RNA Binding Properties of Bunyamwera Virus Nucleocapsid Protein and Selective Binding to an Element in the 5′ Terminus of the Negative-Sense S Segment

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The genome of Bunyamwera virus (BUN) (family Bunyaviridae, genus Bunavirus) comprises three negative-sense RNA segments which act as transcriptional templates for the viral polymerase only when encapsidated by the nucleocapsid protein (N). Previous studies have suggested that the encapsidation signal may reside within the 5′ terminus of each segment. The BUN N protein was expressed as a 6-histidine-tagged fusion protein in Escherichia coli and purified by metal chelate chromatography. An RNA probe containing the 5′-terminal 32 and 3′-terminal 33 bases of the BUN S (small) genome segment was used to investigate binding by the N protein in vitro using gel mobility shift and filter binding assays. On acrylamide gels a number of discrete RNA-N complexes were resolved, and analysis of filter binding data indicated a degree of cooperativity in N protein binding. RNA-N complexes were resistant to digestion with up to 1 μg of RNase A per ml. Competition assays with a variety of viral and nonviral RNAs identified a region within the 5′ terminus of the BUN S segment for which N had a high preference for binding. This site may constitute the signal for initiation of encapsidation by N.

Bunyamwera virus (BUN) is the prototype of the genus Bunavirus and the family Bunyaviridae and possesses a single-stranded negative-sense tripartite RNA genome. The three RNA segments, termed L (large), M (medium), and S (small), encode six proteins. The L segment codes for the L protein, the viral RNA-dependent RNA polymerase, which is responsible for both transcribing and replicating the genome RNAs. The M segment encodes the two virion glycoproteins, G1 and G2, and a nonstructural protein, NSm, as a polyprotein which is probably cotranslationally cleaved by host proteases. The S segment encodes the nucleocapsid (N) protein and, in an overlapping reading frame, a second nonstructural protein called NSs (reviewed in reference 8). In common with other negative-sense RNA viruses, the bunyavirus genome RNA segments are replicated via full-length cRNAs termed antigenomes. Both the negative-sense genome and positive-sense antigenome RNAs are encapsidated by the N protein and are associated with the viral polymerase in ribonucleoprotein complexes called nucleocapsids. It is only within the nucleocapsid that the RNA is transcriptionally active. Bunyavirus genome and antigenome RNAs contain highly conserved, complementary terminal sequences that may form panhandle structures in vivo and are probably responsible for the circular appearance of isolated nucleocapsids (19, 20, 22, 26, 28).

Full-length genome and antigenome segments are usually the only RNAs that are encapsidated in the infected cell. Viral mRNAs, which are not encapsidated, are truncated at the 3′ end and contain a nontemplated capped primer on the 5′ terminus (2, 4, 9, 14, 21). It is therefore likely that the terminal sequences of the genome and antigenome RNAs are involved in the encapsidation process. This theory is supported by the observation that an antisense chloramphenicol acetyltrans-
Sall sites of pQE30 (Qiagen). Recombinant N was expressed as an N-terminally 6-histidine-tagged protein in Escherichia coli strain M15 by induction with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The bacteria were lysed by freeze-thawing and sonication, and N protein was purified under native conditions (23) by Ni-nitrilotriacetic acid (NTA) column chromatography (Bio-Rad Ecosys- tem). Protein immobilized on the Ni-NTA column was washed with 50 mM sodium phosphate buffer (pH 6.0) containing 300 mM NaCl and 10% glycerol, and N was eluted with a linear gradient of 0 to 500 mM imidazole in wash buffer. Fractions containing large amounts of N were eluted at around 250 mM imida- zole, pooled, and dialyzed at 4°C against 10 mM Tris-HCl (pH 8.0). The dialyzed protein was concentrated using a Vivaspin concentrator (molecular weight cutoff, 10,000) (Vivascience) and stored at 4°C.

