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Evidence of a Preresonance Raman Effect of the Base Residue in a Nucleic Acid

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The Ramam spectra of β-uridine-5′-phosphoric acid (UMP), its deuterated product, β-uridine-3′-β-uridine-5′-phosphoric acid (U_pU), and polyribouridylic acid (poly U) have been observed in their aqueous solutions; and the Raman lines assignable to the phosphate group and to the base residue have been indicated. For UMP, the intensity ratio of a Raman line of the base residue *versus* that of the phosphate group was found to become greater on changing the exciting line from the 6328 Å line of a helium-neon laser to the 4880 Å line of an argon ion laser. This fact has been taken as indicating that a preresonance Raman effect takes place of the base residue with the effective absorption at 2600 Å.

In the course of our investigation on the Raman effects of the nucleic acids, we noticed that the apparent intensity ratio of a Raman line assignable to a base residue *versus* that assignable to the phosphate group was often found to be markedly greater in the spectrum obtained with an argon ion laser than that in the spectrum obtained with a helium-neon laser. We suspected that this fact is ascribed to the so-called preresonance Raman effect. As is now well known, each base residue in a nucleic acid has a strong absorption band at about 2600 Å (molar extinction coefficient at 2600 Å is about 10^4), whereas the phosphate group has no strong absorption band in the spectral region from 7000 Å down to 1800 Å. Therefore, it seems quite probable that a great contribution comes from the electronic excited state corresponding to the 2600 Å band (*i.e.* a preresonance with the 2600 Å band takes place) in some Raman lines of base residues while it does not in the Raman lines of the phosphate group. In general, the intensity (I) of a Raman line is given, in an approximation,[1] by

$$I = C\mu^4_{eg}(\nu_0-\nu)^4(\nu^2_{eg}+\nu_0^2)^2/(\nu^2_{eg}-\nu_0^2)^4. \qquad (1)$$

Here, C is a constant, ν_0, ν, and ν_{eg} are the frequencies

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[1] J. Behringer, in "Raman Spectroscopy", ed. by H. A. Szymanski, Plenum Press, New York (1967), p. 191.

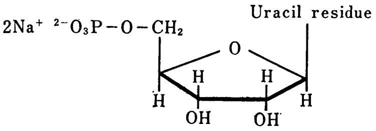
respectively of the exciting light, of a molecular vibration, and of the transition from the ground state to the lowest exicted electronic state; and μ_{eg} is the corresponding transition moment. If ν_{eg} is assumed to be 3.846×10^4 cm^{-1} (2600 Å) for the base residue and 5.556×10^4 cm^{-1} (1800 Å) for the phosphate group, the intensity ratio I(base)/I(phosphate) may be greater for $\nu_0=2.0487\times10^4$ cm^{-1} (4879.9 Å) line of argon ion laser than that for $\nu_0=1.5798\times10^4$ cm^{-1} (6328.2 Å) line of helium-neon laser. If the same value of μ_{eg} is assumed for the phosphate group as that for the base, for example, the value of ϕ defined by

$$\phi=\frac{[I(\text{base})/I(\text{phosphate})]_{\text{Ar}^+}}{[I(\text{base})/I(\text{phosphate})]_{\text{He-Ne}}} \tag{2}$$

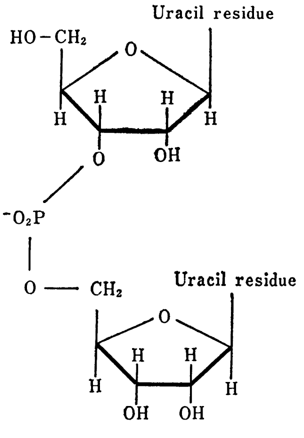
should be 1.6. Thus, the ϕ value is expected to be useful in characterizing each Raman line observed—it would tell whether the 2600 Å band is responsible for the Raman line in question, and if so how much. We attempted, in the present work, a measurement of these quantities for several Raman lines of β-uridine-5′-phosphoric acid in a neutral aqueous solution.

