CHANGES IN THE INFRARED SPECTRA OF SOLUTIONS OF DEOXYPENTOSE NUCLEIC ACID IN RELATION TO ITS STRUCTURE

by

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The infrared spectrum of deoxypentose nucleic acid (DNA), when obtained on a dry film or fiber[1,2], shows two main groups of absorption bands, that between 1550 and 1750 cm^{-1} associated with the purine and pyrimidine components, and that between 900 and 1300 cm^{-1} associated with the sugar-phosphate moiety. In the solid state it is possible to differentiate DNA from ribose nucleic acid (RNA) by virtue of a small band at 1025 cm^{-1} [1], but the spectra of solid samples of DNA of varying molecular weights show no difference provided the degree of salt formation is the same. Thus an explanation of some apparent differences in some DNA spectra[1,2] lies in the fact that the spectra of solid samples of DNA do vary in the region of 1600 cm^{-1} depending on the degree of salt formation.

In order to study DNA in a state more closely approximating that which exists in nature, we have examined its infrared spectra in aqueous solutions using a technique previously described[3] and which has been applied to proteins[4]. In this paper we report the effect of (a) molecular weight, (b) desoxyribonuclease action, (c) pH changes and (d) heat treatment on the infrared spectra of DNA solutions***.

EXPERIMENTAL

Materials. Four different samples of DNA were used in this investigation.

DNA-1 was prepared in this laboratory from calf thymus (by M. ABBATE) using the method of KAY, SIMMONS AND DOUNCE[5]. It is a fibrous material which contained less than 0.1% protein.

DNA-2 was prepared in the Department of Chemistry, Harvard University, from calf thymus (by Drs. DOTY AND VARIN). It is a fibrous material which has a molecular weight of about four million[6].

DNA-3 was purchased from Worthington Biochemical Corporation where it was prepared from calf thymus by the method of MIRSKY AND POLLISTER[7]. It is a fibrous material.

DNA-4 was purchased from Nutritional Biochemicals Corporation where it was prepared from herring sperm using the hot alkaline extraction method of LEVENE[8]. The product is a powder and does not give viscous solutions. It is of low molecular weight.

Technique. A Perkin-Elmer Model 21 double beam spectrometer with a sodium chloride prism and slit widths of approximately 0.072 mm at 2000 cm^{-1}, 0.125 mm at 1400 cm^{-1}, and 0.350 mm at 850 cm^{-1} were used. Solutions in D_2O were examined in the region 1400 to 2000 cm^{-1}, and solutions in H_2O were used in the region 700 to 1400 cm^{-1}.

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The D_2O solutions of DNA-4 were prepared by simply dissolving the substance in D_2O. The presence of a large amount of absorbed water in the high polymeric materials (DNA-1, 2 and 3) made a preliminary deuteration necessary. Therefore these samples were dissolved in D_2O (approximately 2% by weight), lyophilized, and redissolved in D_2O. Since it is necessary to use relatively thin cells (0.025 mm to 0.075 mm), the concentration of the DNA must be of the order of 7%. At this concentration the high polymers form transparent gels. The pH upon solution, of all preparations, was near 5.

RESULTS

As in the solid state, the infrared spectra of DNA in solution show two main groups of absorption bands: one between 1550 and 1750 cm^{-1} and the other between 900 and 1300 cm^{-1}.

(1) *The 1550 to 1750 cm^{-1} region.* In this region solutions of DNA in D_2O show complicated absorption spectra which differ markedly with molecular weight and pH (*cf.* Figs. 1A, 2A, 2B.)

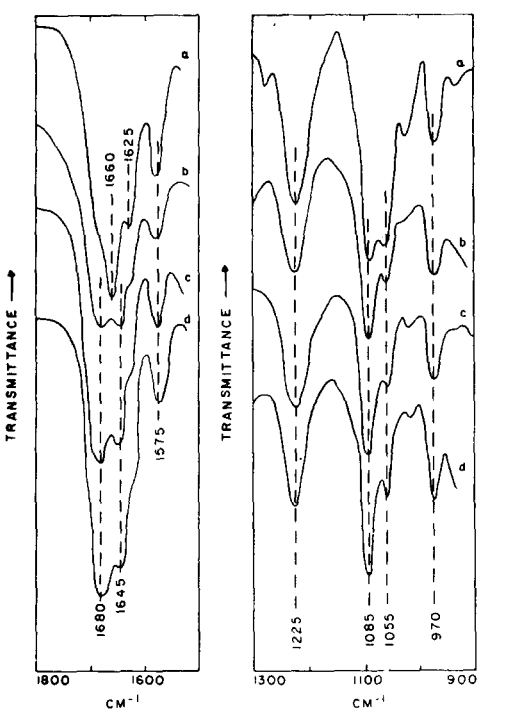

Fig. 1A. D_2O solutions: (a) DNA-4; (b) DNA-3; (c) DNA-2; (d) DNA-1.

