Vol. 81, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1978 Pages 907-914

A COMPARATIVE STUDY OF THE STRUCTURE OF TOBACCO MOSAIC VIRUS AND CUCUMBER VIRUS 4 BY LASER RAMAN SPECTROSCOPY

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Received January 19,1978

SUMMARY. The results of the study of the structure of two related rod-like viruses, tobacco mosaic virus and cucumber virus 4, by laser Raman spectroscopy are presented. It is found that the structure of the protein subunits of the two viruses differ significantly: the TMV protein has more α-helix and less β-structure than the CV protein. All tryptophan residues in the proteins of both viruses are buried in the hydrophobic environment, and out of the four tyrosine residues present in both proteins, two participate in strong H-binding with COO^--groups of acidic amino acids and the two others are exposed to the solvent. Intravirus RNAs of both viruses have the same structure of sugar-phosphate backbone, characterised by the simultaneous presence of at least two different conformations of phosphodiester bonds and of ribose residues. The degree of base stacking in the two intravirus RNAs is much smaller than in helical regions of free RNAs.

In spite of spectacular results achieved recently in the studies of the structure of RNA and protein in TMV * particles (1), many aspects of this problem remain unresolved. Also, very little is known about the subtle differences in the structure of different viruses of TMV group. Low chemical reactivity of RNA and protein *in situ* and artifacts frequently occurring due to particulate nature of the virus preparations commonly render chemical modifications or optical methods ineffective in studies of TMV-like viruses.

The abundance of detailed structural information and the abscence of light-scattering distortions have made Raman spectroscopy a very useful method for investigating the structure of many diffe-

* Abbreviations: TMV- tobacco mosaic virus; CV - cucumber virus 4.

0006-291X/78/0813-0907$01.00/0

rent viruses (2-6). Although TMV-like viruses have a rather low RNA content (about 5 per cents), it may be hoped that also in this case the Raman spectroscopy will help obtain interesting information on the structure of protein and RNA in situ and on the nature of RNA-protein interactions. Here we report some preliminary results of a laser Raman spectroscopy study of the two closely related TMV group viruses, common strain of TMV and cucumber virus 4. These two viruses are very similar in morphology (7), but differ in the amino acid content of coat proteins (7,8) and, probably, in the nature of RNA-protein interactions (9).

MATERIALS AND METHODS. TMV and CV were grown on plants of Nicotiana tabacum and Cucumis sativus, respectively, and purified as described previously (9). For Raman spectra measurements the virus suspensions in 0.01 M K-Na phosphate buffer pH 7.2 were centrifuged for 80 min at 105.000 g, one - two drops of the buffer solution were added to the virus pellet and 10 µl of the resultant paste were placed into Kimmax glass capillaries (d= 1 mm). The concentration of the virus in the paste, as determined by UV absorption (9), was 300 to 350 µg/µl.

Raman spectra were taken in a Ramalog 5 spectrometer (Spex Industries) at room temperature. The spectra were excited by the 5145 A line of an argon laser (Spectra Physics) employing 200 to 300 mw power at the sample. The spectra were taken at a scan speed of 25 cm^{-1}/min, with a resolution of 10 cm^{-1}, a rise time 5 sec and a sensitivity of 10 K counts/sec.

RESULTS AND DISCUSSION. The Raman spectra of TMV and CV in 300 to 1800 cm^{-1} region (Fig. 1) are dominated by contributions of protein that amount to 95 per cents of the dry weight of these viruses. Numerous studies of the Raman spectra of different polypeptides and proteins have demonstrated (10,11), that α-helix is characterized by an intense Amide I peak at 1650 ± 5 cm^{-1}, by a weak Amide III line at 1270 to 1300 cm^{-1} and by C-C-(N) stretching vibration line of medium intensity at 935 ± 5 cm^{-1}; β-structure reveals itself as an intense Amide I peak at 1665 ± 5 cm^{-1} and as an intense Amide III line at 1235 ± 5 cm^{-1}; unordered structure is characterised by a broad intense Amide I peak at 1665 ± 5 cm^{-1}, by an Amide III line

