Raman Spectra of DNA in Aqueous Solution

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The infrared absorption spectra of DNA and polynucleotide have been studied by several workers,[1-3] but little work has been reported on the Raman spectra of an aqueous solution of DNA. In this note the author will report on the Raman spectra of an aqueous solution of DNA.

The calf-thymus DNA was purchased from the Sigma Chemical Co. The concentrations used were *ca.* 2 mg/25 m*l* tris buffer solution at pH=7.4. In order to cancel out the background due to the solvent molecules, tris buffer solutions were used as the reference substances. Before measurement the solutions were centrifuged at 1×10^4 g for 60 min. All the measurements were made at room temperature. The excitation wavelengths were in the vicinity of 260 mμ and of 400 mμ. A slightly-modified Hitachi photoelectric spectrofluorometer was used for both the selection of the excitation ray and the analysis of the scattering spectrum. No fluorescence was observed in these measurements. To obtain the Raman spectra, the correction for Rayleigh scattering was made by the use of Eq. (1)[4,5]:

$$\left.\begin{aligned} &I_{\theta=(\pi/2)}(\lambda) = \\ &\left\{\frac{2\pi^2 n_0^2}{Nr^2\lambda^4}\right\}\left(\frac{dn}{dc}\right)^2 CM^2 2/z^2\{z-1+e^{-z}\}I_0(\lambda) \\ &z = 2/3\left\{\frac{2\pi Rn_0}{\lambda}\right\}^2 \sin^2\left(\frac{\pi}{4}\right) \end{aligned}\right\} \quad (1)$$

where $I_{\theta=(\pi/2)}(\lambda)$ is the relative intensity of the Rayleigh scattering in the direction of $\theta=\pi/2$; $I_0(\lambda)$ the relative intensity of the incident ray; λ, the wavelength; r, the constant determined from the instrument; N, the Avogadro number; c, the concentration of the sample solution (g/m*l*); M, the molecular weight; R, the end-to-end distance of the random-coiling DNA,[6] and n_0 and n, the refractive indices of the solvent and of the solution, respectively.

The corrected scattering spectrum of DNA

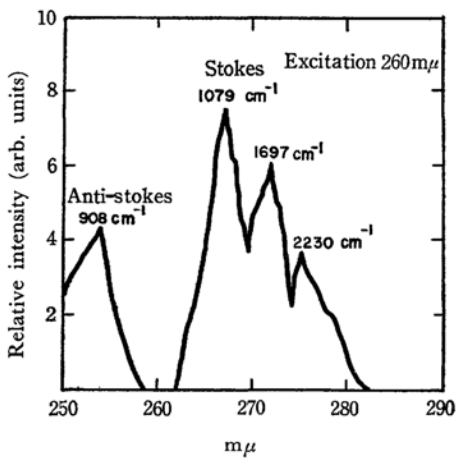

Fig. 1. Raman spectrum of DNA solution.

1) R. S. Morgan and E. R. Blout, *J. Am. Chem. Soc.*, **81**, 4625 (1959).
2) M. Tsuboi, *Seibutu-Buturi*, **3**, 95 (1963).
3) E. M. Bradbury, W. C. Price and G. R. Wilkinson, *J. Mol. Biol.*, **3**, 301 (1961).
4) L. Rayleigh, *Proc. Roy. Soc.* (*London*), **A90**, 218 (1914).
5) P. Debye, *J. Phys. Colloid Chem.*, **51**, 18 (1947).
6) M. E. Reichmann, S. A. Rice, C. A. Thomas and P. Doty, *J. Am. Chem. Soc.*, **76**, 3047 (1954).

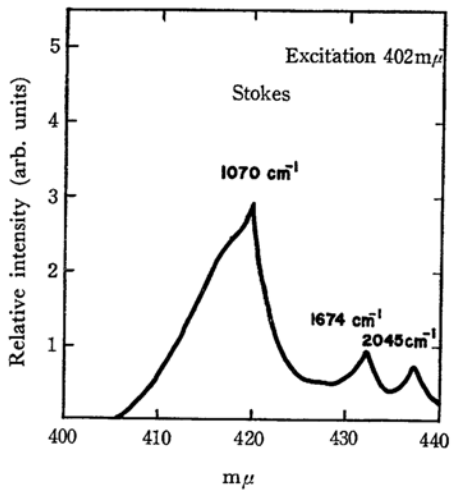

Fig. 2. Raman spectrum of DNA solution.

excited at 260 mμ is illustrated in Fig. 1, in which four frequencies, $\tilde{\nu}_1 \simeq 908\ cm^{-1}$, $\tilde{\nu}_2 \simeq 1079\ cm^{-1}$, $\tilde{\nu}_3 \simeq 1697\ cm^{-1}$ and $\tilde{\nu}_4 \simeq 2230\ cm^{-1}$, are characteristic. In Fig. 2 a similar spectrum excited at 402 mμ is displayed. From the figure it is obvious that three frequencies, $\tilde{\nu}_2 \simeq 1070\ cm^{-1}$, $\tilde{\nu}_3 \simeq 1674\ cm^{-1}$ and $\tilde{\nu}_4 \simeq 2045\ cm^{-1}$, are very similar to those of Fig. 1. These facts lead us to the conclusion that the corrected scattering spectra presented here are the Raman spectra of DNA in solution. That the anti-Stokes line, $\tilde{\nu}_1 \simeq 908\ cm^{-1}$, was not observed in the spectra may be attributed to the error arising from the correction of Rayleigh scattering, because the spectral intensity of the scattering ray deviates from the Gaussian distribution. On the basis of these results, the Raman frequencies of DNA in a tris buffer solution were estimated to be as shown in Table 1. The assignment of the frequencies listed in Table 1 based on the data of the infrared spectra of the purine base,[7] poly-A, DNA,[8,9] and on the Raman spectra of dimethyl phosphate anions.[10]

Table 1. Raman frequencies of DNA in an aqueous solution

Frequency, cm^{-1}		Assignment
$\tilde{\nu}_1$	908	Sym. [OPO] stretching
$\tilde{\nu}_2$	1070—1079	Sym. $[PO_2]^{-1}$ stretching
$\tilde{\nu}_3$	1674—1697	C=C, C=N stretching in the purine ring
$\tilde{\nu}_4$	2045—2230	?

The Raman frequencies listed in Table 1 correspond well to the infrared absorption maxima of poly-A or DNA.[2] On the contrary, the chart produced by the commercial Raman spectrophotometer did not give any significant frequencies for these solutions. Therefore, the measurement of the Raman scattering described here would be a very useful tool for the study of macrobiomolecules in solution.

7) C. H. Willits, J. C. Decius, K. Diele and B. E. Christensen, *ibid.*, **77**, 2569 (1955).
8) M. Tsuboi, *Prog. Theoret. Phys., Supple.*, **17**, 99 (1961).
9) M. Tsuboi, *J. Am. Chem. Soc.*, **79**, 1351 (1957).
10) T. Shimanouchi, M. Tsuboi, Y. Kyogoku and J. Duchesen, ed., "The Structure and Properties of Biomolecules and Biological Systems," Interscience Publishers, London (1964), p. 476.