Experimental

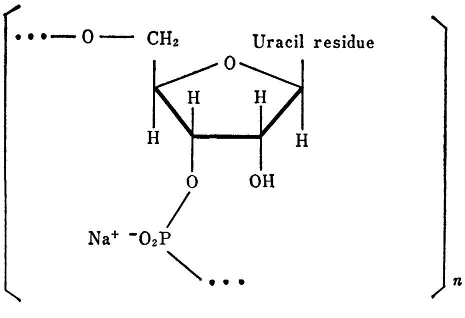
In the present paper the following three compounds are subjected to our examination: di-sodium salt of β-uridine-5′-phosphoric acid (5′ UMP),



sodium salt of β-uridine-3′-β-uridine-5′-phosphoric acid (U_pU),



and polyribouridylic acid,



The sample of 5′UMP was a gift from Takeda Chemical Industries, Ltd., and that of U_pU was purchased from Miles Chemical Company. The purity of these samples was confirmed by a chromatographic method. Poly U with the sedimentation constant $S_{20,w}=6.95$ S was purchased from Miles Chemical Company. For the observation of the Raman spectrum, about 0.2 m*l* of an aqueous solution of each nucleic acid was prepared. The concentration was about 5%. A small amount of NaOH was added in the solvent, and the pH of the solution was adjusted to 7.5 in every case. Each solution was placed in a cylindrical glass tube of 2 mm in inside diameter and 15 mm in length. The tube was placed vertically, and the laser beam was sent along the length of the tube through the flat glass plates at the lower and upper ends of the cylinder (see Fig. 1). The Raman light scattered along a direction perpendicular to the excited beam (*i.e.* along a horizontal direction) was examined by the use of a JRS-U1 spectrophotometer of Japan Electron Optics Laboratory Co., Ltd. The polarization directions of the excited and scattered beam are shown in Fig. 1. The depolarization ratio ρ is defined by $\rho=I_\perp/I_{//}$, where $I_\perp$ and $I_{//}$ are the intensities of the scattered beams whose polarization directions are ⊥ and // to that of the excited beam (see Fig. 1).

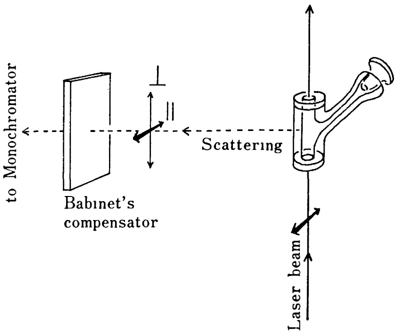


Fig. 1. Raman cell for aqueous solutions of nucleic acids. Polarization directions of excited light and scattered light.

Results and Interpretations

First, in order to provide the data of assignments of the Raman lines of uridylic acids, those of 5′UMP, U_pU, and poly U (*i.e.* monomer, dimer, and polymer of uridylic acid) are shown in Fig. 2. For 5′UMP, Raman spectrum was observed not only in a H_2O solution but also in a D_2O solution, and the result is given in Fig. 2b. Frequencies, approximate intensities, and depolarization ratios of the Raman lines observed are listed in Table 1.

In a solution of pH (or pD) = 7.5, the phosphate group of 5′UMP takes the form PO_3^{2-} with three P⩵O bonds having equal amounts (about one-third) of double-bond character. The phosphate group of U_pU or poly U, on the other hand, takes the form PO_2^- with two P⩵O bond having equal amounts (about one-half) of double-bond character. The characteristic vibrations of these PO_3^{2-} and PO_2^- groups were previously examined in detail,[2,3] and it was shown that symmetric

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[2] M. Tsuboi, *J. Amer. Chem. Soc.*, **79**, 1351 (1957).

