STUDIES ON THE ORGANIZATION OF NUCLEIC ACIDS WITHIN NUCLEOPROTEINS[1]

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INTRODUCTION--Despite recent advances in knowledge of the size, shape, and secondary structure of isolated nucleic acids (1,2) little is known about the organization of nucleic acids within nucleoproteins. Moreover, there is evidence that some nucleic acids, when removed from the constraint imposed by the packing of protein molecules around them, assume thermodynamically stable, secondary and tertiary configurations different from those occurring in the intact particles. For example, the physical properties of the ribonucleic acids (RNA) isolated from tobacco mosaic virus (TMV) and bushy stunt virus (BSV) are similar although the former is derived from rod-like particles and the latter from spherical ones. A further illustration is available from studies of deoxyribonucleic acid (DNA) isolated from various bacteriophages and Shope papilloma virus (SPV) which show that the isolated DNA's are stiff elongated macromolecules with equivalent hydrodynamic volumes larger than those of the particles from which they were obtained.

Discussions of the secondary structure of the nucleic acids often are based on spectrophotometric measurements which show changes in the ultraviolet absorbance due to configurational alterations of the macromolecules. The well known hypochromicity due to base-base interactions within the macromolecules is markedly diminished when nucleic acids are denatured by exposure to acid, alkali, low ionic strength, or high temperature. This report describes

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investigations of the spectra of different nucleoproteins before and after degradation into protein and nucleic acid. From these measurements the configurational hypochromicity within the intact structures is evaluated, thereby providing a basis for inferences about the intraparticle organization of the nucleic acids. Although analyses of the spectra of some nucleoproteins have been reported previously (eg 3,4,5,6), with one exception (6) no consideration was given to the organization of the nucleic acids.

EXPERIMENTAL--Degradation was achieved by the action of sodium dodecyl sulfate (SDS) at a concentration of 1-2% on solutions of the nucleoproteins with an optical density (O.D.) of 1.0 to 2.5 at 260 mμ. In some cases degradation occurred at room temperature; in others the solutions were warmed to 55° C. The buffers were 0.1 - 0.2 M sodium phosphate at pH 7 for all materials except BSV for which acetate buffer at pH 4.4 was used. The completeness of degradation was verified by ultracentrifugation with both schlieren and ultraviolet absorption optics. Absorption spectra were obtained with a Cary Model 11 spectrophotometer. For some experiments differential spectra were obtained directly by measuring the absorbance of a solution of the degraded nucleoprotein (obtained by heating) against that of an identical solution of the intact particles (maintained at low temperature). When the material degraded at room temperature, SDS was omitted from the reference cell. Since the observed O.D. is attributable not only to absorption by the protein and nucleic acid components but also to light scattering, corrections for the latter were necessary. Data for these corrections were obtained from O.D. measurements in a 10.0 cm cell. The O.D. at wave lengths (λ) between 320 mμ to 700 mμ was plotted against λ on a log-log scale and the straight line portion was extrapolated to 260 mμ. The resultant value was then subtracted from the observed O.D. to give the true absorbance at 260 mμ and other wave lengths. Plots of log O.D. vs. log λ were generally straight above 320 mμ with slopes equal to 4 or slightly lower as expected from the Rayleigh scattering law and modifications of it for large particles. Some measurements of the dependence of O.D. on temperature were made in a

Beckman DU spectrophotometer equipped with thermospacers. Viscosity measurements were made in Ostwald type viscometers with an average shear gradient of 300 sec^{-1} in a constant temperature bath at 25 $\pm$ 0.005° C. The ribonucleoproteins, TMV, BSV, and 80 S particles from yeast, and the deoxyribonucleoproteins, T6 bacteriophage and SPV, were prepared by well-established centrifugal procedures. The authors are indebted to C. A. Knight and S. Kass for a sample of SPV (7) and to H. Fraenkel-Conrat for a sample of native TMV protein.

RESULTS--Representative spectral data for TMV and 80 S particles are given in Fig. 1 and the results for five nucleoproteins are summarized in Table I.

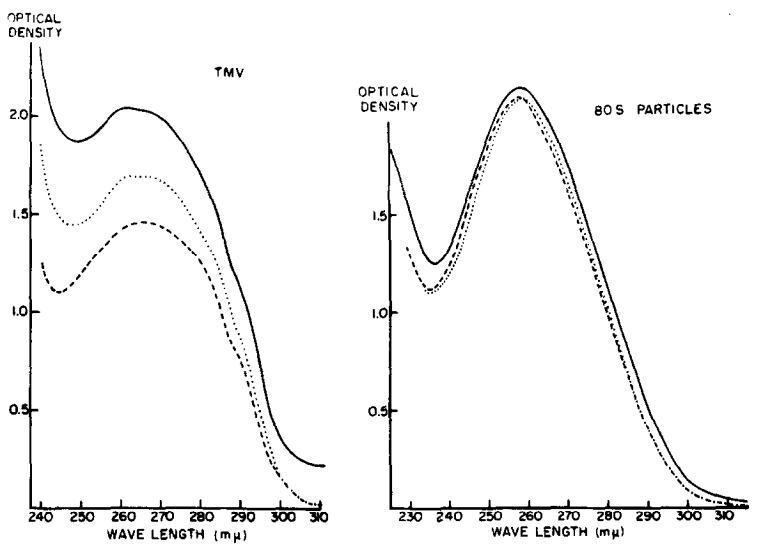

Fig. 1. Ultraviolet absorption spectra of TMV (left) and 80 S particles (right) before and after degradation into RNA and protein. Solid and dotted curves are for the intact nucleoproteins, the former giving the observed O.D. values and the latter showing the curve resulting after light scattering corrections were applied. The dashed curves were obtained after degradation into the RNA and protein.

