The Molecular Weight of Yeast P1 Double-stranded RNA*

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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 $3.5 \pm 0.1 \times 10^6$ by equilibrium sedimentation. Methylmercucic-agarose gel electrophoresis yielded a value of $1.67 \pm 0.11 \times 10^6$ for the single-stranded form. The sedimentation coefficient, $s_{20, w}$, was determined by boundary sedimentation to be $15.6 \pm 0.13$ S. As part of the equilibrium sedimentation work, the effective density gradient for dsRNA in CsSO$_4$ was determined.

By electron microscopy, the length of the dsRNA relative to the replicative form of the DNA phage G4 was found to be $0.70 \pm 0.01$. This represents the first determination of the contour length of a dsRNA molecule of 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 $7.5 \times 10^3$, $5.3 \times 10^3$, and $3.7 \times 10^3$, 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 $2.5 \times 10^6$. However, Wickner and Leibowitz (9), using the same technique but with bacteriophage $\phi 6$ dsRNA as a standard, obtained a value of $3.0 \times 10^6$ for P1 dsRNA. More recently, Herring and Bevan (5) have revised their $2.5 \times 10^6$ value to $3.3 \times 10^6$ 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 $1.6 \times 10^6$ 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 $\text{d}[^{15}N]$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 adjusted and aliquots containing 10⁶ cells added to each of two flasks containing 1 liter of Williamson and Fennel's (12) modification of Wickerham's (11) minimal medium containing $\text{d}[^{15}N]$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 15 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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§ 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.
Ultracentrifugation—Sedimentation analyses were carried out as previously described using a Beckman model E analytical ultracentrifuge equipped with a UV scanner (14, 15). Boundary sedimentation of P1 dsRNA was done in SSC buffer at 40,000 rpm and 20°C. The sedimentation coefficients were corrected to \( s_{20, w} \) using a density of 1.0056 g ml\(^{-1}\) and a relative viscosity of 1.0291 at 20°C for SSC and a factor for dsRNA of 0.53 g ml\(^{-1}\); the last was taken from Bruner and Vinograd (16). The buoyant density was determined by equilibrium sedimentation in CsSO\(_4\) at 44,000 rpm at 25°C using TMV RNA as a marker. TMV RNA precipitates in CsSO\(_4\) and forms a hypershifted band at a density of 1.640 g ml\(^{-1}\) (17).

The molecular weight was determined by the bandwidth at equilibrium in CsSO\(_4\), as described by Hearst and Schmid (18). The results were calculated as in our previous work with this method (14, 15). An isotope substitution experiment (19) was carried out to obtain the effective density gradient, \( (dp/dr)_{eff} \), for dsRNA in CsSO\(_4\), since this quantity has never been determined. This experiment was done by sedimenting a mixture of [\(^{15}\)N]dsRNA and [\(^{14}\)N]dsRNA to equilibrium and measuring the separation of the peaks which differ by a known density calculated from the isotope substitution. The [\(^{15}\)N]dsRNA was obtained by isolating VLPs from a stationary phase culture of S7 grown from a small inoculum in minimal medium containing [\(^{15}\)NH\(_4\)]SO\(_4\), and the mixture was centrifuged to equilibrium at 44,000 rpm at 25°C. The band-sedimenting experiments were carried out using phenol-extracted dsRNA. They were done at a series of concentrations and centrifuged to equilibrium (about 48 h) at 44,000 rpm at 25°C.

**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 the 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 RNA 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 CsSO\(_4\)—** The buoyant density of P1 dsRNA in CsSO\(_4\) 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 with phenol-extracted dsRNA. In order to calculate a molecular weight from band-sedimenting, a value for the effective density gradient, \( (dp/dr)_{eff} \), 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 [\(^{15}\)N]dsRNA as described under “Experimental Procedures.” This co-sedimentation of [\(^{15}\)N] and [\(^{14}\)N]dsRNA yields the value of the related quantity, \( G \), defined by Hearst and Schmid (18) as:

\[
G = \frac{(1 + \gamma)}{(dp/dr)_{eff}} = \frac{\Delta m_p \gamma}{m \Delta \rho G}
\]

where \( \gamma \) is the preferential interaction in grams of water/g of nucleic acid, \( P \) is the buoyant density, \( m \) is the mass of the average cesium nucleotide, and \( \Delta \rho \gamma \) is the increase in \( m \) due to \(^{15}\)N 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\(^{-1}\).

It was used in the equation:

\[
M_{app} = \frac{RTP_{o,C}}{\epsilon G w' G w' G w'}
\]

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 \( \epsilon \), the standard deviation of the experimental Gaussian band, was obtained using a computer program that gives a moment 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 × 10\(^4\) 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.
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shown for the most concentrated dsRNA solution used with G4-RF as described under “Experimental Procedures.” A picture of several typical molecules along with standards is presented in Fig. 5. All the dsRNA molecules were linear and formed a single homogeneous size class. Over

4. Methylmercuric hydroxide-agarose gel electrophoresis. A, Nondenaturing (no CH$_2$HgOH) 1.2% agarose gel; P1 dsRNA and HeLa RNA following co-electrophoresis at 3 mA/gel for 4 h. B, CH$_2$HgOH (5 mM)-agarose (1.2%) gels. The same samples were run under conditions identical with A. Both gels were cut into slices (1 mm) for scintillation counting.

100 RNA molecules were measured; their mean length was found to be 0.70 ± 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 ± 0.1 x 10^6. Agarose gel electrophoresis yielded a value of 1.67 ± 0.11 x 10^6 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 $G$; 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 the effective density gradient by 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 $G$ 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. The one suggested by Franklin (30) for dsRNA is based on such unreliable data, excepting those for R17 replicative form, that it is of little

Electron Microscopy—The dsRNA was prepared and co-electrophoresed 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

Methylmercuric Hydroxide-Agarose Gel Electrophoresis—In order to determine the single-stranded molecular weight of P1 dsRNA, we used agarose gel electrophoresis with CH$_2$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. CH$_2$HgOH as a denaturing agent (20). P1 dsRNA extracted from whole cells labeled with [2-3H]adenine (see “Experimental Procedures”) was subjected to electrophoresis with $^{32}$P-labeled HeLa cell rRNAs (21, 22) in 1.2% agarose gels containing 5 mM CH$_2$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$_2$HgOH. In Fig. 4A, it can be seen that native P1 dsRNA migrated much more slowly than the 28 S HeLa rRNA. However, the denatured (single-stranded) form in the CH$_2$HgOH gel (Fig. 4B) migrated slightly faster than the 28 S 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 28 S, 18 S rRNAs (28, 29), we obtain a single-stranded molecular weight of P1 RNA of 1.67 ± 0.11 x 10^6. This is in good agreement with the data of Herring and co-workers (5, 6) obtained by formamide 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-electrophoresed 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

value for calculating $M_c$ from $s^0$. An adjustment of the calibration of the DNA curve by Freifelder (31) has the form $(s^0 - 2.8) = 0.00834 M_c^{0.579}$. 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 3.8 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.  

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-

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Fig. 5. Electron micrograph of P1 dsRNA. Several of the linear molecules of dsRNA are shown with circular phage G4-RF molecules.

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3 G. N. Godson, personal communication.
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mentation (3.4 × 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 PI 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.

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