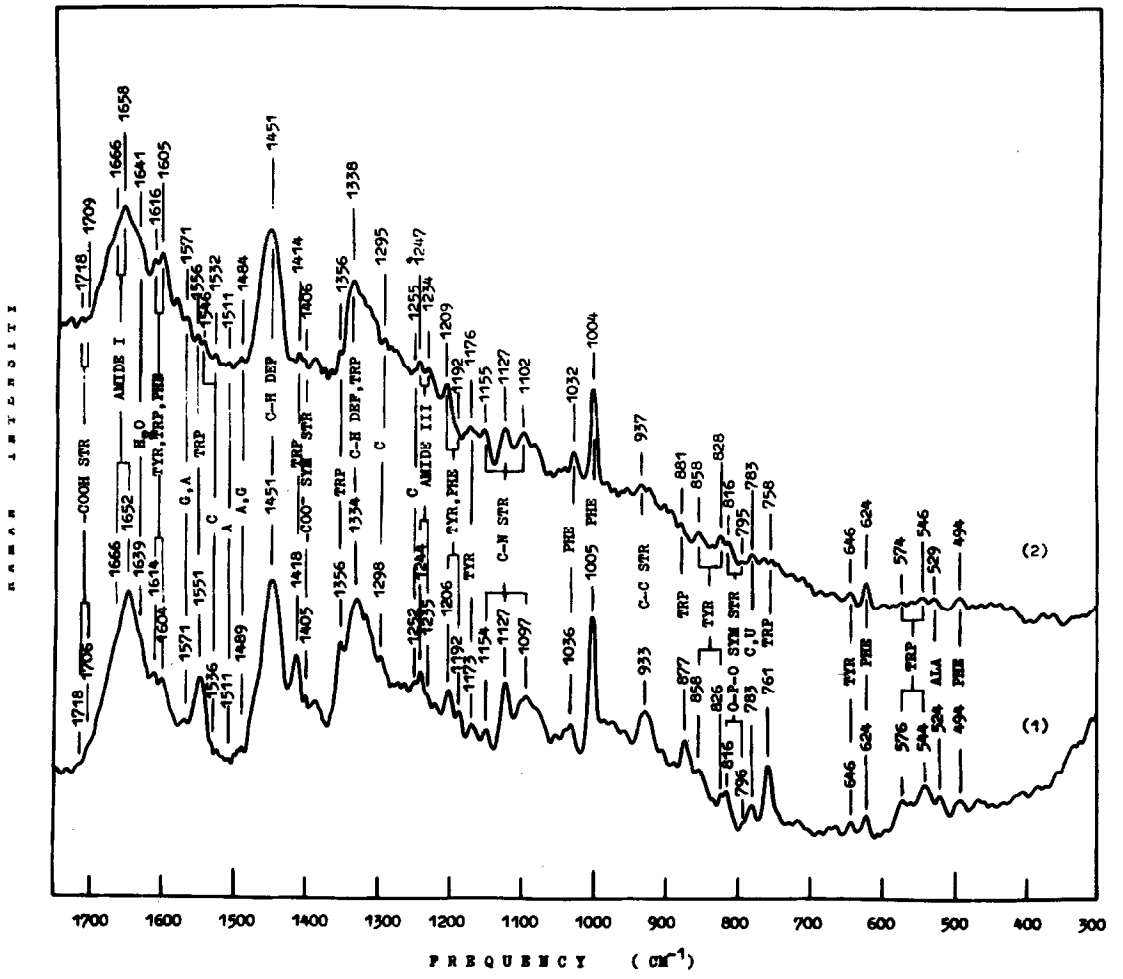

Fig. 1. Raman spectra of TMV and CV in 0.01 M phosphate buffer pH 7.2 at room temperature. Excitation wavelength = 5145 Å, radiant power 200-300 mW, slit width 10 cm^{-1}, scan rate 25 cm^{-1}/min, rise time 5 sec, sensitivity 10 K counts/sec, curve I - TMV, curve 2 - CV.

of a medium intensity at 1248±5 cm^{-1} and by a line of C-C-(N) stretching vibrations at 960±5 cm^{-1}.

