JOURNAL OF VIROLOGY, Apr. 1967, p. 302-307
Vol. 1, No. 2

Purification and Properties of Reovirus Ribonucleic Acid

W. J. IGLEWSKI AND R. M. FRANKLIN

The Public Health Research Institute of The City of New York, Inc., New York, New York 10009

Received for publication 22 November 1966

$NaClO_4$ was employed in a technique for the rapid extraction of reovirus ribonucleic acid (RNA). The extracted RNA, which was purified in a Cs_2SO_4 equilibrium density gradient, had a buoyant density of 1.61 g/cm^3 and a sedimentation coefficient of 15*S* in a 7 to 20% sucrose gradient. It was 90% resistant to ribonuclease in a solution of high ionic strength (0.1 M NaCl). The sensitivity of reovirus RNA to ribonuclease increased with decreasing ionic strength. The thermal denaturation transition of the RNA began at 78 C and was complete at 85 C. The T_m of the transition was 81 C in 0.01 M tris(hydroxymethyl)aminomethane buffer (*p*H 7.2) containing 0.001 M ethylenediaminetetraacetate. Thermal denaturation of reovirus RNA resulted in the formation of three ribonuclease-sensitive fractions. Denaturation at 25 C in the presence of dimethyl sulfoxide resulted in the formation of two ribonuclease-sensitive fractions.

Purified viral ribonucleic acid (RNA) is commonly prepared by extracting the RNA from previously purified virus with such agents as phenol or guanidine hydrochloride, by heating, by exposure to extremes of *p*H, or by dialyzing the virus against high salt. These methods of extraction, plus several others, were unsuccessfully employed by Gomatos and Stoeckenius (6) in the extraction of intact infectious reovirus RNA.

By analysis of the chemical composition of reovirus, Gomatos and Tamm (7) have estimated the minimal mass of RNA per virion to be 10.2×10^6 daltons. The $NaClO_4$ method of extracting and concentrating nucleic acids from bacteriophage (4) was modified by Dunnebacke and Kleinschmidt (2) for preparation of reovirus RNA for observation in an electron microscope. They found a small percentage of the strands to have a maximal length of 7.7 μ. Granboulan and Niveleau (8) have studied the reovirus RNA from virions disrupted during spreading for electron microscopy. The mean length of the molecules was 5.1 μ in their experiments. These lengths correspond to molecules of a molecular weight of 10×10^6 to 14×10^6, and therefore these observations suggest that reovirus RNA exists as a single molecule in the virion.

The present communication describes a procedure in which $NaClO_4$ is used for the rapid extraction and purification of large amounts of reovirus RNA without preliminary purification of the virus. The extraction and purification procedure takes advantage of the fact that reovirus RNA is double stranded. The properties of the $NaClO_4$-extracted and purified RNA are described.

MATERIALS AND METHODS

Growth and concentration of the virus. Reovirus 3, kindly provided by P. Tournier, Villejuif, France, was grown in suspension cultures of L cells as described by Gomatos and Tamm (7). Two modifications were made in the procedure. A suspension culture of L cells containing 4.5×10^5 to 6.0×10^5 cells per milliliter was sedimented at 35 to 40 $\times$ *g* for 20 min. The cells were infected by resuspending the pellet in 50 to 70 ml of a reovirus stock solution at an input multiplicity of 10 and then rotating the suspension on a roller drum for 2 hr at 37 C. During the 2-hr adsorption period, the cell density was 1.2×10^7 to 1.8×10^7 cells per milliliter. The infected cells were subsequently resuspended in Eagle's minimal essential medium (MEM) containing 2% fetal calf serum to a final cell concentration of 4.5×10^5 cells per milliliter and incubated in 2-liter spinner flasks at 37 C for 72 hr before harvesting the virus. The titer of the harvested virus was 1.2×10^8 plaque-forming units (PFU)/ml. Virus replication occurred in the presence of 0.2 μc/ml of ^{3}H-uridine. After maximal virus production had occurred (72 hr postinfection), 1 liter of saturated ammonium sulfate, neutralized to *p*H 7, was added to 2 liters of the virus-infected cell suspension and incubated with stirring for 1 hr at 5 C. The precipitate and cellular debris were sedimented at 9,000 rev/min for 20 min in a Sorvall GSA rotor. The pellet was resuspended in 25 ml of a standard buffer solution (STE) containing 0.1 M NaCl, 0.001 M ethylenediaminetetraacetate (EDTA) and