Fig. 1B. H_2O solutions: (a) DNA-4; (b) DNA-3; (c) DNA-2; (d) DNA-1.

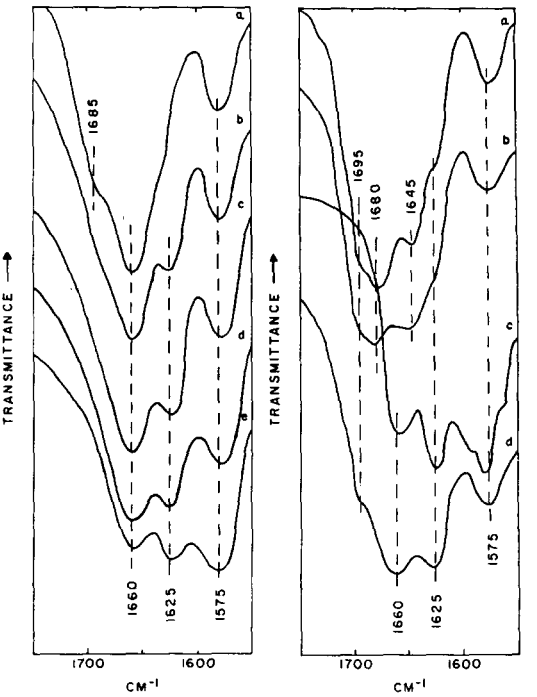

Fig. 2A. DNA-4 in D_2O solutions: (a) pH $\sim$ 2 (cloudy solution); (b) pH $\sim$ 4; (c) pH $\sim$ 7; (d) pH $\sim$ 10; (e) pH $>$ 11.

Fig. 2B. DNA-1 in D_2O solutions: (a) pH $\sim$ 5; (b) pH $\sim$ 9; (c) pH $>$ 11; (d) solution c (pH $>$ 11) brought to pH $\sim$ 6.

A. *The low polymer.* D_2O solutions of DNA-4 near neutrality show three distinct bands: 1660 cm^{-1} (v.s.), 1625 cm^{-1} (s.) and 1575 cm^{-1} (m.) (Fig. 2A). From pH 4 to pH 10 practically no change appears in the absorption curves, but by pH 11 the 1625 cm^{-1} and 1575 cm^{-1} bands have increased markedly in intensity. At pH 2 the 1625 cm^{-1} band

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disappears and a new absorption appears in the form of a shoulder at 1685 cm^{-1} (Fig. 2A, curve a). These changes are reversible. The intensity of the 1660 cm^{-1} band appears to remain unchanged with changes in pH.

In the region between 1550 and 1750 cm^{-1} the spectrum of low polymer DNA in D_2O solution differs markedly from the spectrum of the same material in the dry state. In order to determine the respective effect of deuteration and the solvation we have examined deuterated and non-deuterated DNA-4 in the solid state. In this state the absorption bands of the deuterated neutral material lie at the same frequencies as in neutral D_2O solution (1660, 1625 and 1575 cm^{-1}), whereas the non-deuterated solid material shows absorption bands at higher frequencies (1700, 1660 and 1600 cm^{-1}). It is thus apparent that although deuteration produces spectral changes in low molecular weight DNA, solvation of the deuterated material does not change its infrared spectrum in this region (*vide infra*).

B. *The high polymers.* The high molecular weight samples (DNA-1, DNA-2, and DNA-3) in D_2O solution show distinctly different absorption maxima from the low molecular weight sample (DNA-4). Near pH 5, the two strong bands in all the high polymers are at 1680 cm^{-1} and 1645 cm^{-1} (Fig. 1A, curves b, c, and d), as compared with 1660 cm^{-1} and 1625 cm^{-1} for the low polymer. The band at 1575 cm^{-1} is constant in frequency. In addition two weak shoulders are observed at 1695 cm^{-1} and 1625 cm^{-1} (Fig. 2B, curve a). The presence of 1M sodium chloride does not affect the position of these absorption bands.

In contrast to the spectra obtained with the low molecular weight sample, DNA-4, all the high molecular weight samples have spectra in D_2O solution which differ from those obtained with the same materials in the deuterated solid state. In the deuterated *solid* state the bands in both the high and low polymers lie at 1660, 1625 and 1575 cm^{-1}, whereas in solution in D_2O a shift to higher frequencies (bands at 1680 and 1645, 1575 cm^{-1}) is observed in *only* the high molecular weight samples.

