Structure and Biological Function of Ribonucleic Acid from Tobacco Mosaic Virus

THE finding that isolated ribonucleic acid from tobacco mosaic virus is infective[1] has led to further studies on its biological and physical properties.

The whole ribonucleic acid content of a single tobacco mosaic virus molecule corresponds to a molecular weight of $2-3 \times 10^6$. The experiments to be described suggest that the biological activity depends on the integrity of this core.

Ribonucleic acid prepared by the phenol method[1] contains a component (A) which is homogeneous in the sedimentation diagram (Fig. 1). In addition, there are slower migrating, seemingly degraded components (B). The mean molecular weight[2] of the ribonucleic acid is around 10^6. In order to obtain the molecular weight of component A, the sedimentation constant, the intrinsic viscosity, and the corresponding mean molecular weight have been studied with the ribonucleic acid in the original state and in various states of degradation. In this way, a relation
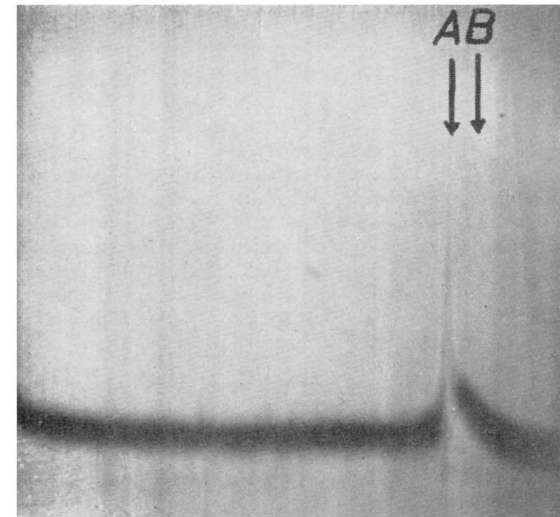

Fig. 1. Sedimentation diagram of 0·24 per cent ribonucleic acid in 0·02 M phosphate buffer, pH 7 at 5° C., 50,000 rev./min. Peak A, homogeneous component; B, slower migrating, poly-disperse component. The sedimentation constant of component A is 19 S at this concentration

Table 1. Decrease of intrinsic viscosity, $[\eta]$, mean molecular weight (weight average) m, and infectivity, L, with time. The decrease was induced by $1 \cdot 5 \times 10^{-5}$ μgm./ml. ribonuclease at 22° C. in a solution of 0·16 per cent ribonucleic acid in 0·02 M phosphate buffer, pH 7. The infectivity, L, is given as the number of lesions per 25 leaves of *Nicotiana glutinosa* inoculated with 20 μgm./ml. ribonucleic acid in 0·1 M phosphate

t (min.)	$[\eta]$ (c.c./gm.)	m	L (lesions)
0	300	$1 \cdot 20 \times 10^6$	980
5	274	$1 \cdot 10 \times 10^6$	786
11	254	$1 \cdot 02 \times 10^6$	383
17	232	$0 \cdot 93 \times 10^6$	487
22	214	$0 \cdot 86 \times 10^6$	272
28	196	$0 \cdot 78 \times 10^6$	242
37	168	$0 \cdot 67 \times 10^6$	62
65	115	$0 \cdot 46 \times 10^6$	1

between molecular weight and sedimentation constant was found and led to a value of about $2 \cdot 1 \times 10^6$ for the homogeneous component A, corresponding within the limits of accuracy to the intact ribonucleic acid core.

The sedimentation constant of the infective component is the same as that of component A within ± 25 per cent. This was determined by differential ultracentrifugation followed by testing the specific infectivity of the ribonucleic acid in the supernatant. The molecular weight of the infective component is thus about $2 \cdot 1 \times 10^6$.

