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RESONANCE RAMAN EFFECTS OF NUCLEOTIDES

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Synopsis. Raman spectra of β-uridine-5'-phosphoric acid, β-cytidine-5'-phosphoric acid, and β-guanosine-5'-phosphoric acid have been observed in their dilute (10^{-4} M) neutral aqueous solutions by the use of the 257.3 nm beam of a frequency doubled argon ion laser.

Common bases of nucleic acid have strong absorption bands (molar extinction coefficient 10^4) in the 260 nm region, and therefore a resonance Raman effect is expected to be observed of a nucleic acid by the use of an exciting beam in this spectral region. For β-adenosine-5'-phosphoric acid (AMP), this has been shown to be the case by Pézolet, et al.[1] They observed strong Raman lines at 1338 and 1485 cm^{-1} of AMP 10^{-4} molar in pH 7 aqueous solution with 257.3 nm excitation. Of β-uridine-5'-phosphoric acid (UMP), however, they failed to observe resonance Raman spectrum with the 257.3 nm excitation, and on the basis of this fact they suggested that the strong UMP Raman lines (1230 cm^{-1}, for example) do not derive their intensities from the 260 nm band. We have found that resonance Raman effects in the 260 nm bands do appear not only for AMP, but also for UMP, and also for the other two common nucleotides: β-cytidine-5'-phosphoric acid (CMP) and β-guanosine-5'-phosphoric acid (GMP).

The samples of UMP, CMP, and GMP were purchased from Kohjin Chemical Co. Distilled water was used as the solvent. The concentration was 10^{-4} molar and pH was 6.0∿6.2. Each of these sample solutions was placed in a silica glass rotating cell. For excitation a 257.3 nm CW laser beam was used. This was obtained by the use of a Coherent Radiation Model 52 argon ion laser (514.5 nm beam) in combination with a Coherent Radiation Model 440 UV Generator. For recording Raman spectra, we could use a Spex 1401 monochromator, a HTV R585 photomultiplier, and a photon-counting device, through the kindness of Professor T. Shimanouchi and Dr. H. Hamaguchi.

The recorded curves are shown in the figure. Of these, the following significance is now realized.

(i) This technique provides a new, non-destructive analytical tool for nucleic acid biochemistry. The sample concentration needed is so low as what is required for the ultraviolet absorbance measurement. Yet there appears a number of peaks at different positions for different base residues.

(ii) It is evident that strong Raman lines of the uracil residue observed here at 1679, 1634, 1474, 1399, and 1233 cm^{-1}, those of the cytosine residue at 1651, 1528, 1292, and 1239 cm^{-1}, those of the guanine residue at 1582, 1487, and 1328

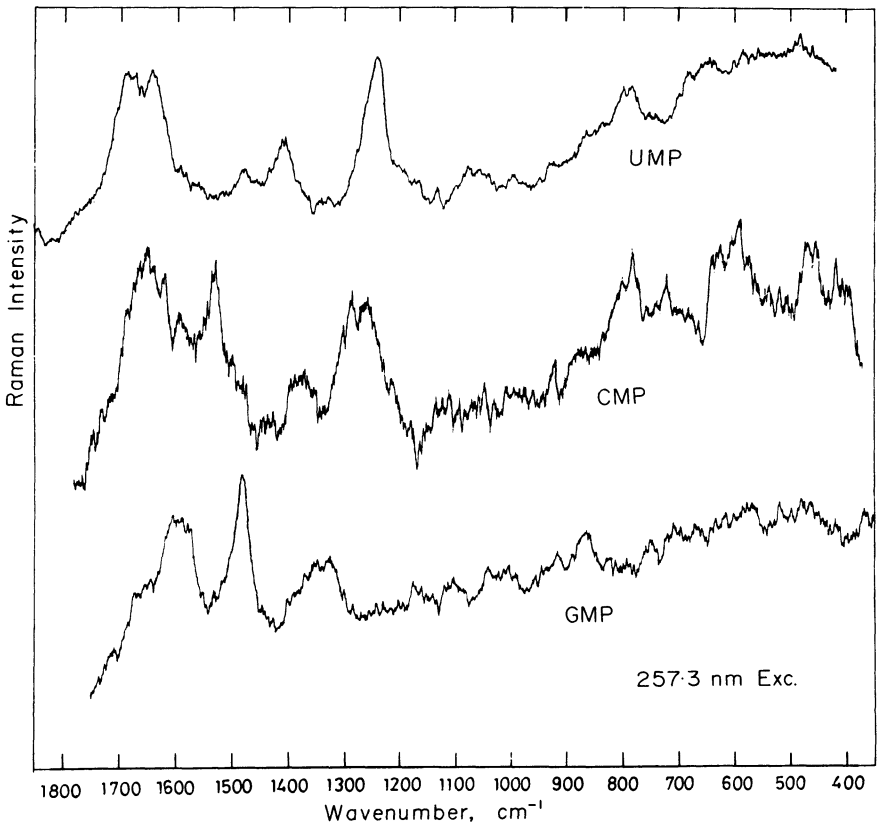

Fig. 1. Resonance Raman spectra of nucleic acids 10^{-4} molar or 0.004% in neutral aqueous solutions with frequency doubled argon ion laser light at 257.3 nm.

cm^{-1}, as well as those of the adenine residue at 1580, 1484, and 1340 cm^{-1} are caused by the electronic excited states corresponding to the bands in the 260 nm region of the base residues. The intensity of each Raman line should be proportional to the square of the absorption intensity. Therefore, if a base stacking causes a hypochromic effect in a 260 nm absorption, this should appear in each Raman scattering intensity in an amplified manner. Such a Raman line would be useful in detecting a conformational change of a nucleic acid in which a stacking or destacking of base residues takes place.

(iii) Some pieces of information can now be obtained of the excited state geometry of the base residues. The equilibrium conformation of each base residue is considered to be distorted, on going from the ground electronic state to the excited state now in question, along the normal coordinate for each of the above mentioned Raman lines.[2,3] The results of our study along this line will be detailed elsewhere.

References

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