(a) *The action of desoxyribonuclease.* The addition of a trace of desoxyribonuclease to a gel of DNA-1 (pH 5) at 37° C liquefies the gel completely in $2^1/_2$ hours. Concomitant with the liquefaction of the gel the infrared spectrum changes markedly in that the 1680 and 1645 cm^{-1} bands disappear and bands at 1660 cm^{-1} and 1625 cm^{-1} appear (Fig. 3A). The new spectrum is the same as that of DNA-4 in this region.

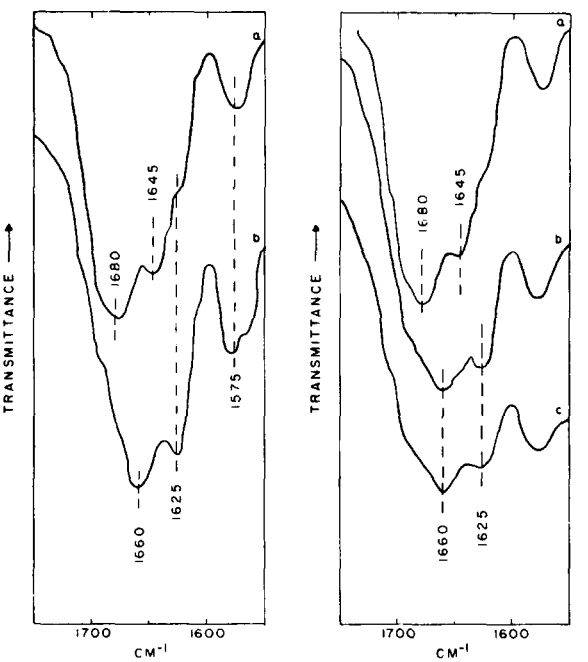

Fig. 3A. (a) DNA-1 in D_2O pH ~ 5 (gel); (b) DNA-1 gel a, treated with desoxyribonuclease for 2 ½ hours at 37° C pH ~ 5.

Fig. 3B. (a) DNA-1 in D_2O pH ~ 5 (gel); (b) DNA-1 gel a, heated 8 minutes at 100° C; (c) DNA-1 gel a, heated 60 minutes at 100° C.

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(b) *The effect of pH changes.* In the concentrated DNA solutions we use for spectral studies, we were not able to prepare a solution more acidic than pH 5 with the high molecular weight samples. With increasing pH the gel becomes more fluid, but until pH 9 the spectrum is practically unmodified. However, at pH $>$ 9 the bands at 1680 and 1645 cm^{-1} disappear and the 1660 and 1625 cm^{-1} bands appear along with an increase in intensity of the 1575 cm^{-1} band (Fig. 2B, curve c). At this point, the spectrum is similar to that of DNA-4 at the same pH (Fig. 2A, curve e). If the highly alkaline solution is neutralized to pH 6 the original spectrum at that pH is not observed. After going through the cycle pH 5 $\rightarrow$ pH $<$ 11 $\rightarrow$ pH 6 the spectrum resembles that obtained with DNA-4 at pH values between 4 and 7.

(c) *The effect of heat.* By heating a gel of 7% DNA-1 (pH 5) in D_2O at 100° C for 8 minutes, a stronger, more elastic gel is produced. Heating for as long as 60 minutes at 100° C did not further change the appearance of the material. However, the infrared spectrum of a sample so treated is different from that of the original gel. The bands at 1680 and 1645 cm^{-1} are weakened, and two strong bands at 1660 and 1625 cm^{-1} appear (Fig. 3B).

(2) *The 900 to 1300 cm^{-1} region.* All the DNA samples which we have examined showed similar absorption spectra in this region. The spectrum shows three main bands—one strong band at 1225 cm^{-1}, a doublet at 1055 and 1085 cm^{-1} of which the 1085 cm^{-1} component is very strong, and a less strong band at 970 cm^{-1}. In addition there is a small band at 1025 cm^{-1} (Fig. 1B). Aqueous solutions of RNA show a strong shoulder at 1120 cm^{-1}. The differences shown in this region easily distinguish RNA from DNA in solution.

The absorption bands of DNA in this region lie at the same frequencies despite variations in the molecular weight of the samples examined. Furthermore we have not observed any change in the spectra in this region of the high molecular weight DNA samples upon varying the pH through the range 3 to 12, or upon heating the samples in solution to 100° C, or with the action of desoxyribonuclease for relatively short time intervals.