0.05 M tris(hydroxymethyl)aminomethane (Tris) *p*H 7.2. The concentrated virus suspension was dialyzed against STE at 5 C.

$NaClO_4$ extraction of reovirus RNA. Cellular debris was removed from the concentrated virus suspension by centrifugation at 500 rev/min for 20 min at 5 C. Removal of cellular debris by high-speed centrifugation was avoided because of loss of virus attached to debris (11). A 30-ml sample of the supernatant fluid containing the virus was placed on a gradient composed of 0.75 ml of 30% sucrose in STE which floated upon 1.0 ml of STE containing 5 M $NaClO_4$. The gradient was centrifuged at 25,000 rev/min for 4 hr at 12 C in a Spinco SW 25.1 rotor. The $NaClO_4$ fraction containing the extracted reovirus RNA was collected, centrifuged to remove debris, and dialyzed against STE at 5 C. A 3-ml sample of the concentrated and partially purified reovirus RNA solution, containing Cs_2SO_4 of $\bar{\rho} = 1.60$ was centrifuged at 33,000 rev/min for 72 hr at 5 C in a Spinco SW 39 rotor. After centrifugation, 0.2-ml fractions were collected and diluted in STE; the radioactivity per fraction was determined directly on 0.02 ml of the diluted fractions. The fractions containing reovirus RNA were pooled and dialyzed against STE at 5 C.

Sucrose gradient analysis. A 0.20- to 0.25-ml sample of reovirus RNA was placed on a 4.4-ml sucrose gradient (7 to 20% sucrose) containing STE. The gradient was centrifuged at 39,000 rev/min for 4.5 hr at 12 C in a Spinco SW 39 rotor. After centrifugation, 0.2-ml samples were collected in 0.8 ml of STE. The RNA was precipitated by adding 0.1 mg of carrier yeast RNA and 0.2 ml of 60% trichloroacetic acid. The precipitate was collected on a membrane filter (Millipore Filter Corp., Bedford, Mass.), and was washed once with 10% trichloroacetic acid and then with absolute alcohol. The radioactivity adhering to the dried filter was counted in 2,5-diphenyloxazole–1,4-bis-2-(5-phenyloxazolyl)benzene (PPO-POPOP) toluene in a Packard scintillation counter.

$NaClO_4$-sucrose gradient analysis. A 7 to 20% sucrose gradient containing $NaClO_4$ was employed for both extraction and sedimentation analysis of reovirus RNA. The $NaClO_4$ content of the gradient was varied from 0.025 to 2.5 M, according to the requirements of the experiment. Alternate layers of concentrated reovirus and $NaClO_4$, at twice the concentration employed in the gradient, were layered on top of the gradient and incubated for 45 min at 5 C. The gradient was then centrifuged at 36,000 rev/min at 12 C in a Spinco SW 39 rotor. The centrifugation time was dependent upon the $NaClO_4$ concentration of the gradient, since an increased $NaClO_4$ concentration decreased the sedimentation rate of the sample.

Removal of $NaClO_4$ from extracted reovirus RNA. Reovirus RNA was also extracted by mixing 1 volume of reovirus with 1 volume of 2 M $NaClO_4$. The $NaClO_4$ was precipitated by the addition of 1.5 volume of 2 M KCl at 0 C. Under these conditions, a highly insoluble $KClO_4$ precipitate was formed which was removed by centrifugation. The reovirus RNA in the supernatant fluid was precipitated at −20 C after the addition of 2 volumes of ethyl alcohol and 0.1 volume of 2 M potassium acetate. The precipitated RNA was resuspended in STE and centrifuged on a 7 to 20% sucrose gradient.