In the Raman spectrum of TMV there are an intense Amide I peak at 1652 cm^{-1} with a shoulder at 1666 cm^{-1}, a medium intensity Amide III line at 1244 cm^{-1} with a weak shoulder at 1235 cm^{-1} and a broad medium intensity line at 933 cm^{-1} (Fig.1, curve 1). The TMV spectrum shows that the polypeptide chain of its coat protein has a very

high content of α-helix and unordered structures and only a very small amount of β-structure. The recent X-ray diffraction studies show (1) that TMV protein *in situ* has about 50 per cents of amino acid residues in α-helical regions.

The Raman spectrum of CV displays an intense Amide I peak at 1658 cm^{-1} with a weak shoulder at 1666 cm^{-1} (shoulders at 1641 and 1678 cm^{-1} are produced by water and RNA, respectively), medium intensity Amide III lines at 1234 and 1247 cm^{-1} and C-C-(N) stretching vibration lines at 937 and 958 cm^{-1} (Fig.1, curve 2). The higher intensity of 1234 cm^{-1} line, the lower intensity of 937 cm^{-1} line and the shift of the Amide I peak to the greater wave numbers compared to the TMV spectrum, testify to a significantly higher β-structure content and lower α-helix content in CV protein than in TMV protein. Taken together with the classical X-ray diffraction data (12) on lower electron density at low radii in CV protein subunit as compared to TMV subunit, this result can mean that the differences in the α-helix content of TMV and CV proteins *in situ* reside at least partially, in the internal (low radius) regions of the virion. As shown recently, it is the internal α-helical regions of TMV protein that interact with the intravirus RNA, protect RNA molecule from the central canal side and play a major role in the virus assembly process (1). So the differences in the α-helix content of TMV and CV proteins may be responsible for the specificity of RNA-protein recognition during TMV and CV assembly (13), for different accessibility of intravirus TMV and CV RNA to formaldehyde (14) and for differences in RNA-protein interactions in these two viruses (9).

The TMV protein contains three tryptophan residues per subunit and CV-protein only one such residue (7,8), therefore the Raman spectra of these viruses differ significantly in the intensity of

tryptophan lines at 544, 576, 761, 877, 1356, 1418 and 1551 cm^{-1}. It was shown (15-17) that the strong peak at 1360 ± 5 cm^{-1} is typical of tryptophans buried in a hydrophobic environment, so a well resolved peak at 1356 cm^{-1} in the TMV spectrum should indicate a hydrophobic environment of two or even all three tryptophans in the TMV protein. The same applies to the single tryptophan in the CV protein.

The ratio of intensities of the tyrosine lines at 858 and 826 cm^{-1} characterise the state of the side chains of tyrosine residues in proteins (18). If tyrosine is buried in a hydrophobic environment and forms a strong H-bond between its OH-group and a negatively charged acceptor, I_{858}: I_{826} = 0.3 to 0.5, and if tyrosine is exposed to a solvent, I_{858}: I_{826} = 1.25 to 1.40. The relative intensity of these two lines in the TMV and CV spectra is equal to 0.75 (both proteins contain four tyrosine residues). So, in both viruses two tyrosine residues are buried and form a strong H-bond with a negative acceptor and the other two are exposed to the solvent. As in TMV particles tyrosine residues are localized far from the RNA backbone (1), the PO^-_2-group of RNA cannot be an acceptor in these H-bonds, and COO^--groups remain the sole candidates for this role.

The existence of ionised carboxyl groups of glutamic and aspartic acids in TMV and CV proteins is confirmed by the presence of a weak line of symmetrical COO^--stretching vibrations at 1405 cm^{-1} in the virus spectra (17). On the other hand, the lines of -COOH groups carbonyl stretching vibrations at 1706 and 1718 cm^{-1} show that protonated aspartic and glutamic acids are also present in TMV and CV. This second group should include amino acid carboxyls with anomalous pK values, which were shown to exist in TMV (12)

The data on the state of the side chains of aromatic and aci-

dic amino acids in TMV protein reported in this work correlate well with the results obtained by the other methods (19-21).