RNA transcription plasmids. pT7BUNSS(32/3)(33) contains cDNA to the precise 5′-terminus plus 33 and 33 3′-terminal bases of the negative-sense segment, linked by a 5-base sequence which creates a SmaI site, under control of a T7 promoter in pUC19. BUNS5(32/3)(33) RNA was transcribed following linearization with BstNI, and BUNS5(32/3) RNA was transcribed following linearization with SmaI.

pT7BUNSS(32/3)(33) contains cDNA to the precise 33 3′-terminal bases of the negative-sense BUN S segment under control of a T7 promoter and was constructed by inserting a 63-bp SmaI-BglII fragment from pT7BUNS(32/3)(33) into pT7Z12. pT7BUNS(32/3)(33) RNA was transcribed following linearization of the plasmid with BstNI.

pT7BUNSCAT contains a negative-sense chloramphenicol acetyltransferase gene flanked by the entire untranslated regions of the BUN S segment in pUC19 (7). BUNS5(65) RNA was transcribed following linearization of this plasmid with FflI, and BUNS5(135) RNA was transcribed following linearization with Tsp509I.

The template for transcription of BUNS5(32/3) RNA was generated by annealing two oligonucleotides, 5′-CTAATAGCAGCTACATATAATAT (modified T7 promoter) and 5′-CTAAAATCATTATTTATAGTATTTAATATATAGTGATCTATTTAG, representing bases 23 to 56 of the BUN S 5′ terminus. ORF(1+) RNA was generated from a PCR product produced by amplification of the ORF1F from nucleotides 577 to 800 of the cDNA, to which a T7 promoter was incorporated, using primers 5′-CTAAATAGCAGCATACTATATAATATATAGTGATCTATTTAG and 5′-CTGAATTCGTAGTGATCTCAGAGG and 5′-CTGCGGATCCATCATATGTTTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT
RNA binding by N protein. The terminal sequences of each of the three bunyavirus genome segments have been implicated in encapsidation and are proposed to contain the site for nucleocapsid assembly (25). A 69-base riboprobe designated BUNSS(32)/3(33) was generated to investigate whether the recombinant N protein would bind the S-segment termini. This RNA corresponds to the exact terminal 32 bases of the 5′ end and 33 bases of the 3′ end of the genome-sense S segment (Fig. 1). Samples (100 pg) of radiolabeled BUNSS(32)/3(33) riboprobe were incubated with increasing concentrations of N under reaction conditions based on those described by Götte et al. (11) and then analyzed by polyacrylamide and agarose gel electrophoretic mobility shift assays (GEMSA), filter binding assays, and Northwestern blotting. When GEMSA was performed using an acrylamide gel (Fig. 3), at low concentrations of N a small proportion of riboprobe was shifted into a single band of higher molecular mass. At higher concentrations of N the riboprobe was shifted into multiple higher complexes, forming a ladder-like profile, which suggests sequential filling of binding sites on the RNA (3). The multiple bands are interpreted as the result of riboprobe being bound by discrete numbers of N molecules. Further addition of protein resulted in saturation of the riboprobe, which was shifted toward the top of the gel. To investigate the saturated complexes further, identical reactions were analyzed by agarose GEMSA (Fig. 4). In this assay the saturated complexes ran into the gel and could be seen to reach a finite maximum size. To confirm that the mobility shifts of the riboprobe were due to interaction with N, the protein was shown to bind BUNSS(32)/3(33) RNA directly by Northwestern blot analysis (Fig. 2, lane 5). No binding of the riboprobe was observed in a mock expression lane in which bacteria containing the empty parent vector had been subjected to an identical induction and purification regime (lane 6), suggesting that the shifts observed in the GEMSA were not attributable to interaction with a native bacterial protein. When N was replaced by bovine serum albumin in binding reactions, no shift of the riboprobe was observed, indicating that the mobility shift was specific for the N protein (Fig. 4).