Ribonuclease resistance. The resistance of reovirus RNA to 1 μg/ml of ribonuclease (pancreatic ribonuclease A, Worthington Biochemical Corp., Freehold, N.J.) was determined by incubation in STE at 37 C for 10 min. The ribonuclease-treated samples were chilled before being precipitated with 10% trichloroacetic acid in the presence of carrier yeast RNA. The precipitate was collected on a membrane filter, and the radioactivity of the acid-precipitable nucleic acid was counted.

Thermal denaturation. Reovirus RNA was dialyzed against 0.01 M Tris buffer (*p*H 7.2) containing 0.001 M EDTA (TE). The RNA was heated for 3 min in a water bath at the desired temperature and then quickly cooled in a dry ice bath. The sedimentation profile of the denatured RNA was examined on a 7 to 20% sucrose gradient, and the ribonuclease resistance was determined.

Denaturation with dimethylsulfoxide (DMSO). Reovirus RNA was dialyzed against TE for 24 hr at 5 C. One part of this RNA was mixed with 6 parts of DMSO to yield a final DMSO concentration of 85.7% (9). The DMSO-reovirus RNA solution was incubated at 25 C for various periods of time and then was quickly cooled in an ice bath. NaCl was added to a final concentration of 0.1 M, calculated on the basis of the aqueous volume. The denatured RNA was precipitated at −20 C after adding 2 volumes of ethyl alcohol and 0.1 volume of 2 M potassium acetate. The precipitated RNA was suspended in TE and dialyzed against TE for 24 hr before sedimentation analysis in a 7 to 20% sucrose gradient. The gradient was fractionated, and the ribonuclease resistance of the denatured RNA was determined.

RESULTS

The RNA extracted from reovirus by the $NaClO_4$ method had a buoyant density of 1.61 in a Cs_2SO_4 equilibrium density gradient. This buoyant density is a characteristic of the double-stranded reovirus RNA (13). The double-stranded RNA, previously purified on the Cs_2SO_4 gradient, had a sedimentation coefficient of 15*S* in a 7 to 20% sucrose gradient (Fig. 1). A double-stranded RNA with a sedimentation coefficient of 15*S* would be expected to have a molecular weight of about 2.5×10^6 to 3.1×10^6, as estimated from the relation between molecular weight and sedimentation coefficient (1) and by comparison with the double-stranded replicative form (RF) of the RNA containing R17 bacteriophage (3). Therefore, the extracted reovirus RNA was approximately one-quarter the size predicted from the chemical analyses of Gomatos and Tamm (7) and the electron microscope studies of Dunnebacke and Kleinschmidt (2) and Granboulan and Niveleau (8).

To eliminate the possibility of shearing the RNA molecule during experimental manipula-

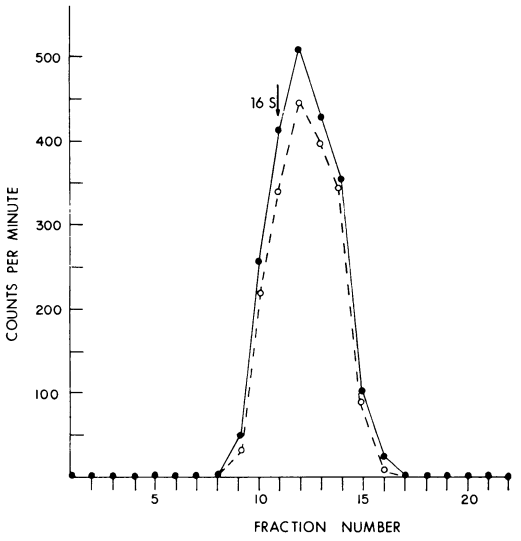

FIG. 1. *Sucrose gradient sedimentation analysis of reovirus RNA extracted in a $NaClO_4$ gradient and further purified in a $CsSO_4$ gradient. Purified ^3H-labeled RNA was mixed with 16S Escherichia coli ribosomal RNA and then layered on a 4.4-ml sucrose gradient (7 to 20% sucrose) containing STE, placed in a SW 39 rotor, and centrifuged for 4.5 hr at 39,000 rev/min in a Spinco model L2 ultracentrifuge with the temperature set at 12 C. Equal fractions were collected and divided into two portions: ●, untreated ○, treated with ribonuclease (1.0 μg/ml, 10 min, 37 C).*