Shown in the figure are the observed curves of O.D. vs. λ and the true absorption spectra after correcting for scattered light. As seen in Table I where all of the data for 260 mμ are normalized to an O.D. of 1.0, the scattering correction varies from 2% for the 80 S particles to 33% for T6 bacteriophage. Also shown in Fig. 1 are the absorption spectra of the nucleoproteins after degradation into protein and nucleic acid. Since

the experimental conditions, low temperature and moderately high ionic strength were such that free RNA or DNA would be hypochromic, a decrease in the absorbance upon degradation provides a direct indication of the lack of hypochromicity of the nucleic acids within the intact nucleoproteins.

Table I

Sample	Sedimentation Coefficient[1]		Absorption at 260 mμ		
	Intact Nucleoprotein	After Degradation[2]	Intact Nucleoprotein		Nucleic Acid and Protein[2]
	S	S	Observed	True Absorbance[3]	
TMV	187	31	1.0	0.82	0.69
80 S	80	30, 18	1.0	0.98	0.97
BSV	130	30[4]	1.0	0.89	0.83
T6	1100	37	1.0	0.67	0.67
SPV	284	18, 24	1.0	0.73[5]	0.73

[1]Sedimentation coefficients were determined with ultraviolet absorption optics. When more than one sedimentation coefficient is listed the first corresponds to the principle component. In nearly all samples examination of the sedimentation pattern with schlieren optics showed that the protein sub-units had $s_{20,w}$ of 2 - 4 S.

[2]These were measurements on unfractionated, degraded nucleoproteins in buffer solutions.

[3]These figures were obtained by subtracting the scattering correction from the observed optical density.

[4]Other components were also observed depending upon the experimental conditions.

[5]The scattering which is greater than expected theoretically is due in part to aggregated material.

TMV--The decrease in the true absorbance upon degradation showed clearly that the RNA within TMV is non-hypochromic. Heating the solution of degraded TMV caused an increase in absorbance to the value of the undegraded virus. This corresponds to a 25% increase in the absorbance of RNA, a result in agreement with other studies (2) on purified RNA. If TMV was degraded by SDS in the absence of buffer ions the liberated RNA was non-hypochromic and the absorbance (260 mμ) of the degraded virus was virtually identical to

that of the undegraded virus. Moreover, addition of salt to the former caused an immediate lowering of the absorbance to that resulting directly from SDS action in salt solution. That the spectral changes in Fig. 1 were due principally to alterations in the state of the RNA rather than protein was demonstrated by studies on pure TMV protein. Aggregation of the protein to rods (8) when the temperature or pH was changed produced a difference spectrum with maxima at 283 and 290 mμ. A similar effect was caused by addition of SDS to pure protein. But the change in absorbance at 260 mμ due to these protein interactions was negligible (1-2%) compared to that caused by degradation of TMV into its components.

80 S Particles--Except for a slight shift in the spectrum the absorbance of the degraded particles was virtually the same as that of the intact 80 S particles showing that the RNA within the particles was hypochromic. Heating the solution to 80° C caused a 25% increase in absorbance due to the rupture of the secondary structure of the liberated RNA.

BSV--Degradation of the virus caused a slight decrease in the absorbance, thereby showing that the RNA within the BSV is not fully hypochromic. Further evidence for this was obtained by heating the degraded virus, which caused a 23% increase in absorbance to a value greater than that of the intact particles. From these data it is concluded that the RNA within BSV is only partially hypochromic.

T6 Bacteriophage--The absorbance of the mixture of DNA and phage protein in SDS was virtually identical to that of the intact virus. Thus the DNA in the virus is fully hypochromic. Heating the degraded virus caused an increase of 30% in absorbance at 260 mμ due to the melting of the DNA. As in the case of the 80 S particles the differential technique revealed a slight shift in λ_{max} upon degradation.

SPV--The absorbance of the DNA plus protein was virtually identical to that of the intact SPV, thereby indicating that the DNA was hypochromic in the virus. Studies of this DNA to determine if it was doubly stranded were limited by the extremely small amounts of SPV available, and only preliminary

results were obtained. Electron micrographs by J. Toby and R. C. Williams showed fibrous particles. The reduced viscosity was about 20 dl/g. The absorbance was independent of ionic strength from 0.001 to 0.1 but, upon heating, increased sharply by more than 35% with a melting point of 85° C. This transition, unlike that observed for DNA from other sources, was reversible as shown by sedimentation, viscosity, and O.D. measurements; even after the solution was heated to 95° C both sedimenting boundaries were extremely sharp with unaltered sedimentation coefficients. From the physical data it is concluded that the DNA is doubly stranded and has a molecular weight of $3 - 4 \times 10^6$, a value consistent with the particle weight of SPV and its DNA composition. The nature and significance of the 24 S component remains as a problem for further study.

Unless we make the assumption that large changes in ultraviolet absorbance might be caused by base-protein interaction, it can be concluded that the nucleic acids from 80 S particles, T6, and SPV are packed in the intact particles in a manner involving base-base interaction to the same degree as found for the isolated nucleic acids. At the other extreme is RNA from TMV. Here packing apparently precludes such interaction as would be expected from recent x-ray diffraction evidence (9). BSV represents an intermediate case in terms of the organization of its RNA.

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