Effect of ionic concentration on binding. Binding reaction mixtures were assembled in the presence of different concentrations of NaCl (0.15 to 2 M) and of MgCl₂ (0 to 20 mM), and the products were analyzed by agarose GEMSA (Fig. 5). Complex formation was not impaired by up to 0.5 M NaCl; at higher salt concentrations there was no evidence of dissociation of the complex, indicated by the lack of free riboprobe, but radioactivity was smeared further up the gel. We assume that this is an electrophoresis artifact caused by the high salt concentration in the sample. Binding of N to the riboprobe was unaffected at high concentrations of N, the riboprobe was shifted toward the top of the gel.
Complexes are resistant to RNase. The RNA in bunyavirus nucleocapsids is relatively resistant to digestion by “reasonable concentrations” (12, 16) of RNase A but is digested by 100 µg of RNase A per ml (19). To investigate whether in vitro-assembled N-RNA complexes were resistant to RNase A, BUNSS'(32)/3'(33) riboprobe was incubated in binding reactions either with or without N and then different amounts of RNase A were added and incubation was continued for 10 min at 37°C. All reaction mixtures contained 20 U of RNasin (Promega), which is needed to inhibit any RNases present in the N protein stock (and thus would otherwise digest the RNA before N was able to bind); this amount of RNasin was considered to exert a nominal effect on the amount of RNase A added (1 U of RNasin inhibits 5 ng of RNase A by 50%; Promega). After phenol extraction the RNAs were analyzed by electrophoresis on a denaturing, sequencing-type polyacrylamide gel. As shown in Fig. 6, RNA complexed with N was resistant to 1 µg of RNase A per ml whereas the naked riboprobe was almost fully digested at this concentration. Neither complexed nor naked riboprobe was resistant to 2.5 µg of RNase A per ml or higher concentrations.

**Kinetics of N binding.** The kinetics of binding of N to BUNSS'(32)/3'(33) RNA was measured by filter binding assays with a wider range of concentrations of N than that used in GEMSA. Binding-reaction mixtures containing 100 pg of radiolabeled riboprobe were passed through nitrocellulose membranes under vacuum. Whereas free riboprobe passed through the membrane, riboprobe complexed with N was immobilized on it. The degree of binding could thus be measured as the proportion of radiolabeled RNA retained on the membrane by its association with N (Fig. 7a). Maximal binding was obtained with 180 ng of N in the reaction, equivalent to a molar ratio of 1:1,500 (RNA to protein). The dissociation constant ($K_d$), which was calculated as half-maximum binding, was approximately $7 \times 10^{-8}$ M. Analysis of binding kinetics using a Hill plot provides a mathematical calculation of the degree of cooperativity in the binding event (3). The gradient of the resulting line serves as a measure of the number of sites that are bound cooperatively. Analysis of the binding data in Fig. 7a by this technique gave the results shown in Fig. 7c and yielded a gradient of approximately 1.2, indicating that the binding event showed a degree of cooperativity (3).

**Competitive binding assays.** The encapsidation signal for bunyavirus genome and antigenome segments is proposed to reside within the 5'-terminal sequences (12, 16, 25). We used a panel of RNAs in competitive filter binding assays to investigate the presence of such a signal. Binding-reaction mixtures were assembled containing radiolabeled BUNSS'(32)/3'(33) riboprobe in the presence of a 1,000-fold mass excess of unlabeled competitor RNAs, which were mixed prior to the addition of N (Fig. 8). These experiments would therefore provide a measure of the degree of selectivity of N for the competitor N.
RNA over BUN5(32)/3(33) RNA. The results were expressed as the percentage of competition shown by the competitor RNA for binding to N; a low value indicates that most of N is binding the riboprobe, and a high value indicates that there is competition for binding by the unlabeled RNA. To ensure that any competition observed was not due to loss of labeled riboprobe through RNase degradation, the competitor RNAs were tested for RNase contamination by incubation with the riboprobe, under binding conditions, followed by electrophoresis on a denaturing polyacrylamide gel and autoradiography. No evidence of RNase degradation of the riboprobe was observed (data not shown).

A 1,000-fold molar excess of the homologous RNA, BUN5(32)/3(33), gave about 50% competition, whereas yeast RNA competed to less than 20% (Fig. 8). Higher levels of competition were observed with competitor RNAs containing the 5' end of the BUN S segment, BUN5(32) and BUN5(135) RNAs, comprising the terminal 32 and 135 bases, respectively, reduced retention of riboprobe by about 75 to 80%. BUN5(65) RNA consists of the 5' terminal 65 bases of the BUN negative-sense S segment and is thus a similar length to BUN5(32)/3(33). In the presence of a 1,000-fold excess of this competitor, the proportion of riboprobe retained dropped by approximately 97%, indicating a high degree of competition. However, an RNA comprising an internal region of the 5' end, BUN5 SL2 (bases 23 to 56), showed only 20% competition.