tion, ammonium sulfate-precipitated reovirus was extracted, and its RNA was sedimented simultaneously on a single $NaClO_4$-sucrose gradient. The sedimentation coefficient of the reovirus RNA extracted in this manner was also 15*S*, as compared with a 16*S* ribosomal RNA marker (Fig. 2). Decreasing the $NaClO_4$ concentration in the sucrose gradient from 2.5 to 0.025 M did not alter the sedimentation coefficient of reovirus RNA. The decrease in the $NaClO_4$ concentration did, however, decrease the amount of RNA released from the virus, as might be expected (5). Also, since exposure of bacteriophage R17 replicative form or *E. coli* ribosomal RNA to 5 M $NaClO_4$ did not alter their sedimentation coefficients, $NaClO_4$ did not degrade double- or single-stranded RNA.

According to Dunnebacke and Kleinschmidt (2), shorter exposure times of reovirus to 2 M $NaClO_4$ produced a larger number of longer RNA filaments. In an attempt to simulate their conditions, reovirus was exposed to 1 M $NaClO_4$, followed by immediate addition of KCl to form a precipitate of $KClO_4$. The reovirus RNA extracted by this method also had a sedimentation coefficient of 15*S*.

Purified reovirus RNA was 90% resistant to 1 μg/ml of ribonuclease when incubated in STE at 37 C for 10 min. There was only a slight decrease in the resistance of the RNA as the concentration of the enzyme was increased to 10 μg/ml (Table 1). Also, reovirus RNA became significantly less resistant to ribonuclease at low ionic strength (0.5 × STE and 0.1 × STE), as previously reported (13). The concentration of reovirus RNA was very low during the ribonuclease digestion, since only tracer

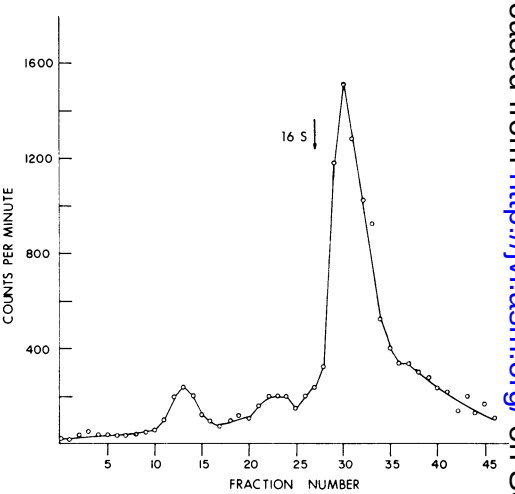

FIG. 2. *$NaClO_4$-sucrose gradient analysis of reovirus RNA. Ammonium sulfate precipitated reovirus was suspended in STE and dialyzed against STE at 5 C. Alternate 0.1-ml layers of concentrated virus and 5 M $NaClO_4$ in STE were layered on 4.4-ml sucrose gradient (7 to 20% sucrose) containing STE and 2.5 M $NaClO_4$. The gradient was centrifuged at 36,000 rev/min for 16 hr at 12 C in the SW 39 rotor of a Spinco ultracentrifuge.*

TABLE 1. *Effect of ribonuclease on reovirus RNA*

RNAase concn[a]	Per cent resistance of reovirus RNA to ribonuclease			
	RNA in STE	RNA in 0.5 × STE	RNA in 0.1 × STE	RNA[b] heated to 75 C
μg/ml				
0.1	93	—	—	92
1.0	89	58	3	84
2.5	85	—	—	70
5.0	82	—	—	58
10.0	80	—	—	39

[a] Samples incubated with ribonuclease for 10 min at 37 C.

