Characterization of a Mumps Virus Nucleocapsidlike Particle

Robert Cox,1 Todd J. Green,1 Shihong Qiu,1 Jungsoon Kang,1 Jun Tsao,1 Peter E. Prevelige,1 Biao He,2 and Ming Luo1*

Department of Microbiology, University of Alabama at Birmingham, 1025 18th Street South, Birmingham, Alabama 35294,1 and Department of Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, Pennsylvania 168022

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and the inner diameter is 5.6 nm. The thickness of the ring is 6.7 nm. The shape of the ring, with one end narrower than the other, is similar to the shape of rings of other nucleocapsidlike particles. This number of subunits, 13, is consistent with that of the twist in the nucleocapsid of measles virus, another member of paramyxovirus family; the diameter of the ring and its hollow center are consistent with those of the nucleocapsid of measles virus (3).

To confirm the size determined with the averaged EM image, the sedimentation coefficient ($s_{20,w}$) was determined to be 32.191 S by ultracentrifugation by using the programs Sednterp and Sedfit (13, 18) (Fig. 2C). The molecular mass calculated from the sedimentation coefficient is 853,000 Da. This molecular mass is within the experimental error when it is compared to the calculated molecular mass of a 13-subunit MuV NP-RNA ring (830,960 Da), assuming that the molecular mass of a MuV NP subunit is 62 kDa and that of six nucleotides per subunit is 1.92 kDa.

FIG. 1. MuV NP and P proteins were coexpressed in E. coli and purified as described in the text. (A) Lanes 1 to 4 represent total soluble proteins postinduction, in the Ni column flowthrough, in the binding buffer wash, and in the washing buffer wash of the Ni column, respectively. Lane 5 shows a fraction containing the NP-P complex eluted by the linear imidazole gradient from the Ni affinity column. (B) The washing buffer wash contained a large amount of NP protein. It was dialyzed to 200 mM NaCl and further purified on a HiTrap Q HP (GE Healthcare) ion-exchange column. Lanes 1 to 5 contain the MuV NP-RNA complex. (C) Fractions containing the NP-RNA complex from the ion-exchange column were further purified by size exclusion chromatography. Lanes 1 and 2 represent the molecular weight markers (in thousands) and fractions containing the MuV NP-RNA complex, respectively. (D) Profile of MuV NP-RNA eluting from the ion-exchange column. The largest peak represents the NP-RNA complex. (E) Profile of MuV NP-RNA eluting from the size exclusion column. The single large peak represents the NP protein.

Similar structures of other recombinant NSRV NP protein complexes often contain encapsidated RNA. We measured the ratio of the optical density at 260 nm to that at 280 nm for the MuV NP protein complex and found the value to be 1.26, indicating that the MuV NP protein complex has encapsidated RNA. To characterize the encapsidated RNA, materials extracted with Trizol (Invitrogen) from the purified NP protein complex were examined by electrophoresis under denaturing conditions (Fig. 3A, lane 4). The gel clearly showed that RNA is present in the complex. However, the length of RNA is more variable than what has been observed for the VSV, RABV, or RSV NP-RNA complexes (6, 9, 19). Assuming that each NP subunit accommodates six nucleotides, the RNA in a fully occupied 13-subunit ring of the MuV NP-RNA complex should have a length of 78 nucleotides. In fact, a dominant RNA band corresponding to this length was shown in the RNA gel (Fig. 3A, lane 4, band I). Additionally, a large number of RNA pieces smaller than band I were observed (Fig. 3A). The ap-
appearance of a dominant band, band II, which was actually part of a population of small RNA pieces, could be an artifact of ethidium bromide or SYBR gold (Invitrogen) staining. This observation suggests that the RNA piece encapsidated by the MuV NP protein during heterologous expression was not fully protected during purification, which is supported by the observation that the RNA in the MuV N-RNA complex is susceptible to digestion when incubated with RNase A for 1 h at 37°C (Fig. 3A, lane 6).

In order to determine the effect of pH, salt, and temperature on the susceptibility of NP-bound RNA, the MuV NP-RNA ring was incubated under a variety of different conditions. The
conditions examined included pH 4, pH 4.5, pH 5, pH 5.5, pH 6.0, pH 6.5, pH 7.5, pH 9, pH 11, 1 M NaCl, 2 M NaCl, 42°C, and 60°C. The NP-RNA ring was incubated for 1 h under each condition and then returned to normal buffer conditions (50 mM Tris [pH 7.5], 470 mM NaCl). After incubation at a pH lower than 5, all RNA pieces were lost. This level of RNA protection is significantly less than that of the VSV NP-RNA ring (6). At a pH above 6, no further loss of RNA was observed, which is similar to findings with the VSV and RABV NP-RNA rings (6, 9). When the MuV NP-RNA ring was subjected to 1 M NaCl, the RNA was also lost, similar to the situation when the nucleocapsid of influenza A virus was treated with high salt concentrations (8). The RNA in the VSV or RABV NP-RNA complex was not

FIG. 4. EMs of the MuV NP-RNA complex were negatively stained with 2% uranyl acetate (magnification, ×42,000). (A) The MuV NP-RNA complex incubated in 50 mM Tris pH 8.3 plus 1 M NaCl; (B) the MuV NP-RNA complex as a precipitant in 0.1 M citric acid-sodium citrate (pH 4) plus 470 mM NaCl; (C) the MuV NP-RNA complex incubated at pH 4 and resolubilized by increasing the pH back to approximately 7.5. The ring structures in panels A, B, and C were comparable to that of the untreated MuV NP-RNA ring (Fig. 2A) after the RNA was lost. These ring structures could be considered “empty-capsid”-like structures. The scale bar in the bottom right corner of each micrograph corresponds to 100 nm.

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affected by high salt concentrations (6, 9). Finally, the RNA was lost when the MuV NP-RNA ring was heated to 60°C for 1 hour. No information on heat treatment of other NSRV NP-RNA rings was available at the time this study was done. The loss of RNA at a pH of less than 5.0 or under high-salt or high-temperature conditions suggested that associations between the MuV NP subunits may be weaker than between the NP subunits of other NSRVs.

EM images were taken of samples that had been subjected to different conditions as described above (Fig. 4). These images (Fig. 4A and B) showed that the loss of RNA from the MuV NP-RNA ring after various treatments was not due to denaturation of the NP protein. The ring structures remained intact after RNA was lost and looked like untreated MuV NP-RNA rings. These ring structures could be considered "empty-capsid"-like structures. One interesting observation is that the MuV empty-capsid-like rings formed rodlike structures after being subjected to pH 4.0 and then reneutralized (Fig. 4C). It seems that the MuV rings could easily make top-and-bottom interactions as would be present in the superhelical structure of the paramyxovirus nucleocapsid (4, 17).

Several NSRV nucleocapsidlike rings have recently been described (2, 7, 9). Coexpression of the MuV NP and P proteins in E. coli has resulted in the production of nucleocapsidlike rings with morphologies similar to those seen in other NSRVs. The MuV NP-RNA ring contains 13 NP subunits and contains RNA. There are multiple sizes of RNA contained within the NP ring, which is not seen with other NSRVs such as the NP-RNA ring of VSV, suggesting that the RNA in the MuV NP ring is less protected (6). The study of the MuV nucleocapsidlike particle will lead to further understanding of the unique features of the paramyxovirus nucleocapsid.

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REFERENCES


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