[3] T. Shimanouchi, M. Tsuboi, and Y. Kyogoku, in Advances in Chemical Physics, Vol. VII, "The Structure and Properties of Biomolecules and Biological Systems," ed. by J. Duchesne, Wiley-Interscience, New York (1964), pp. 435—498.

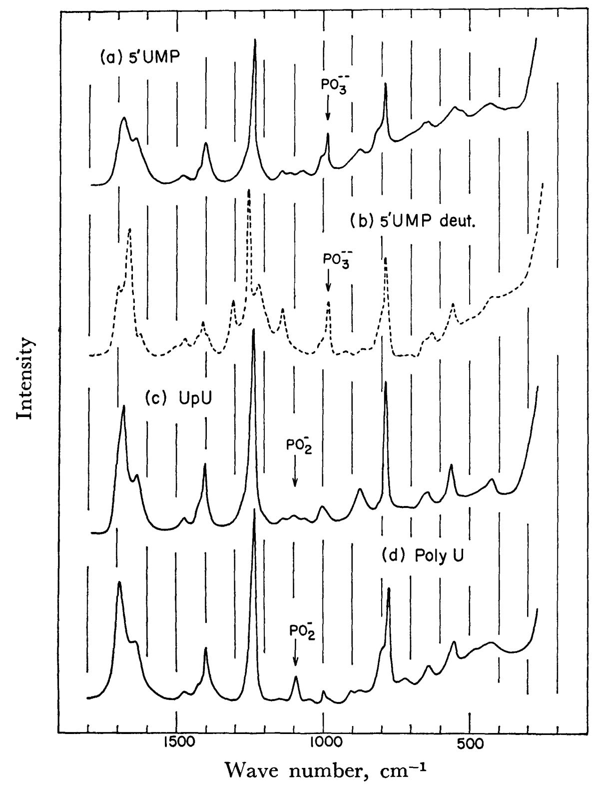

Fig. 2. Raman spectra of (a) 5'UMP, (b) deuterated 5'UMP, (c) U$_p$U, and (d) poly U in their 5% aqueous solutions at room temperature and at pH 7.5. Observed by the use of an argon ion laser (4880 Å line).

stretching vibrations of the PO$_3^{2-}$ and PO$_2^-$ groups give strong Raman lines respectively at 980 and 1100 cm^{-1}. This is found to be the case for 5'UMP, U$_p$U, and poly U. Thus, a sharp Raman line at 980 cm^{-1} is found for both of undeuterated and deuterated 5'UMP but not for U$_p$U and poly U, while a peak at 1100 cm^{-1} is observed only for U$_p$U and poly U. It is certain that the former is assignable to the PO$_3^{2-}$ symmetric stretching vibration and the latter to the PO$_2^-$ symmetric stretching vibration. The relative intensity of the 1100 cm^{-1} line is lower in U$_p$U than that in poly U. This is understandable because in U$_p$U only one half PO$_2^-$ group is involved per uracil residue, whereas in poly U one PO$_2^-$ group per uracil residue.

Most of the other Raman lines observed for 5'UMP are found also for U$_p$U and poly U at nearly the same frequencies (Fig. 2). Their relative intensities and depolarization ratios are also almost equal to those of the corresponding Raman lines of 5'UMP (Fig. 2, Table 1). This fact may be taken as indicating that these Raman lines are all assignable to vibrations in the uracil residue. At the same time, the fact indicates that the intra-molecular interactions, if any, are so weak between the uracil residues in the U$_p$U and poly U molecules, that they are hardly detected in the Raman spectra.