[b] RNA heated to 75 C for 3 min in TE, chilled in a dry ice bath, ionic strength adjusted to that of STE, and ribonuclease added.

quantities of RNA without any carrier RNA were being used.

A decreased rate of sedimentation was observed when reovirus RNA was treated with increasing concentrations of ribonuclease. Treatment of the RNA with 50 μg/ml of ribonuclease for 10 min at 37 C decreased the sedimentation coefficient of the RNA to 6S. Low concentrations of ribonuclease (0.2 μg/ml) had little if any effect on the sedimentation coefficient of the RNA.

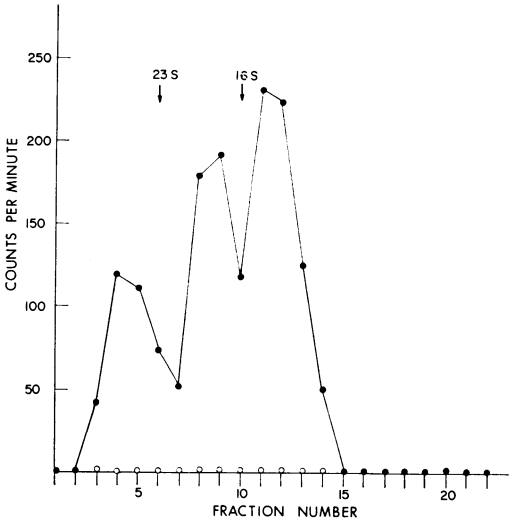

FIG. 3. *Sedimentation analysis of thermally denatured RNA. Purified ^{3}H-labeled reovirus RNA was dialyzed against TE, heated to 95 C for 3 min, and rapidly cooled in a dry ice bath. The denatured RNA was placed on a 4.4-ml sucrose gradient (7 to 20% sucrose) and centrifuged at 39,000 rev/min for 4.5 hr at 12 C in a Spinco SW 39 rotor. Acid-insoluble ^{3}H-labeled RNA, (●) untreated and (○) treated with ribonuclease (1.0 μg/ml, 10 min, 37 C), was measured as described in Materials and Methods.*

The thermal denaturation transition of reovirus RNA was determined by the ribonuclease sensitivity of the heated RNA. The RNA was first dialyzed against TE to reduce the ionic strength of the suspending medium before denaturation experiments. The RNA began to denature at 78 C and was completely denatured at 85 C in TE. The T_m of the thermal transition was 81 C. These results are in agreement with those of Shatkin (13), who determined the thermal transition of reovirus RNA by the hyperchromic effect. Sedimentation analysis of the RNA which had been dialyzed against TE indicated that the double-stranded RNA was stable in the solution of low ionic strength. When reovirus RNA in TE was heated to 75 C, there was no alteration of its rate of sedimentation on a 7 to 20% sucrose gradient. As noted in Materials and Methods, all sucrose gradients contained STE. The RNA was significantly less resistant to increasing concentrations of ribonuclease, however (Table 1). Thus, at 75 C there may be a partial denaturation of the double strands without actual separation of them. When heated to 95 C, the double-stranded RNA was converted to three ribonuclease-sensitive fractions with sedimentation coefficients of 26S, 18S, and 14S (Fig. 3). The molecular weight of these single-stranded RNA molecules would be about 1.45×10^6, 0.67×10^6, and 0.39×10^6, as calculated from $M = 1{,}550\ S^{2.1}$ (14). In morphological studies, Dunnebacke and Kleinschmidt (2) have reported

