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# Determination of the Backbone Structure of Nucleic Acids and Nucleic Acid Oligomers by Laser Raman Scattering

**(calf-thymus DNA/yeast transfer RNA/guanylyl-3′:5′-cytidine)**

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ABSTRACT **Raman spectra of fibers of DNA that have been prepared in the A, B, and C forms are presented and compared with Raman spectra of DNA and RNA in dilute solution. It is shown that the phosphate vibrations in the region 750–850 $cm^{-1}$ are very sensitive to the specific conformation of the phosphate group in the backbone chain and are virtually independent of all other factors. Thus, a very simple method for the determination of the specific conformation of the backbone chain of nucleic acids, at least so far as the sugar-phosphate chain is concerned, appears available. The method is applied to short oligomers and dimers of ribonucleosides. It is found that at low temperatures, at pH 7, the phosphate group goes into the geometry of the A conformation when the stacking forces between the bases are sufficiently strong.**

The most reliable method for the determination of the structure of nucleic acids and polynucleotide helical chains appears to be that of x-ray diffraction (1, 2). However, this method is only applicable to nucleic acids in highly concentrated fibrous form. In general, it is not applicable to dilute nucleic acid solutions, although meaningful progress in the interpretation of x-ray scattering from fairly concentrated solutions has recently been reported (3). Thus, it would seem helpful to have available a method that could be used to obtain structural information, both on fibers and on dilute solutions, where these materials naturally occur. In this paper, we wish to report the observation of several Raman bands that arise from the vibration of the sugar-phosphate backbone, in both ribonucleic acids (RNA) and deoxyribonucleic acids (DNA) whose frequencies and intensities are directly related to whether or not the material is in the A, B, or C form, as designated by the x-ray crystallographers (1, 2), and are virtually independent of all other parameters, such as the base composition, the presence or absence of the 2′-hydroxyl, etc. Furthermore, these bands can be observed in single-chain structures and oligomers, so that the geometry of the phosphate group in these substances can, under favorable circumstances, be determined.

Recently, work in several laboratories has shown the existence of a Raman band at about 810–814 $cm^{-1}$ that is always present in ribonucleic acid structures, when these structures are in an ordered or partially ordered form (4–6). This band is plainly evident in the Raman spectrum of yeast transfer RNA shown in Fig. 1, and has also been observed in ribosomal RNA (5). Upon raising the temperature of the solution, so that the secondary structure vanishes, this band at 814 $cm^{-1}$ inevitably vanishes (4, 5). Since this band is completely independent of base composition and is present in all ordered ribo-structures, it may be due to the sugar-phosphate diester symmetric (4, 5) stretch or antisymmetric (6) stretch. The band at 814 $cm^{-1}$ is highly polarized, so that the former assignment seems somewhat more reasonable. Recent work in this laboratory (4) has shown that in aqueous solution, deoxyhomopolymers and DNA never show this band at 814 $cm^{-1}$. This can be seen from the spectrum of calf-thymus DNA in Fig. 1, where the 814 $cm^{-1}$ is obviously absent, but a broader peak at about 835 $cm^{-1}$ is present.

## RAMAN SPECTRA OF NUCLEIC ACIDS

One possible explanation for the difference in DNA and RNA in this backbone vibration is that it is due to the basic structural difference between the backbone conformations of RNA and DNA, i.e., the configuration of the phosphate group in the A form (1, 2), characteristic of RNA, gives rise to the strong highly polarized vibration at 814 $cm^{-1}$, while the phosphate conformation in the B form (1, 2), characteristic of DNA in solution, does not.

In an effort to test this hypothesis, we have made DNA fibers in the A form, following the direction of Cooper and Hamilton (7), using low salt (NaCl) content and 75% humidity. As we suspected, under these conditions a band essentially the same as the 814 $cm^{-1}$ band in RNA solutions appears in the DNA fiber. This rather sharp, strong Raman band is slightly shifted about 5–7 $cm^{-1}$ to a slightly lower frequency of about 807 $cm^{-1}$ in the A form of DNA. Since the uncertainty in our measurements is no more than 5 $cm^{-1}$, this shift is apparently real and appears to represent a slight difference in the vibrational frequency of the phosphate diester stretch due to the replacement of the ribose in ribonucleic acid structures by deoxyribose. Upon raising the humidity of the air surrounding the fiber from 75 to 98%, the DNA changes to the B form after equilibrium is reached, and the Raman spectrum changes so as to become virtually identical to the Raman spectra of DNA in solution. These results are shown in Fig. 2, where the completely reversible disappearance of the 807 $cm^{-1}$ band that is humidity dependent and the appearance of the 835 $cm^{-1}$ band is shown.