DISCUSSION

The two main groups of infrared absorption bands shown by DNA have been correlated in a general way with (1) the purine and pyrimidine components (1550–1700 cm^{-1}) and (2) the sugar-phosphate parts of the molecule (900–1300 cm^{-1})[1]. This general correlation is in accord with studies on simpler molecules containing phosphate groups [9,10,11] as well as those containing purine and pyrimidine rings[12–16].

The most interesting phenomenon which has been observed with *high molecular weight DNA in solution* is that it *has a characteristic infrared spectrum which may be changed by physical, chemical, or enzymic treatment.* Furthermore, it is important to note that after the action of very different agents, the resulting altered DNA's all show the same altered spectrum.

It is known that the changes in DNA brought about by short alkaline treatment[17] at room temperature, or by desoxyribonuclease[18], result in a decrease in the viscosity and the molecular weight. Therefore it seems logical to associate the observed change in the infrared spectrum, upon treatment with alkali or desoxyribonuclease, with decreases in molecular weight. However, the action of heat at pH's near neutrality, which also changes the infrared spectrum of DNA in the same manner, results in no change in molecular weight but rather a change in configuration of the molecule.[19]

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It may be concluded therefore, that the observed changes in the infrared solution spectrum of DNA, although related to its molecular weight, are more particularly related to its configuration. Furthermore, since the characteristic observed change in the spectrum lies in the region of strong absorption by the purine and pyrimidine components of DNA, this is indicative of the important role these bases must play in the configuration of the molecule.

The exact physical meaning of the shift in the frequency of the bands (1680 and 1645 $cm^{-1} \rightarrow$ 1660 and 1625 cm^{-1}) with the action of the above-mentioned agents, is difficult to interpret. The strong bands in this region are caused by the component purines and pyrimidines involving principally the C = O, C = N and $-NH_2$ bonds. These groups are almost certainly hydrogen bonded and if this shift in frequency is correlated with the strength of the hydrogen bonds, it must be concluded that in the altered DNA's the purines and pyrimidines are more strongly bonded than in the original high molecular weight DNA. This may be an unwonted conclusion because in the high polymer, it is thought that structure is maintained by very specific and strong intermolecular hydrogen bonding[20]. However, since the infrared spectrum of the original high polymer DNA and the altered DNA's in the deuterated *dry* state show the same bands at 1660 and 1625 cm^{-1}, it is suggested that the characteristic absorption of the high polymer at 1680 and 1645 cm^{-1} we observe *in solution* involves modification by hydration or solvation. If we assume that the double stranded helical configuration of solid DNA[20] is maintained in solution, then hydration of the outer parts of the helix (the sugar and phosphate) might result in a force on the intermolecular purine-pyrimidine bonds which would tend to increase their absorption frequency. *On treatment with heat, alkali, or desoxyribonuclease, we postulate that the specific helical configuration is altered* (with or without change in the molecular weight) *and this change in the configuration allows hydration of the molecules without a distorting force on the purine-pyrimidine bonds.* Spectral changes such as those observed, *i.e.* from higher to lower frequency, are generally associated in hydrogen bonded species with increased strength of the hydrogen bond. Then, on this basis, one must assume that in the altered DNA samples either the purine-pyrimidine hydrogen bonds are stronger than in the original material (because of of removal of a distorting force as just discussed) *or* that the purine-pyrimidine hydrogen bonds are ruptured by the treatments and new bonds (purine-D_2O and pyrimidine-D_2O) are formed which lie at the lower frequencies.

A comparison of the infrared solution spectra of DNA and its component nucleotides and nucleosides [1] gives some information about the origin of the bands at 1660, 1625, and 1575 cm^{-1}. The band at 1575 cm^{-1}, which shows medium intensity in acidic or neutral D_2O solution and strong intensity in alkaline D_2O solution, can be attributed to guanine since both deoxyguanosine and deoxyguanylic acid show a similar band with the same behavior. The other nucleotides present in DNA do not show this band. The bands at 1660 and 1625 cm^{-1} probably are associated principally with thymine. Thymidine and thymidylic acid show the same strong bands with similar pH behavior. However, it is probable that adenine also contributes to the absorption at 1625 cm^{-1} since the adenylic acids in D_2O solution show a strong and sharp band at this frequency[21]. It may be that cytosine also participates in the absorption in this region, but we have noted that the absorption maxima of deoxycytidylic acid solutions do not correspond exactly with any observed DNA absorption band.