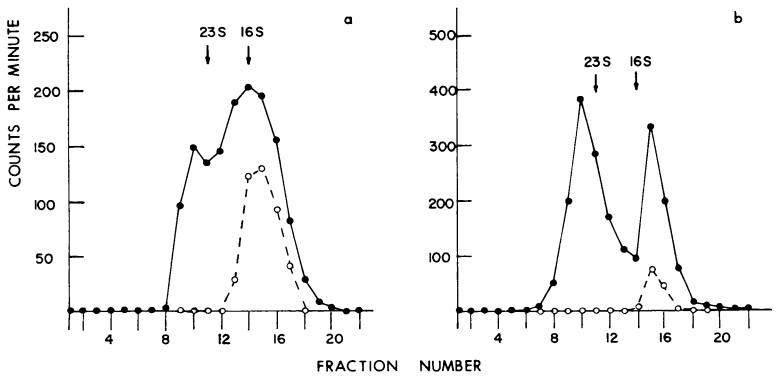

FIG. 4. *Sedimentation analysis of reovirus RNA denatured in DMSO. Purified ^{3}H-labeled RNA was dialyzed against TE and then mixed with DMSO (1:6, v/v). The mixture was incubated at 25 C for (a) 5 min or (b) 10 min, and then quickly cooled in an ice bath. NaCl was added to a final concentration of 0.1 M, and the RNA was precipitated with 2 volumes of ethyl alcohol. The RNA was resuspended in TE, layered in a 4.4-ml 7 to 20% sucrose gradient, and centrifuged at 39,000 rev/min for 3 hr at 12 C in a Spinco SW 39 rotor. Acid-insoluble ^{3}H-labeled RNA, (●) untreated and (○) treated with ribonuclease (1.0 μg/ml, 10 min, 37 C), was measured as described.*

the presence of similar populations of molecules after thermal denaturation in the presence of formaldehyde.

Since the high temperature required for thermal denaturation may cause degradation of the RNA, DMSO was used to denature the RNA. Reovirus RNA can be melted at 25 C in 85.7% DMSO. Incubation of reovirus in DMSO for 5 min produced two RNA fractions having sedimentation coefficients of 26*S* and 15*S* (Fig. 4a). The 26*S* fraction was completely sensitive to ribonuclease and appeared to be the single-stranded denaturation product of 15*S* double-stranded reovirus RNA. Since the 15*S* fraction of Fig. 4a was 65% resistant to ribonuclease, it may have been partially denatured double-stranded 15*S* reovirus RNA. Increasing the time of incubation of reovirus RNA in DMSO to 10 min produced an increased accumulation of the 26*S* ribonuclease-sensitive fraction and an increase to 80% in the sensitivity of the 15*S* fraction to ribonuclease (Fig. 4b). Even after incubating reovirus RNA in DMSO for 30 min at 25 C or increasing the temperature of incubation to 37 C, the partially ribonuclease-resistant 15*S* fraction was still observed, along with the 26*S* ribonuclease-sensitive fraction.

DISCUSSION

Extraction of reovirus RNA with $NaClO_4$, followed by purification on a Cs_2SO_4 equilibrium density gradient, offers a rapid method for the preparation of purified reovirus RNA, a method which completely eliminates the necessity for previous purification of the virus. Other advantages associated with the $NaClO_4$ extraction procedure are: (i) simultaneous concentration of the nucleic acid; (ii) minimization of shearing forces during extraction; (iii) partial purification in the 30% sucrose solution; and (iv) inhibition of ribonuclease by $NaClO_4$. The reovirus preparations concentrated with ammonium sulfate contained low levels of contaminating ribosomal RNA and possibly soluble RNA (Fig. 2). This contaminating RNA is probably removed from the virus RNA preparations by the 30% sucrose band employed in the $NaClO_4$ extraction gradient. The high sedimentation rate of reovirus permits it to penetrate the 30% sucrose band while the molecules of lower sedimentation rate, such as ribosomal and soluble RNA, are retarded. Deoxyribonucleic acid (DNA) is not removed during extraction of the reovirus in the $NaClO_4$ gradient. Reovirus-infected L cells were labeled with ^{3}H-thymidine and then the virus was extracted in $NaClO_4$ as described. A large quantity of ^{3}H-thymidine of this extracted material was located at the top of a Cs_2SO_4 equilibrium density gradient ($\bar{\rho}$ = 1.60).

