The Molecular Weight of Yeast P1 Double-stranded RNA*

(Received for publication, May 22, 1978)


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The molecular weight of the double-stranded RNA (P1 dsRNA) always associated with the virus-like particles that occur in the yeast Saccharomyces cerevisiae was determined to be 3.5 ± 0.1 × 10^6 by equilibrium sedimentation. Methylmercuric-agarose gel electrophoresis yielded a value of 1.67 ± 0.11 × 10^6 for the single-stranded form. The sedimentation coefficient, s_{20,w}, was determined by boundary sedimentation to be 15.6 ± 0.13 S. As part of the equilibrium sedimentation work, the effective density gradient for dsRNA in Cs2SO4 was determined.

By electron microscopy, the length of the dsRNA relative to the replicative form of the DNA phage G4 was found to be 0.70 ± 0.01. This represents the first determination of the contour length of a dsRNA molecule in known molecular weight relative to a dsDNA molecule. Such a relative contour length, when compared with our molecular weight data, implies a relative base pair separation along the helix (dsRNA/dsDNA) of 0.79 under our spreading conditions. This value may be used in future determinations of the molecular weight of an unknown dsRNA molecule from a co-spread DNA standard if similar spreading conditions are used. The relative length measurement and the molecular weight values were used in assessing the configurations of the dsRNA and dsDNA molecules. These suggest that the RNA is in an A-like configuration and the DNA is in a B-like configuration, or that both differ proportionately from their respective fiber structures.

A virus-like particle containing double-stranded RNA has been detected in many laboratory strains of the yeast Saccharomyces cerevisiae (1, 2). The dsRNA molecule, P1 dsRNA, which is invariably associated with these VLPs is assumed to represent the viral genome and has been widely studied (for review, see Ref. 3). It is important to determine the molecular weight of the P1 dsRNA because an estimate of its coding capacity is essential to our study of the development of the yeast VLP. We previously have demonstrated that three proteins, V, B, and D, of molecular weights 7.5 × 10^6, 5.3 × 10^6, and 3.7 × 10^6, respectively, are associated with this VLP and are present in the ratio 10V:1B:1D (4). Herring and Bevan (5) recently have demonstrated that RNA polymerase activity is associated with the yeast VLP. The number of molecules per virion of any polymerase would be expected to be less than the number of the V, B, or D proteins. It is of interest to know whether the VLP genome has the capacity for encoding the V, B, and D proteins together with the polymerase, or whether one or more of these proteins must be encoded by yeast nuclear DNA.

The estimates of the molecular weight of P1 dsRNA which have been made to date have yielded a range of values. Estimates of the double-stranded molecular weight by co-electrophoresis of P1 dsRNA in polyacrylamide gels with marker dsRNAs from the Aspergillus foetidus virus (1, 6), the Penicillium chrysogenum virus (7), and reovirus (8) have all given a value of 2.5 × 10^6. However, Wickner and Leibowitz (9), using the same technique but with bacteriophage φ6 dsRNA as a standard, obtained a value of 3.0 × 10^6 for P1 dsRNA. More recently, Herring and Bevan (5) have revised their 2.5 × 10^6 value to 3.3 × 10^6 on the basis of new information on the molecular weight of the A. foetidus virus dsRNAs which they used as standards. These workers have also obtained a value of 1.6 × 10^6 for the single-stranded molecular weight of P1 RNA using formamide polyacrylamide gels with yeast rRNAs as molecular weight standards (5, 6).

In this paper, we report the application of several different methods to the determination of the molecular weight and other physical properties of the P1 dsRNA.

**Experimental Procedures**

**Organism**—Saccharomyces cerevisiae S7, a "sensitive," haploid strain containing elevated amounts of P1 dsRNA, but no P2 dsRNA, has been described previously (4).

**Growth Conditions**—For routine preparation of dsRNA or of VLPs, S7 was grown to late stationary phase in YM-1 + 2% (w/v) glucose (10) at 23°C.

When ds[^14]N]RNA was required, the following procedure was adopted. A single colony of S7 was picked from an agar plate and the cells were suspended in 10 ml of Wickerham's minimal medium (11) without ammonium sulfate. The cell density of this suspension was determined and aliquots containing 10^8 cells added to each of two flasks containing 1 liter of Williamos and Fennell's (12) modification of Wickerham's (11) minimal medium containing [^14]NH4]SO4. Growth was allowed to proceed at 23°C for 4 days, by which time stationary phase had been reached and the cells had passed through approximately 13 generations.

