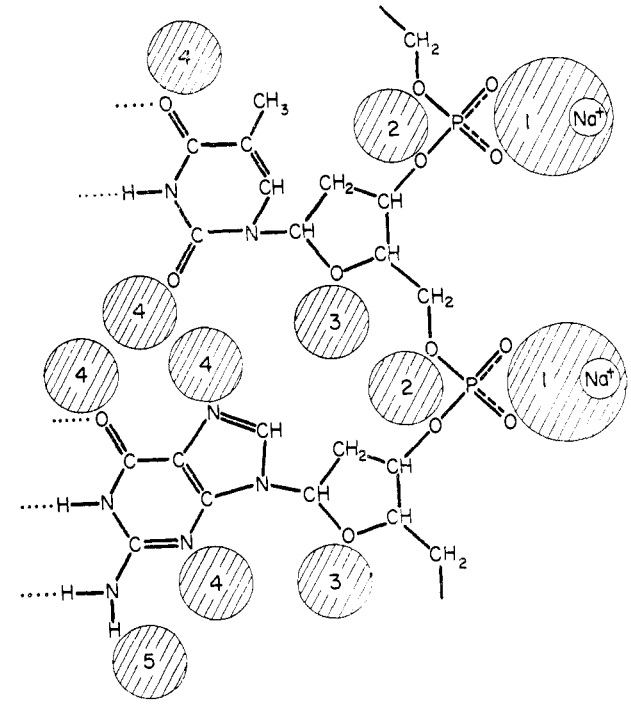

Fig. 6.—A schematic drawing of one strand of DNA containing thymine and guanine bases, indicating sites for possible water adsorption as shaded areas.

2. Sequence in Which Different Molecular Sites Become Hydrated.—All three molecular subgroups in DNA (the heterocyclic bases, deoxyribose, and the diesterified phosphate groups) provide sites where water molecules can be adsorbed. The five possible hydration sites, indicated schematically in Fig. 6, may of course contain more than one water molecule and conversely one water molecule may be attached to more than one site. Since the frequencies and intensities of the infrared bands associated with the various molecular subgroups are sensitive to environmental effects, particularly to hydrogen bonding, the r.h. range in which these subgroups become hydrated can be determined from the behavior of the bands associated with these groups. However one must distinguish carefully between the changes in the spectrum due directly to hydration and those due to structural changes induced by hydration.

The assignment of the 1220–1240 cm.$^{-1}$ band to the antisymmetric stretching vibration of the PO_2^- group is certain.[5,6,18] The frequency lowering of 20 cm.$^{-1}$ between 0 and 65% r.h. is a direct indication that the PO_2^- group becomes hydrated in that r.h. range. A similar lowering occurs upon hydration in $K^+H_2PO_2^-$,[18] $Ba^{++}[(CH_3O)_2PO_2^-]_2$[5] and $Na^+[(CH_3O)_2PO_2^-]$.[6] At 65% r.h. 5 to 6 water molecules are adsorbed per nucleotide.[12] These water molecules may be said to form a hydration shell about the $PO_2^-Na^+$ groups (sites labeled "1" in Fig. 6) since no further change in the 1220–1240 cm.$^{-1}$ band is observed above 65% r.h.

Two bands in the 1100 to 900 cm.$^{-1}$ region shift below 60% r.h. and no band shows a detectable shift above 60% r.h. The bands that shift occur at 962–970 cm.$^{-1}$ and at 1066–1052 cm.$^{-1}$ and have been assigned by Tsuboi[9,10] to P–O and C–O stretching vibrations, respectively. Assuming these assignments to be correct we conclude that sites "2" and probably also sites "3" (Fig. 6) become hydrated below 60% r.h. Thus some of the water molecules forming the "hydration shell" of the $PO_2^-Na^+$ group also hydrate the P–O–C oxygen

(18) M. Tsuboi, *J. Am. Chem. Soc.*, **79**, 1351 (1957)

atoms, and possibly also the C–O–C oxygen atoms of the deoxyribose ring.

The absorption in the 1550–1720 cm.$^{-1}$ region in the spectrum of deuterated DNA is due to C=O and ring stretching vibrations of the purine and pyrimidine bases. The additional band observed at 1690–1712 cm.$^{-1}$ in the non-deuterated spectrum has a contribution from the bending vibration of the NH and NH_2 groups. Since changes in these bands have just begun to occur at 65% r.h., we conclude that sites "4" and "5" do not begin to hydrate until near 65% r.h.

Conclusions.—The hydration behavior of DNA may be summarized: between 0 and 60 to 65% r.h. water adsorbs on sites provided by the $PO_2^-Na^+$ portions of the DNA backbone. The P–O–C and C–O–C oxygens probably also become hydrated below 65% r.h. At 65% r.h. about 6 water molecules are adsorbed completing the hydration of the phosphate group.[19] The C=O groups and ring nitrogen atoms become hydrated above 65% r.h. A transition from a low r.h. structure to the high r.h. helical form of DNA occurs between about 60 and 75% r.h.[11,20] Above 75% the water molecules hydrating the C=O groups and ring nitrogen atoms fill the grooves of the DNA helix. This process is complete by about 80% r.h. and further hydration of DNA is accompanied by swelling.[12] All exposed hydration sites are probably filled at this point.