The reovirus RNA prepared by $NaClO_4$ extraction followed by Cs_2SO_4 equilibrium density gradient centrifugation appears to have the same properties as the RNA prepared by more elaborate methods (6, 13). The RNA is highly resistant to ribonuclease, and has a T_m of 81 C and a buoyant density of 1.61.

The chemical data of Gomatos and Tamm (7) and the electron microscope studies of Granboulan and Niveleau (8) and Dunnebacke and Kleinschmidt (2) suggest that the molecular weight of reovirus RNA is between 10×10^6 and 12×10^6. In the present study, the RNA obtained by $NaClO_4$ extraction had a sedimentation coefficient of 15*S*. According to the calculations of the molecular weight of a DNA molecule which are based upon its sedimentation coefficient (1), a double-stranded nucleic acid molecule with a sedimentation coefficient of 15*S* would be expected to have a molecular weight of 3.14×10^6. According to Gomatos and Stoeckenius (6), the relationship between molecular weight and sedimentation constant for double-stranded DNA leads to values which are too high when applied to the double-stranded RNA of reovirus. Franklin (3) has also shown that the molecular weight of a double-stranded RNA molecule (RF of bacteriophage R17) is slightly less than that predicted for a DNA molecule with the same sedimentation coefficient. Therefore, we estimate the molecular weight of the 15*S* reovirus RNA to be about 2.5×10^6 to 3.1×10^6. Thus, the reovirus RNA extracted with $NaClO_4$ may be quarter molecules. The 15*S* reovirus RNA has also been found by other investigators using other methods of extraction of the RNA (10, 12, 13). The appearance of 15*S* Reovirus RNA might be due to shearing forces involved during preparation. The procedure involving the $NaClO_4$-sucrose gradient should, however, eliminate shear, since the molecule is not physically manipulated from the time it is extracted from its protein coat to the time its sedimentation coefficient is determined. The resulting RNA has, however, a sedimentation coefficient of 15*S*.

Electron microscope studies (2, 8) have eliminated the possibility that fragmentation of reovirus RNA results from a configurational stress caused by the removal of the protein coat of the virus. The possibility still exists, however, that fragmentation of the RNA molecule may be caused by the chemical agents employed to extract the RNA. Even though bacteriophage R17 replicative form and *Escherichia coli* ribosomal RNA are not degraded by $NaClO_4$, the possibility remains that reovirus RNA may contain bonds which are attacked by $NaClO_4$. Treatment of reovirus with 5 M $NaClO_4$ for time

intervals ranging from several seconds to 4 hr always resulted in the appearance of 15S RNA. Also, treatment of reovirus with $NaClO_4$ concentrations ranging from 0.025 to 5 M always resulted in the appearance of 15S RNA fragments. It would appear that, after the cleavage of these hypothetical $NaClO_4$-sensitive bonds, the remainder of the molecule is stable in $NaClO_4$. The presence of $NaClO_4$-sensitive bonds is also suggested by the morphological studies of Dunnebacke and Kleinschmidt (2), who reported a reduced number of long RNA filaments following prolonged exposure to $NaClO_4$. If $NaClO_4$-sensitive bonds do exist in reovirus RNA, they must be located in a nonrandom manner along the length of the molecules. The 15S fragments resulting from extraction with $NaClO_4$ suggest three sensitive bonds per RNA molecule, located at quarter-length intervals along the molecule.