Clearly, a comparison of Figs. 1 and 2 in the region of 760–840 $cm^{-1}$ shows that RNA and DNA in aqueous solution are in the A and B forms as determined in the fibers. Although this conclusion has been shown earlier by workers in x-ray diffraction (1–3) and circular dichroism (8, 9), the present method offers many advantages, since it is independent of both base composition and sugar composition, and the form

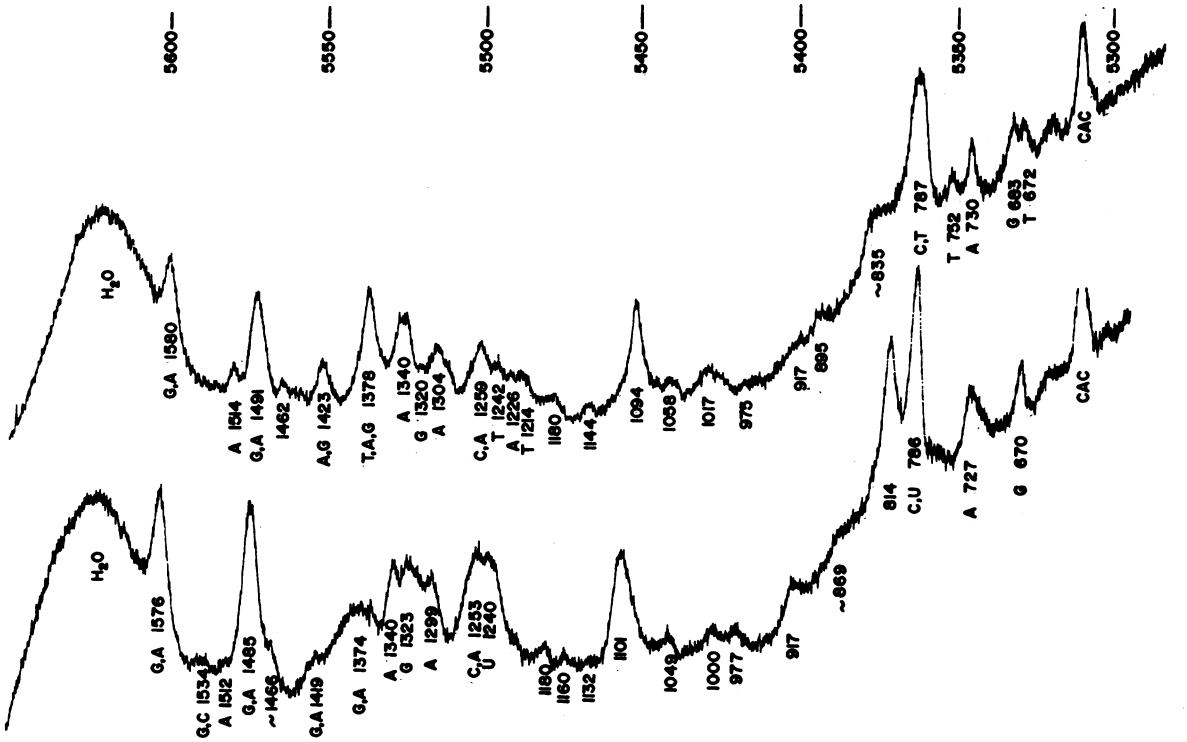


FIG. 1. Raman spectra of calf-thymus DNA in 2.5% aqueous solution at pH 7.2 (*upper*) and yeast transfer RNA in a 2.5% aqueous solution at pH 7 (*lower*) with 0.01 M cacodylate buffer.

(i.e., fiber or solution) of the material. Furthermore, the present method may be applied to single-chain structures and oligomers.

In addition to the changes in the 800–840 $cm^{-1}$ region, parallel changes are easily observed in the 1100 $cm^{-1}$ symmetric dioxy-stretch of the phosphate group. When the nucleic acid backbone is in the A form, this frequency lies at 1101 $cm^{-1}$, while in the B form it lies at 1094 $cm^{-1}$; how-