Due to the low RNA content, most RNA lines in the Raman spectra of TMV and CV are rather weak (Fig. 1). Among the RNA lines, which are resolved, two, a strong line at 816 cm^{-1} and a weak line at 796 cm^{-1}, deserve special attention. The intensity of the 816 cm^{-1} line is comparable to the intensity of the pyramidine residues line at 783 cm^{-1}. The analogous line at 812 cm^{-1} was observed by us in the infrared spectrum of hydrated TMV films (data not shown). Such a line is present in the Raman and infrared spectra of all studied helical polynucleotides, RNAs and DNAs in A-form. (22,23). On A → B transition of DNA and on disordering of RNA, this line is replaced by a 790 cm^{-1} line (22,23). Accordingly, the 815 cm^{-1} line was presented to be indicative of the A-form of nucleic acids.

Calculations of the normal vibrations for dimethylphosphate (24) and approximate calculations for A and B forms of DNA (25) showed that the 815 cm^{-1} line is produced by symmetrical stretching vibrations of O-P-O group, when phosphodiester bond has a gauche$^-$-gauche$^-$ conformation and sugar residue has a C(3')-endo conformation. The shift of this line to 790 cm^{-1} may be caused by a change in the conformation of the phosphodiester bond or by a transition of the sugar residue to a C(2')-endo conformation.

According to such an interpretation, simultaneous presence of the 816 and 796 cm^{-1} lines in the Raman spectra of TMV and CV should mean that nucleotides in both intravirus RNAs may exist in different conformations. A part of the nucleotides should have a gauche$^-$-gauche$^-$ conformation of phosphodiester bond and a C(3')-endo conformation of ribose and the remaining nucleotides should have some other conformation of phosphodiester bond and/or a

C(2')-endo conformation of ribose. It should be stressed, however, that such a conclusion is not in full accord with the model of intravirus TMV RNA structure recently proposed on the basis of 4 Å X-ray diffraction data (1). In this model, all the nucleotides of the intravirus RNA have a C(2')-endo conformation of ribose and do not have gauche$^-$-gauche$^-$ conformation of phosphodiester bond.

In any case, judging by the X-ray data (1,12), the structure of RNA in TMV-like viruses differ most strongly from the structure of the A-form of RNA in solution. So the data reported here show that the presence of the 815 cm^{-1} line in the vibrational spectra is not sufficient for ascribing an A-form to the nucleic acid in question.

The 1298 (1295) and 1536 (1532) cm^{-1} lines in the TMV and CV spectra show that at least some of cytosine residues in the two intravirus RNAs are unprotonated (26). At the same time, the presence of some protonated cytosine residues is not excluded either as the Raman spectra show lines at 1252 (1255) and 1546 cm^{-1}.

It was shown (4,6) that denaturation of RNA is accompanied by an increase in the intensity of a 1531 cm^{-1} line and by a decrease of the ratio of intensities of the purine peaks at 1485 and 1575 cm^{-1}. A well resolved peak at 1536 (1532) cm^{-1} is seen in the Raman spectra of TMV and CV in spite of the low RNA content of these viruses and the I_{1489}: I_{1571} ratio for both intravirus RNAs is significantly lower than for free RNAs (4). So it may be deduced that the bases in intravirus TMV and CV RNA are involved in stacking interactions to a much smaller extent than bases in helical regions of free RNAs. This conclusion is in accord with the recently proposed model of intravirus TMV RNA structure (1). The Raman data obtained so far show no difference between the TMV and CV RNA structure *in situ*.

The results reported here show that Raman spectroscopy can prove rather useful in the studies of the structure of viruses with a low RNA content and in elucidating subtle structural differences between related viruses. It follows also that even closely related viruses may be differentiated on the basis of their Raman spectra.

ACKNOWLEDGMENT. The authors wish to thank Mr. R.Worbs (Spex Industries) for the use of Ramalog 5 spectrometer.

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