In Fig. 3, the result of our experiment for determining the ϕ values (defined by Eq. 2) of these Raman lines due to the base residue is shown. The two curves were recorded for the Raman scatterings from the same sample (20% 5'UMP in H$_2$O) by the use of the same

Table 1. Raman lines observed for neutral H$_2$O solution of 5'UMP, D$_2$O solution of 5'UMP, H$_2$O solution of U$_p$U, and H$_2$O solution of poly U

5' UMP			5'UMP deuterated		U$_p$U			Poly U		
Frequency (cm^{-1})	Intensity	Depolarization ratio	Frequency (cm^{-1})	Intensity	Frequency (cm^{-1})	Intensity	Depolarization ratio	Frequency (cm^{-1})	Intensity	Depolarization ratio
395	0	0.8[a]								
425	1	0.8[a]	425	1	422	1		430	0	0.6[a]
563	1	0.6[a]	553	2	559	2	0.4	559	1	
			623	1						
651	1	?	639	0	640	1	0.5[a]	640	1	0.5[a]
								724	0	
785	6	0.2	784	8	785	7	0.1	786	6	0.1
815	2	?	806	3				802	3	0.1[a]
873	1	~0	868	0	873	2	0.2	868	0	
			916	0				906	0	
980	4	0.1	979	4						
1002	1	~0[a]	1000	1	999	1	0.3	999	0	0[a]
1066	0				1053	0		1053	0	0.4[a]
					1093	1	~0[a]	1093	1	~0[a]
1133	0	0.8[a]	1138	2	1142	0	0.4[a]	1142	0	0[a]
			1214	3						
1233	10	0.4	1249	10	1235	10	0.3	1234	10	0.3
			1305	3						
1399	4	0.4	1405	2	1399	4	0.5	1400	3	0.2
1474	0	0.8[a]	1464	1	1473	0	0.5[a]	1472	0	0.4[a]
			1620	0						
1634	4	0.4	1659	9	1634	3	0.4	1631	3	0.3
1679	7	0.3	1696	6	1683	7	0.3	1689	6	0.2

a) The value of the depolarization ratio is less accurate than others.