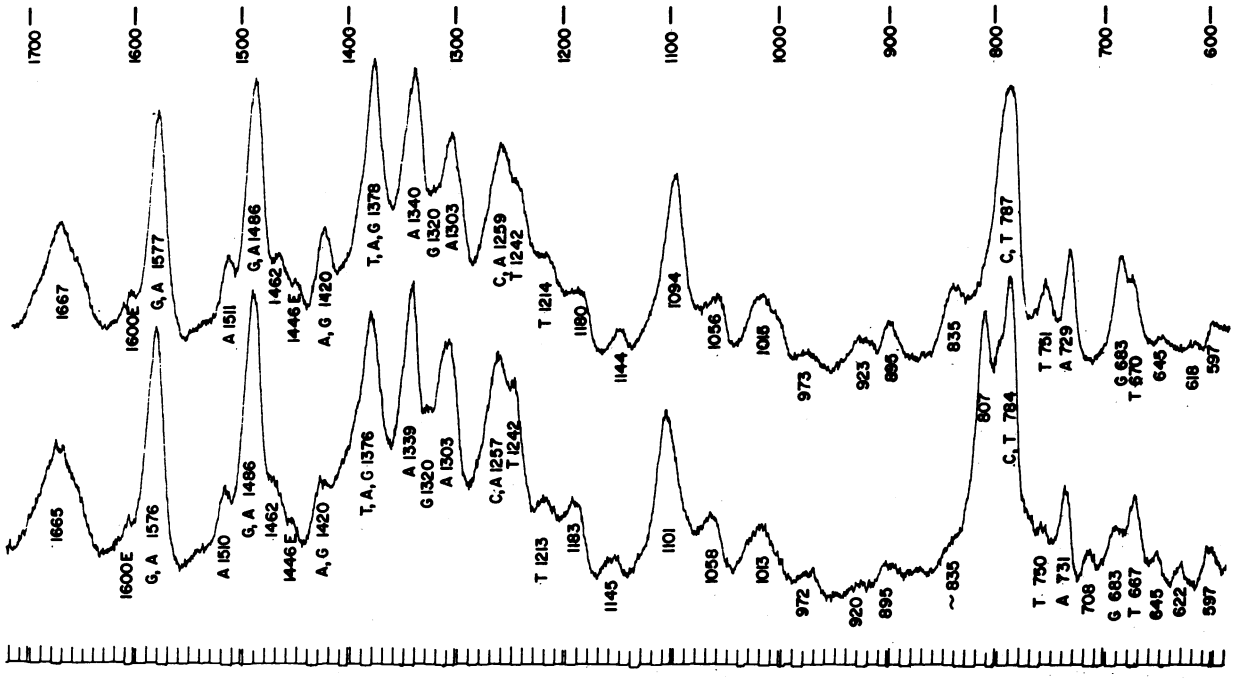


FIG. 2. Raman spectra of a fiber of the sodium salt of calf-thymus DNA at 98 (*upper*) and 75% (*lower*) relative humidity; the spectra are completely reversible with changing humidity.

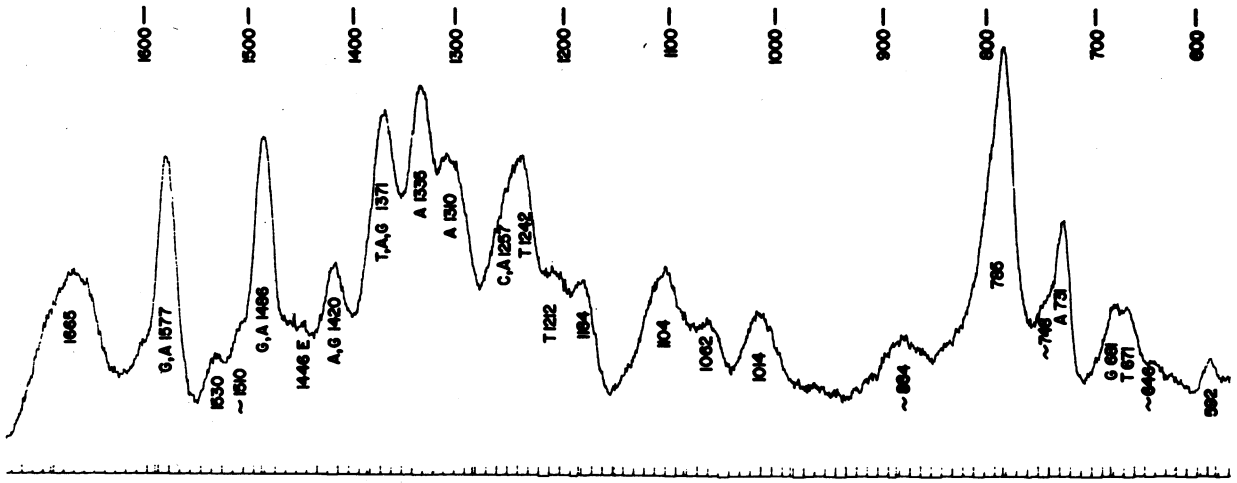


FIG. 3. Raman spectrum of a fiber of the lithium salt of calf-thymus DNA at 32% relative humidity.

ever, this shift is small and may be difficult to observe. Other apparent differences can be seen particularly in the 670 $cm^{-1}$ region, but these are small and more difficult to interpret.