As noted previously the region 900 to 1300 cm^{-1} is primarily associated with the

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sugar-phosphate part of the DNA molecule. The position and intensity of the bands at 970 cm^{-1} and 1085 cm^{-1}, by comparison with model phosphate esters[22], shows that the phosphate group is completely ionized at pH > 5. This is in agreement with the electrometric titration data on DNA[23]. Furthermore the fact that there is no change in these bands with the above described treatments indicates that the phosphate groups do not play a primary role in the molecular configuration of high molecular DNA.

SUMMARY

High molecular weight deoxypentose nucleic acid (DNA) in aqueous solution has a characteristic infrared spectrum which may be changed by physical, chemical, or enzymic treatment. The region of the spectrum in which the most important changes occur is that associated with the purine and pyrimidine components. In particular, two strong absorption bands at 1680 and 1645 cm^{-1} disappear upon (a) raising the pH over 11, or (b) heating the solution for a few minutes at 100° C, or (c) upon short treatment with desoxyribonuclease. Concomitant with the disappearance of the absorption bands at 1680 and 1645 cm^{-1} is the appearance of two new bands at 1660 and 1625 cm^{-1}. We have not been able to reverse these changes in the infrared absorption spectra of DNA in solution once they have occurred.

The observed infrared spectral changes of DNA in solution, with the treatments described above, lie in the region where purine-pyrimidine absorption occurs and are indicative of the important role these bases must play in the molecular configuration of DNA. The nature and extent of the spectral shifts are such as to lead us to postulate that such treatments irreversibly alter the specific double stranded helical configuration of the original high molecular weight DNA.

RÉSUMÉ

Un acide désoxypentosenucléique (DNA) de poids moléculaire élevé possède, en solution aqueuse, un spectre infrarouge caractéristique qui peut être modifié par traitement physique, chimique ou enzymatique. La région du spectre où ont lieu les modifications les plus importantes est celle qui est associée avec les constituants puriques et pyrimidiques. En particulier, deux bandes d'absorption fortes à 1680 et 1645 cm^{-1} disparaissent (a) quand on élève le pH au dessus de 11, ou (b) quand on chauffe la solution quelques minutes à 100° ou enfin (c) après un traitement bref avec la désoxyribonucléase. En même temps que disparaissent les bandes à 1680 et 1645 cm^{-1} apparaissent deux nouvelles bandes à 1660 et à 1625 cm^{-1}. Les auteurs ont observé que ces modifications du spectre infrarouge du DNA en solution sont irréversibles.

Les modifications du spectre infrarouge du DNA en solution, observées au cours des traitements décrits plus haut, se produisent dans la région où absorbent les purines et les pyrimidines et sont un signe du rôle important que ces bases doivent jouer dans la structure moléculaire du DNA. La nature et l'importance des déplacements spectraux sont telles qu'elles permettent de supposer que ces traitements altèrent irréversiblement la configuration spécifique en double hélice du DNA de poids moléculaire élevé initial.

ZUSAMMENFASSUNG

Desoxypentose-Nukleinsäure (DNA) von hohem Molekulargewicht besitzt in Wasserlösung ein charakteristisches infrarotes Spektrum, welches durch physikalische, chemische oder enzymatische Behandlung verändert werden kann. Die bedeutendsten Veränderungen kommen an jenen Stellen des Spektrums vor, welche mit Purin- und Pyrimidinkomponenten verbunden sind. So verschwinden z.B. zwei starke Absorptionsstreifen (1680 und 1645 cm^{-1}), wenn man (a) den pH-Wert über 11 erhöht, oder (b) die Lösung einige Minuten lang auf 100° C erhitzt, oder (c) dieselbe kurz mit Desoxyribonuklease behandelt. Gleichzeitig mit dem Verschwinden der Absorptionsstreifen (1680 und 1645 cm^{-1}) erscheinen zwei neuen Streifen auf 1660 und 1625 cm^{-1}. Diese Erscheinungen im infraroten Absorptionsspektrum von DNA in Lösung konnten nicht mehr rückgängig gemacht werden.

Die beobachteten, durch obige Behandlung hervorgebrachten Veränderungen des infraroten Spektrums von DNA in Lösung befinden sich in der Absorptionsgegend von Purin und Pyrimidin und weisen daher auf die wichtige Rolle hin, welche diese Basen in der molekularen Konfiguration von DNA spielen dürften. Art und Ausmass dieser spektralen Veränderungen führen zu der Annahme, dass die genannten Behandlungen in der spezifischen, gewundenen Doppelschraubenkonfiguration der ursprünglichen DNA mit hohem Molekulargewicht eine unwiderrufliche Verwandlung hervorrufen.

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