Denaturation of reovirus RNA produces either two or three ribonuclease-sensitive fractions, depending on the method of denaturation. Single-stranded 26S RNA is found after both thermal denaturation and denaturation in DMSO. The 26S RNA product would have a molecular weight of 1.45×10^6 based on the relation $M = 1{,}550\ (S_{20,w})^{2.1}$ (14). These would presumably be derived from 15S double strands containing uninterrupted chains, i.e., $2 \times 1.45 \times 10^6 = 2.9 \times 10^6$. The 18S and 14S ribonuclease-sensitive fractions resulting from thermal denaturation are probably degradation products of the 26S RNA. Thermal degradation of RNA usually results in a heterogeneous population of molecules, producing an extremely broad band in a sucrose gradient. However, the 26S RNA appears to degrade to form the distinct 18S and 14S fractions. This degradation pattern may suggest specific thermal sensitive bonds within the 26S RNA molecule. Denaturation at 25 C in the presence of DMSO does not produce the 18S or 14S fractions which are observed when denaturation occurs at 95 C. Denaturation in DMSO does produce a completely ribonuclease-sensitive 26S RNA fraction and a partially ribonuclease-sensitive 15S fraction. The 26S fraction is the single-stranded denaturation product of double-stranded reovirus RNA. The ribonuclease-sensitive 15S fraction is probably partially denatured double-stranded RNA. The strands of the partially denatured RNA may be held together by hydrogen bonding between complementary base pairs at intervals along the length of the molecule. Such a molecule might result from an incomplete denaturation of the complementary strands or a partial renaturation of the complementary strands of the double-stranded reovirus RNA molecule. It appears that the 15S double-stranded RNA molecule is composed of two uninterrupted chains.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-07645 from the National Institute of Allergy and Infectious Diseases and by grant GB 5365 from the National Science Foundation.

We are indebted to Yvonne Edwards for her able assistance.

LITERATURE CITED

1. BURGI, E., AND A. D. HERSHEY. 1963. Sedimentation rate as a measure of molecular weight of DNA. Biophys. J. **3**:309–321.
2. DUNNEBACKE, T., AND A. KLEINSCHMIDT. 1966. Ribonucleic acid from reovirus as seen in protein monolayers by electron microscopy. Z. Naturforsch., *in press*.
3. FRANKLIN, R. M. 1967. Replication of bacteriophage ribonucleic acid: some physical properties of single-stranded, double-stranded, and branched viral ribonucleic acid. J. Virol. **1**:64–73.
4. FREIFELDER, D. 1965. A rapid technique for the preparation of purified bacteriophage DNA or RNA from crude lysates. Biochim. Biophys. Acta **108**:318–319.
5. FREIFELDER, D. 1966. Effect of $NaClO_4$ on bacteriophage: release of DNA and evidence for population heterogeneity. Virology **28**:742–750.
6. GOMATOS, P. J., AND W. STOECKENIUS. 1964. Electron microscope studies on Reovirus RNA. Proc. Natl. Acad. Sci. U.S. **52**:1449–1455.
7. GOMATOS, P. J., AND I. TAMM. 1963. The secondary structure of reovirus RNA. Proc. Natl. Acad. Sci. U.S. **49**:707–714.
8. GRANBOULAN, N., AND A. NIVELEAU. 1966. Étude au microscope électronique du RNA du Reovirus. J. Microscopie, *in press*.
9. KATZ, L., AND S. PENMAN. 1966. The solvent denaturation of double stranded RNA from Poliovirus infected HeLa cells. Biochem. Biophys. Res. Commun. **23**:557–566.
10. KUDO, H., AND A. F. GRAHAM. 1965. Synthesis of reovirus ribonucleic acid in L cells. J. Bacteriol. **90**:936–945.
11. LERNER, M. 1964. Concentration and purification of viruses with special reference to reovirus. Bacteriol. Rev. **28**:391–396.
12. LOH, P. C., AND M. SOERGEL. 1965. Growth characteristics of reovirus type 2: actinomycin D and the synthesis of viral RNA. Proc. Natl. Acad. Sci. U.S. **54**:857–863.
13. SHATKIN, A. J. 1965. Inactivity of purified Reovirus RNA as a template for E. coli polymerase in vitro. Proc. Natl. Acad. Sci. U.S. **54**:1721–1728.
14. SPIRIN, A. S. 1963. Some problems concerning the macromolecular structure of ribonucleic acids, p. 301–345. *In* J. N. Davidson and W. E. Cohn [ed.], Progress in nucleic acid research, vol. 1. Academic Press, Inc., New York.