We have also determined the Raman spectrum of the C form (Fig. 3) of DNA from a fiber of the lithium salt held at the low humidity of 32%, following the directions of Marvin *et al.* (10). In the C form, the strong band at 785 $cm^{-1}$ stands alone, and neither the sharp band at 807–814 $cm^{-1}$ nor the broad band at 835 $cm^{-1}$ is present, although there is an unusual band at 884 $cm^{-1}$ as well as a number of other unusual bands that are difficult to assign as yet. At high humidities, the spectrum of the C form changes back to a spectrum similar to the B form, in agreement with the x-ray result (10).

It seems apparent that the results presented here will have significance in the determination of the backbone structure of nucleic acids in situations where other techniques are inadequate. These include the determination of the structure of nucleic acids in particles such as viruses and in nucleic acid–protein complexes (11), as well as in oligomers and single chain structures.

## RAMAN SPECTRA OF DIMERIC NUCLEOSIDES

One of the fundamental unsolved problems of the nucleic acids is a detailed understanding of the potential energies that cause the backbone of the polymer to be formed into a specific configuration. It is now well-known that base-stacking forces cause oligomers and single-chain polymers to

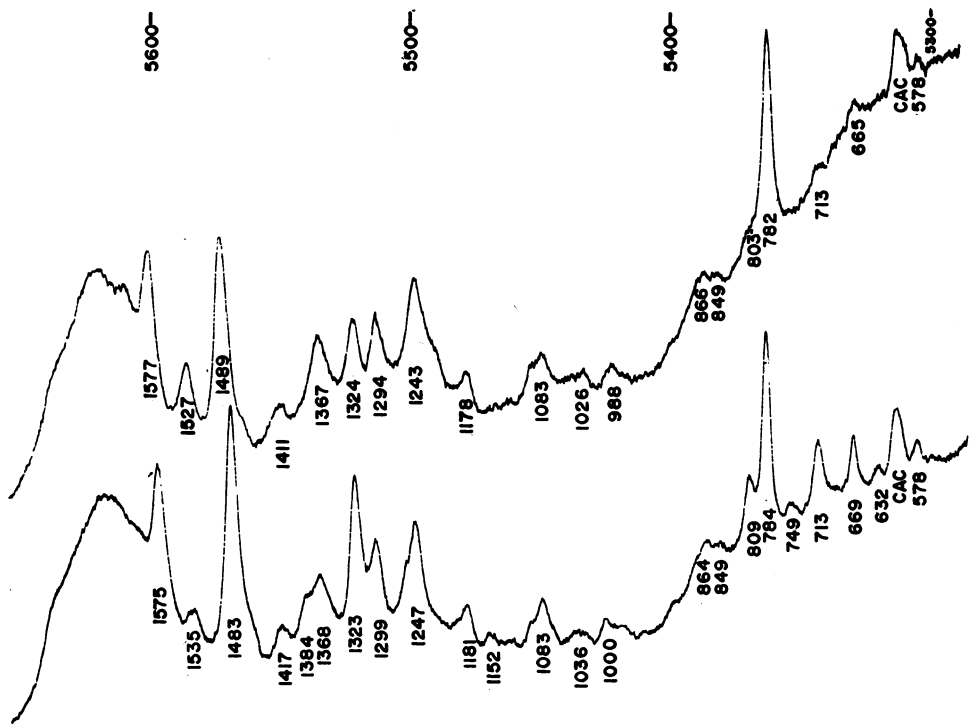


FIG. 4. Raman spectra of 2% guanylyl-3′:5′-cytidine in 0.01 M cacodylate buffer (pH 7) at 6 (*lower*) and 65° (*upper*).

become self-stacked in aqueous solutions as the temperature is lowered (12–17). However, there has been no way up until now for determination of the conformation of the phosphate linkage in these self-stacked structures. Fig. 4 shows the Raman spectra of the diribonucleoside phosphate, guanylyl-3′:5′-cytidine in aqueous solution at pH 7, and at 6 and 65°. The reversible appearance of the 809 $cm^{-1}$ band shows that as the dimer becomes increasingly self-stacked at lower temperatures, the phosphate group assumes the geometry of the A form. Thus, we are brought to the interesting conclusion that in oligoribonucleotides, the stacking forces will tend to take the molecules into the A conformation at low temperatures. Not all diribonucleoside phosphates show this behavior, and a detailed report on many dimers and oligomers will be published elsewhere. However, we can say that there is a strong correlation between self-stacking tendencies as determined by Warshaw and Tinoco (12) from ultraviolet hypochromism measurements and the observation of the 810 $cm^{-1}$ band of the A form at low temperatures.

Sundaralingam (18, 19) has noted a correlation between the preferred (helical) conformation of the backbone of sugar phosphates and the occurrence of base stacking, while Olsen and Flory (20) have begun to investigate the effect of base stacking on the conformation of single polyribonucleotides. Our experimental results should be of help in guiding further theoretical studies into the forces that are responsible for the conformation of nucleic acids and their components.

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