**Isolation of Nucleic Acids**—For the ultracentrifugation studies, P1 dsRNA was extracted from VLPs purified by the method of Oliver et al. (4). Methods of extracting dsRNA from either whole cells or VLPs are also given in Ref. 4. The same technique was used to extract RNA from pure TMV virions (kindly supplied by Dr. S. Wildman of the University of California, Los Angeles). The RF of phage G4 was prepared as previously described (13).

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*This work was supported by a Science Research Council grant (S. G. O.), by grants from the Cancer Research Coordinating Committee of the University of California and from the Burns Family Foundation (C. S. M.), and Grants CA 10628 (C. S. M.), CA 11861 (E. K. W.), and CA 12627 (R. C. W.) from the National Cancer Institute. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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§ The abbreviations used are: dsRNA, double-stranded RNA; VLP, virus-like particle; RF, replicative form; SSC, 0.15 M NaCl, 0.015 M sodium citrate; TMV, tobacco mosaic virus.
RESULTS

Preparation and Purity of dsRNA—For the centrifugation experiments, P1 dsRNA was either extracted from isolated VLPs (4) or VLPs were disaggregated in situ using a detergent Sarkosyl. We previously have shown (4) that VLPs isolated from strain S7 by our CsCl gradient technique contain P1 dsRNA and no other species of nucleic acid. In the present study, the purity of the P1 dsRNA preparation was confirmed both by polyacrylamide gel electrophoresis (4) and by band velocity sedimentation through CsCl (data not shown).

In the other experiments, P1 dsRNA was extracted directly from stationary phase cells of strain S7 (4). The extract was freed of rRNA by LiCl precipitation (25). Double-stranded RNA prepared in this way contains some tRNA which, due to its low molecular weight, did not interfere with our experiments.

Equilibrium Sedimentation in Cs2SO4—The buoyant density of P1 dsRNA in Cs2SO4 at 25°C was found to be 1.611 ± 0.003 g ml⁻¹ based on a density of 1.640 g ml⁻¹ for TMV RNA (17). The same result was obtained using VLPs lysed in the centrifuge with Sarkosyl as well as phenol-extracted dsRNA.

In order to calculate a molecular weight from band-spreading, a value for the effective density gradient, (dp/dr), is required. This parameter depends on the nature of the nucleic acid as well as on the conditions of centrifugation. It was determined by the isotope substitution method (19) using [ds¹⁴N]RNA as described under “Experimental Procedures.” This co-sedimentation of [ds¹⁴N]- and [ds¹⁷N]RNA yields the value of the related quantity, G, defined by Hearst and Schmid (18) as:

\[ G = \frac{(1 + \lambda^2)(dp/dr)_{(ds^{17}N)}}{(dp/dr)_{(ds^{14}N)}} = \frac{\Delta m_{\rho_0}}{m\Delta \rho_0} \]

where \( \lambda \) is the preferential interaction in grams of water/g of nucleic acid, \( \rho_0 \) is the buoyant density, \( m \) is the mass of the average cesium nucleotide, and \( \Delta \rho_0 \) is the increase in \( m \) due to ¹⁷N substitution. All of the quantities on the right hand side are directly measurable from the isotope substitution experiment. \( G \) was found to have a value of 14.7 × 10⁻³ g s⁻¹ cm⁻¹. It was used in the equation:

\[ M_{app} = \frac{RT\rho_0}{\frac{\Delta m_{\rho_0}}{m\Delta \rho_0}} \]

where \( R \) is the gas constant and \( T \) is the absolute temperature, to obtain \( M_{app} \), the apparent molecular weight on an anhydrous basis (18). The value of \( \lambda \), the standard deviation of the experimental Gaussian band, was obtained using a computer program that gives a moments analysis of the equilibrium distribution at each of four concentrations of dsRNA, essentially as described by Schmid and Hearst (26). The resulting values of \( M_{app} \) were converted from the cesium salt of RNA to the standard sodium salt form by multiplying by 0.757, the ratio of the average nucleotide weight in the sodium and cesium salt forms. Thermodynamic nonideality was corrected for by extrapolation to infinite dilution of the macromolecules using the reciprocal plot shown in Fig. 1. The intercept at zero concentration yields a molecular weight of 3.5 ± 0.1 × 10⁶ for the dsRNA.

Sedimentation Coefficient—Boundary sedimentation in SSC buffer was used to determine the sedimentation coefficient, \( s_{20,w} \), of P1 dsRNA at several concentrations. Three scans of the sedimenting boundary are shown in Fig. 2. Fig. 3 shows an extrapolation of \( s_{20,w} \) against concentration that yields an \( s_{20,w} \) of 15.6 ± 0.1 S.
Fig. 2. Boundary sedimentation of P1 dsRNA. A series of scans is shown for the most concentrated dsRNA solution used ($A_{260} = 1.2$). a, 98 min; b, 60 min; c, 84 min.