(19) It is significant that this value corresponds closely to the hydration shell of about 6.5 water molecules per nucleotide for DNA in aqueous solution found by J. H. Wang, *J. Am. Chem. Soc.*, **77**, 258 (1955).

(20) R. E. Franklin and R. G. Gosling, *Acta Cryst.*, **6**, 673 (1953).

[CONTRIBUTION FROM THE SPECTROSCOPY LABORATORY AND THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE, MASSACHUSETTS]

Hydration of Deoxyribonucleic Acid. III. A Spectroscopic Study of the Effect of Hydration on the Structure of Deoxyribonucleic Acid[1]

BY MICHAEL FALK,[2] KARL A. HARTMAN, JR., AND R. C. LORD

RECEIVED SEPTEMBER 24, 1962

The polarized infrared and ultraviolet spectra of oriented films of the sodium salt of deoxyribonucleic acid (DNA) have been investigated as a function of relative humidity (r.h.). Quantitative data are presented for the behavior of the dichroic ratios of the infrared band at 1660 cm.$^{-1}$ and the ultraviolet band at 2600 Å. and for the absorbance of the latter. The curves obtained are congruent, showing one plateau above 75% r.h. and a second below 55% r.h. Between 75 and 55% r.h. there occurs a sharp increase in the dichroic ratios and absorbance at 2600 Å. These changes are reversible. The increase in ultraviolet absorption is similar to that observed when DNA is denatured in aqueous solution by thermal or other means. It is concluded that DNA films are stable in the B configuration at r.h. values as low as 75% and that at still lower humidities a reversible transition occurs to a disordered form in which the bases are no longer stacked one above another and are no longer perpendicular to the axis of the helix. The loss of base stacking upon drying suggests that the B configuration of DNA is stabilized by the stacking of the bases in the presence of water. The B configuration may thus be considered a kind of micelle structure (as postulated by Herskovits, Singer and Geiduschek), in which the number of attractive water–water and base–base interactions is maximized.

Introduction

Numerous studies of the structure of the sodium and lithium salts of deoxyribonucleic acid (DNA) in the solid state have been made using the techniques of X-ray diffraction and several distinct structures have been found to exist in various relative humidity (r.h.) regions.[3–8] Studies using polarized infrared radiation have been made but in these either r.h. was not varied[9] or the investigators were mainly interested in providing infrared evidence for the B⇄A and B⇄C transitions and did not study in detail the behavior of spectra at lower r.h.[10,11]

The ultraviolet spectrum of DNA does not appear to have been studied as a function of r.h.

In this paper are presented the results of a study of the changes occurring in the polarized infrared and ultraviolet spectra of DNA films and of the changes occurring in the absorbance of the band at 2600 Å. as a function of r.h. Such data allow inferences to be drawn concerning structural changes occurring as a function of hydration and provide information about the sources of stabilization energy of the helical configuration of DNA.

Experimental

Infrared.—Apart from the additional techniques described below, samples, hygrostatic cells, and procedures used to obtain polarization spectra were the same as in the preceeding paper.[12] Data are presented only for the sodium salt of deoxyribonucleic acid (NaDNA). Most polarization spectra were recorded on the Perkin–Elmer Model 137 spectrophotometer equipped with a mechanical guide which assured the correct repositioning of the hygrostatic cell on successive runs. Two types of polarizers were used: a wire grid polarizer in the 4000 to 1400 cm.$^{-1}$ region,[13] and a seven-sheet silver chloride polarizer below 1400 cm.$^{-1}$. These were mounted just before the exit slit of the instrument and thus polarized the radiation in both beams. Since each polarizer is essentially 100% effective, the moderate polarizing property of the instrument does not affect the measured dichroic ratio when the polarizer is placed in this position. Spectra were run with the electric vector of the radiation first parallel and then perpendicular to the slits by rotating the polarizer 90°.

Ultraviolet.—The spectra were recorded on a Beckman DK-2 spectrophotometer using a dichroic polarizer generously provided by Mr. A. Makas of the Polaroid Corporation. This polarizer was tested and found to be nearly a perfect polarizer at 2700 Å. and therefore dichroic measurements were made at this wave length. The polarizer was placed at the exit slit and was therefore in both beams. The entire sample chamber of the Beckman instrument was humidified by employing saturated salt solutions.[14]

(1) This work was supported by Grant No. A-2262(C3) from the National Institutes of Health, Public Health Service.

(2) Atlantic Regional Laboratory, National Research Council (Canada), Halifax, Canada.

(3) R. E. Franklin and R. G. Gosling, *Acta Cryst.*, **6**, 673 (1953).

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