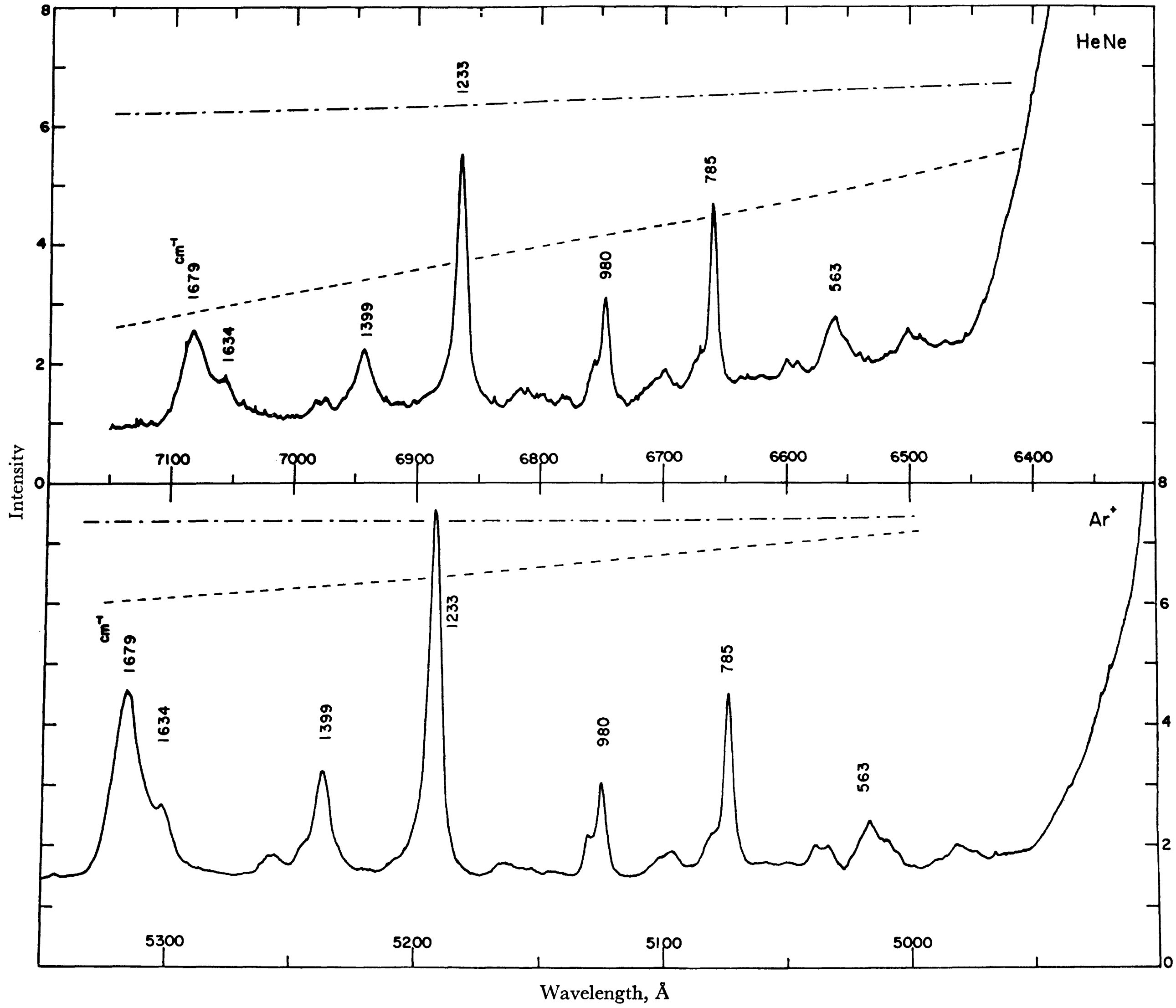

Fig. 3. A reproduction of the recorded curves for the Raman scattering from an aqueous solution (20%) of 5′UMP.
Upper: a helium-neon laser was used as the source of the exciting light (6328 Å beam).
Lower: an argon ion laser was used as the source of the exciting light (4880 Å beam).
Spectral slit width: 10.7 cm^{-1}. —·—·— shows the efficiency of the diffraction grating in the monochromator, and ------ shows the spectral response of the photomultiplier used.

spectrophotometer. One of them (upper) was obtained on exciting with the 6328 Å line of a helium-neon laser, whereas the other (lower) with the 4880Å line of an argon ion laser. The slit widths were adjusted (400 μ for the run with the helium-neon laser and 235 μ for that with argon ion laser) so that the spectral slit widths were approximately equal (10.7 cm^{-1}) for the two runs. As is readily seen in Fig. 3, the relative intensity of the 1233 cm^{-1} peak (base residue), for example, with respect to the 980 cm^{-1} peak (PO_3^{2-}) is greater in the curve (lower) with Ar^+ than that (upper) with He-Ne.

For obtaining the ϕ values (Eq. 2), corrections have been made for the efficiency of the diffraction gratings in the monochromator and for the spectral response of the photomultiplier. The monochromator contains two Jarrell-Ash No. 1297 Gratings (Cat. No. 980-45-20-22, 1180 grooves/mm 5000 Å blaze) and the efficiency-wavelength curve for each grating (reproduced by —·—·— in Fig. 3) was given by the manufacturer. The efficiency of the two gratings at each wavelength was assumed to be the square of the efficiency of one grating. The spectral response of the photomultiplier used (EMI 9558B) is given in EMI Technical Data and is reproduced by ------ in Fig. 3.

In Table 2, the ϕ values thus determined for six

Table 2. The values of ϕ defined by Eq. (2) for some Raman lines of 5′UMP.

Frequency, cm^{-1}	ϕ
563	1.1_2
785	1.2_1
980	(1.00)
1233	1.5_6
1399	1.6_0
1634	1.4_2
1679	1.6_3

stronger Raman lines (all assignable to vibrations in the uracil residue) are given. The ϕ values for the 1233, 1399, 1634, and 1679 cm^{-1} lines are approximately equal to the value 1.6 predicted above on the basis of Eq. (1) with $\nu_{eg}=3.846\times10^4\ \mathrm{cm}^{-1}$ (2600 Å), and therefore it is now evident that a preresonance Raman effect takes place in each of these lines. All of these Raman lines are strongly polarized (depolarization ratio $\ll$ 0.75) and they are assignable to in-plane vibrations in the uracil residue.