Fig. 3. Concentration dependence of the sedimentation coefficient. The $s_{20w}$ value at each of three concentrations is plotted against relative concentration (0.3 $A_{260}$ units = 1) and extrapolated to zero concentration to define an $s_{20w}$ of 15.6 ± 0.1 for the molecule.

**Methylmercuric Hydroxide-Agarose Gel Electrophoresis**—In order to determine the single-stranded molecular weight of P1 dsRNA, we used agarose gel electrophoresis with CH$_3$HgOH as a denaturing agent (20). Methylmercuric hydroxide is a strong, reversible denaturant which is able to dissociate double-stranded nucleic acids completely and to destroy all secondary structure in single-stranded molecules (20). P1 dsRNA extracted from whole cells labeled with [2-^3^H]adenine (see “Experimental Procedures”) was subjected to co-electrophoresis with $^{32}$P-labeled HeLa cell rRNAs (21, 22) in 1.2% agarose gels containing 5 mM CH$_3$HgOH at 3 mA/gel for about 4 h. Preparation of these gels and the subsequent scintillation counting of gel slices was carried out as described in Ref. 20.

Fig. 4 shows the radioactivity profiles of two such gels run with (Fig. 4B) and without (Fig. 4A) CH$_3$HgOH. In Fig. 4A, it can be seen that native P1 dsRNA migrated much more slowly than the 28E HeLa rRNA. However, the denatured (single-stranded) form in the CH$_3$HgOH gel (Fig. 4B) migrated slightly faster than the 28S HeLa rRNA. Ethidium bromide staining (27) confirmed that P1 RNA was, in fact, in the single-stranded form in the denaturing gels. Based on molecular weight values of 1.76 ± 0.15 and 0.68 ± 0.07, respectively, for HeLa 28S, 18S rRNAs (28, 29), we obtain a single-stranded molecular weight of P1 RNA of $1.65 \pm 0.11 \times 10^5$. This is in good agreement with the data of Herring and co-workers (5, 6) obtained by foramide gels and with our own results using similar gels and formamide-denatured material run on nondenaturing polyacrylamide gels.

**Electron Microscopy**—The dsRNA was prepared and co-spread with G4-RF as described under “Experimental Procedures.” A picture of several typical molecules along with G4-RF standards is presented in Fig. 5. All the dsRNA molecules were linear and formed a single homogeneous size class. Over

100 RNA molecules were measured; their mean length was found to be $0.70 \pm 0.01$ G4-RF units.

**DISCUSSION**

This study has established a number of physical parameters of the P1 dsRNA molecule carried by many strains of *S. cerevisiae*. The double-stranded molecular weight for P1 dsRNA determined by the density gradient equilibrium technique was $3.5 \pm 0.1 \times 10^5$. Agarose gel electrophoresis yielded a value of $1.67 \pm 0.11 \times 10^5$ for the single-stranded molecular weight. Both of these methods provide reliable measures of molecular weight. The equilibrium sedimentation method is a primary method and is independent of empirical calibration. Although the agarose gel method depends upon external standards, the standards were well characterized, single-stranded RNAs (28, 29). Furthermore, under the conditions used (20), the molecules were fully denatured.

An advantage of determining the parameter $G$, related to the effective density gradient by the isotope substitution method, is that the molecular weight can then be calculated from a few directly measurable quantities. No assumption is necessary concerning the magnitude of $\Gamma$; the partial specific volume of dsRNA is not needed; and compression effects in the gradient are corrected for. $G$ is a constant for dsRNA in Cs$_2$SO$_4$ and its determined value can now be used in other measurements of the molecular weight of dsRNAs by the band-spreading method. The value of $14.7 \times 10^{-10} \text{g} \text{s}^2 \text{cm}^{-4}$ for G for dsRNA may be compared with that for dsDNA in Cs$_2$SO$_4$ at $25^\circ$C of $18.3 \times 10^{-10}$. This value was calculated from the data in Table 2 of Hearst and Schmid (18) and is based on the isotope substitution experiment by Schmid and Hearst (19). The value for dsRNA is not expected to be the same as for dsDNA. It will reflect differences resulting from a lower $\Gamma$ for dsRNA than for dsDNA as well as differences in the compressibility of the nucleic acid species in the gradient and differences in the values for the compositional gradient due to Cs$_2$SO$_4$ at the specific densities at which the two species are buoyant.