The molecular weight of the smallest infective units can be obtained by studying the correlation between the decrease in mean molecular weight (weight average m) and infectivity L in the course of degradation by ribonuclease. Control experiments revealed that m is nearly proportional to the intrinsic viscosity $[\eta]$, and L to the counts of lesions on *Nicotiana glutinosa*. A typical example of the measured decrease in infectivity, viscosity and mean molecular weight is given in Table 1. The decrease in L is much faster than that in m. This indicates that the molecular weight of the infective component (M) is larger than m. A quantitative correlation can be derived to calculate M:

$$\frac{1}{m_1} - \frac{1}{m_2} = \frac{1}{2M} (\ln L_1 - \ln L_2)$$

The values given in Table 1 lead to $M = 2 \cdot 2 \times 10^6$, whereas the mean value from all measurements of this type is $2 \cdot 5 \times 10^6$.

When the infective unit has received an average of one break of an internucleotide bond through the action of ribonuclease, the infectivity is expected to be reduced by a factor e. The molecular weight can

be calculated if the activity of the enzyme, given as the proportion of bonds split per unit of enzyme concentration and time, is measured under the conditions used for the infectivity test. This was done in two steps. First, it was estimated by titration that a state of degradation corresponding to one break per molecular weight of 7,000 is indicated by an increase in the ultra-violet absorption at 260 mμ by 15 per cent. Then it was found that this state is reached by 260 times the enzymatic action which reduces the infectivity by the factor e. Therefore, the molecular weight of the active unit is about $260 \times 7,000 = 1 \cdot 8 \times 10^6$.

Thus, within the limited accuracy of the determinations described, different approaches lead to the same size of 2×10^6 for the infective unit, corresponding approximately to the entire ribonucleic acid core of a tobacco mosaic virus molecule. Smaller fragments obtained by the action of ribonuclease are inactive. Parts necessary for infection must therefore be distributed along a large part of the ribonucleic acid core. Sections without biological functions in between are not excluded. If small active sub-units exist, they might form strands wound parallel around each other to make up the ribonucleic acid core. A structure of this type, however, is not likely in view of the following evidence.

With respect to the structure of the ribonucleic acid in solution, the following observations are relevant.

(1) The intrinsic viscosity of the ribonucleic acid is found to be proportional to approximately the first power of its mean molecular weight, suggesting a structure intermediate between a random coil and a stiff rod. The whole core appears as a flexible, moderately coiled chain with dimensions of several thousand angstroms.

(2) Ribonucleic acid of high molecular weight has a large optical rotation ($\alpha = 220°$ at 546 mμ) as compared with that of degraded ribonucleic acid ($\alpha < 30°$), whereas the ultra-violet absorption at 260 mμ is less for larger molecules than for smaller ones. The minimum molecular weight for high rotation and low absorption is about 7,000, corresponding to twenty nucleotides. This is evidence in favour of a superstructure of the larger molecules of ribonucleic acid in solution.

(3) Ribonuclease causes a breakdown of the ribonucleic acid that proceeds without a lag phase (Table 1), indicating that single splitting processes determine the rate.

(4) From the absolute activity of the ribonuclease and the induced reduction in the mean molecular weight of ribonucleic acid, it is estimated that any, or nearly any, break of an internucleotide bond causes a molecule to disintegrate.

If the structure should consist of several strands (3) and (4) would imply that splits within different strands are not independent. This being unlikely, the ribonucleic acid is more probably single-stranded. A simple model for a single-stranded superstructure would be a helix with hydrogen bonds between nucleotides of adjacent turns.

A detailed account of this work will be published elsewhere. I am much indebted to Prof. G. Schramm and Prof. H. Friedrich-Freksa for support and advice and to Miss A. Kleih and Mr. W. Mönch for assistance.

ALFRED GIERER

Max-Planck-Institut für Virusforschung,
　　　　　Tübingen.
　　　　　April 12.

[1] Gierer, A., and Schramm, G., *Nature*, **177**, 702 (1956); *Z. f. Naturforsch.*, **11b**, 138 (1956).

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