For the strong Raman line at 785 cm^{-1}, on the other hand, the ϕ value is found to be much smaller (1.2). The depolarization ratio is very small for this line (0.2), and therefore this line is also assignable to an in-plane vibration in the uracil residue. Lord and Thomas⁴⁾ suggested that this line is caused by a ring-breathing type of motion. Eaton and Lewis⁵⁾ observed a vibrational structure in the 2600 Å band of a 1-methyluracil crystal with a spacing of 750 cm^{-1}, and they suggested that this frequency corresponds to the ground state frequency, 785 cm^{-1}. Thus, it is probable that the excited electronic state corresponding to the 2600 Å band of the uracil residue has its potential minimum at a position appreciably shifted from that of the ground state along the normal coordinate corresponding to the 785 cm^{-1} line. If so, one would expect a rather stronger preresonance effect and therefore rather greater ϕ value for the 785 cm^{-1} Raman line, in contrast to what is actually found. Professor T. Miyazawa (Osaka University), in his private communication, suggested that in the normal vibration corresponding to the Raman line at 785 cm^{-1} the atomic movements are not necessarily localized in the base residue. If the 785 cm^{-1} vibration consists of movements of the uracil and ribose groups, for example, and if an appreciable contribution to the intensity of the 785 cm^{-1} Raman line comes from the ribose part, the observed low ϕ value is certainly understandable.

A low ϕ value is found also for the 563 cm^{-1} line. This line shows somewhat greater depolarization ratio (Table 1), and it is probable that this is caused by an out-of-plane vibration of the uracil residue.

It has now been shown that the intensities of the Raman lines at 1233, 1399, 1634, and 1679 cm^{-1} are caused almost exclusively by the excited state of the uracil residue corresponding to the 2600 Å band, while the intensity of the 785 cm^{-1} Raman line is caused only partly by the 2600 Å excited state. In other words, Eq. (1) is nearly valid for the first four Raman lines while it is not for the 785 cm^{-1} line (other terms are necessary in the right side of Eq. (1)). If Eq. (1) is valid, the intensity of the Raman line should be proportional to μ^4_{eg}, *i.e.*, to the square of the ultraviolet absorption intensity of the 2600 Å band. Therefore, when an inter-base interaction (such as a hydrophobic stacking interaction⁶⁾) causes a hypochromicity in the 2600 Å band of a uracil residue, it should cause a much more marked lowering in the intensities of the Raman lines at 1233, 1399, 1634, and 1679 cm^{-1}. Tomlinson and Peticolas⁷⁾ made a similar prediction on the basis of their theory,⁸⁾ but they actually found the intensity of the Raman line at 720 cm^{-1} of adenine residue to show a rather linear dependence on the ultraviolet absorbance.⁷⁾ We suspect that the 720 cm^{-1} line of the adenine residue has a similar nature to the 785 cm^{-1} line of the uracil residue. If so, what they found is understandable, because only a part of its intensity comes from the 2600 Å excited state, and only that portion of the intensity is proportional to μ^4_{eg} (2600 Å).

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4) R. C. Lord and G. J. Thomas, Jr., *Spectrochim. Acta*, **23A**, 2551 (1967).

5) W. A. Eaton and T. P. Lewis, *J. Chem. Phys.*, **53**, 2164 (1970).

6) See for example I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **82**, 4785 (1960).

7) B. L. Tomlinson and W. L. Peticolas, *J. Chem. Phys.*, **52**, 2154 (1970).

8) W. L. Peticolas, L. Nafie, P. Stein, and B. Fanconi, *J. Chem. Phys.*, **52**, 1576 (1970).