There is no established empirical relation of $s^2$ to $M$, for dsRNA such as is widely used for dsDNA. This one suggested by Franklin (30) for dsDNA is based on such unreliable data, excepting those for R17 replicative form, that it is of little

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*1* L. E. Holland and S. G. Oliver, unpublished results.
value for calculating $M_c$ from $s^0$. An adjustment of the calibration of the DNA curve by Freifelder (31) has the form $(s^0 - 2.8) = 0.00834 M_c^{0.479}$. If this equation is applied to dsRNA, it indicates that $M_c = 4.3 \times 10^6$ for $s^0 = 15.63$ S, a value considerably higher than that given by our other measurements on P1 dsRNA. A similar result for the dsRNA of the replicative form of phage R17 was pointed out by Franklin (30). His value of $s^0 = 14.7$ S yields $M_c = 3.6 \times 10^6$ for a dsRNA for which $M_c = 2.2 \times 10^6$. Franklin suggested that this effect results from a greater flexibility of dsRNA than of dsDNA. Thus, the only conclusion that can be reached about the relation between $s^0 = 15.63$ S and $M_c = 3.4 \times 10^6$ for P1 dsRNA is that they are probably consistent.

The molecular weight value based on nondenaturing polyacrylamide gel electrophoresis of Wichner and Leibowitz (9) and the revised value of Herring and Bevan (5) are both within the range of molecular weights we obtained. These values are also in good agreement with single-stranded molecular weights obtained from polyacrylamide gels using formamide as a denaturant (6).

A double-stranded molecular weight of $3.4 \times 10^6$ for P1 dsRNA requires that we increase our estimate of the potential coding capacity of the molecule from 167,000 daltons of protein (4) to 226,000 to 234,000 daltons of protein. Of this potential capacity, the V, B, and D proteins of the VLP account for 165,000 daltons (4). This leaves 61,000 to 69,000 daltons for a protein that may be concerned with dsRNA transcription or replication (5). Here, it should be borne in mind that the yeast mitochondrial RNA polymerase has a molecular weight of only 68,000 (32).

In contour length measurements by electron microscopy, the absolute contour length of DNA varies from spreading to spreading. However, molecules spread on the same film vary in a uniform manner and thus, relative contour length is reproducible and reliable molecular weights can be obtained if a DNA standard of known molecular weight is co-spread with an unknown (33, 34). Since no length standards are available for dsRNA, we have co-spread P1 dsRNA with a dsDNA standard, G4 RF, and have calculated a molecular weight based on the following considerations: 1) our measured value of the contour of P1 dsRNA of 0.70 G4-RF units; 2) a length of G4-RF of 5577 base pairs; 3) an assumption that the ratio of the base pair separation along the helix axis for dsRNA to that for dsDNA is the same in the spreading film as that determined for the predominant x-ray diffraction pattern in oriented fibers. DNA in the B configuration has a base pair separation of 3.38 Å (35). Double-stranded RNA in the A configuration has a separation of 2.73 Å (36). This form, with 11 base pairs per turn of the helix, was favored by Arnott et al. (37) from x-ray crystallographic data and supported by further crystallographic studies by Arnott et al. (36). The ratio of dsRNA to dsDNA thus has the value of 0.81. 4) A molecular weight (sodium salt) of 686 per base pair for P1 dsRNA is assumed. This is based on a G + C content of 45% (38).

Using these values, a molecular weight of $3.3 \times 10^6$ was calculated for the P1 dsRNA. Since the value is in good agreement with our other data, it suggests that the DNA is in a B-like configuration and the RNA is in an A-like configuration, or that both differ proportionately from their respective fiber structures.

These results will be useful in future determinations of RNA molecular weights from contour lengths relative to DNA standards. Such determinations require knowledge of relative base pair separations of RNA and DNA. This relative separation is determined from our data, under our spreading conditions, to be close to the ratio of 2.73 Å for dsRNA in the A configuration to that of 3.38 Å for DNA in the B configuration. More directly, by using a molecular weight for P1 dsRNA averaged from our agarose gels and equilibrium sedi-

\[ \text{G. N. Godson, personal communication.} \]
mentation (3.4 x 10^6) and our relative contour length measurements, we infer for our spreading conditions a relative base pair separation (dsRNA/dsDNA) of 0.79. Alternatively, the P1 dsRNA itself could be used as a contour length standard in determining the molecular weight of other co-spread dsRNAs since it is of known base composition (38) and its molecular weight has been determined here.

Acknowledgments—We are indebted to Dr. Merrill Camien for help with the ultracentrifuge runs, to Pamela Sutherland for expert technical assistance, and to Nathan Chu for helpful advice on the electron microscopy.

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