The binding kinetics of N to BUN5(65) RNA were measured by a filter binding assay (Fig. 7b) and shown to be similar to those of BUN5(32)/3(33). Analysis of the binding data with BUN5(65) RNA yielded a similar Hill plot to that of BUN5(32)/3(33) RNA (Fig. 7c).

BUN5(33/22) consists of the 33 terminal bases at the 3' end of the S segment and some vector sequences, and competed to a low level similar to that of yeast RNA. ORF(−) and ORF(+) RNAs comprise 87-base transcripts, genome and antigenome sense, respectively, representing a region of the N ORF which encodes a highly conserved domain in the N protein. Neither of these RNAs competed more than yeast RNA. dsORF RNA was generated by annealing the two single-stranded ORF transcripts and was used to compare the preference of N for dsRNA and ssRNA. dsORF RNA competed only slightly more than the ssRNAs and did not reach the high levels shown by competitors containing 5' terminal sequences.

**DISCUSSION**

We have described experiments to study the RNA binding properties of BUN nucleocapsid protein in vitro. Recombinant BUN N protein was expressed as a His-tagged protein in bacteria and purified by nickel affinity chromatography. N was reactive to antiserum raised against purified BUN and demonstrated binding activity for RNA containing the terminal sequences of the negative-sense S RNA segment, with a dissociation constant of $7 \times 10^{-8}$ M, similar to the value reported for hantavirus N protein (29). RNA binding reactions in the presence of increasing concentrations of N, followed by polyacrylamide GEMSA, indicated the presence of discrete complexes until the RNA was fully encapsidated. Although the observed pattern might represent binding of RNA to misfolded N protein, we think this unlikely since multiple bands of appropriate sizes are seen on Western blots (Fig. 2), suggesting that N is capable of homo-oligomerizing. Analysis of the filter binding assay data by a Hill plot (Fig. 7) indicated a low (non-simultaneous) degree of cooperativity in the binding of N to the BUN5(32)/3(33) RNA. Richmond et al. (27) reported RNA-binding by recombinant tomato spotted wilt virus N to be a cooperative event, as did Gött et al. (11) for analysis of bacterially expressed hantavirus N protein binding, although neither group presented corroboratory data, for example in the form of a Hill plot.

RNA-N protein complexes showed a finite maximum size when analyzed on an agarose gel (Fig. 4). We interpret this as indicating that N binds along the RNA as opposed to the RNA associating with preformed multimers of N, whose size would be proportional to the concentration of N in the reaction mixture. The RNA-N complexes formed in the binding reactions were shown to be resistant to a level of RNase A digestion similar to that of authentic nucleocapsids, indicating that they possess similar properties.

We observed a discrepancy between the $K_d$ calculated from filter binding data and the equivalent degree of binding in GEMSA: the concentration of N required for 50% maximum binding in the filter binding assay was lower than that required in the gel shift assay. Since this was a consistent observation, we assume that it is because the N-RNA complexes were less stable during electrophoresis, particularly through a polyacrylamide gel. Filter binding was therefore the preferred method for competitive binding assays to investigate the selectivity of binding by N. Interpretation of the results was complicated by the finding that even in the presence of a 1,000-fold excess of homologous unlabeled competitor, the retention of BUN5(32)/3(33) riboprobe was decreased by only 50%. The unexpected lower level of competition observed with the homologous RNA may be due to interactions between the 5' and 3' termini of BUN5(32)/3(33) such that the labeled RNA is able to base-pair with unlabeled homologous RNA to form multimers, thereby reducing the sensitivity of the assay. This phenomenon would occur when the RNA possesses both termini on the same strand.

We were able to identify the first 32 bases of the 5' negative-sense S segment as a region for which N possesses a preference over RNAs lacking this region. Analyses of the RNAs containing 5'-terminal sequences by the Mfold program (18, 35) predict the presence of a stem-loop structure, designated I, near the 5' terminus (Fig. 9). It is possible that the observed preference for binding is provided by this putative stem-loop,
which might act as a signal for N to begin binding the segment RNA. BUNSS5(65) is predicted to form a second stem-loop, II (Fig. 9a), and stem-loops similar to stem-loop II are predicted in the first 65 bases of the 5’ negative-sense termini of the other BUN segments (data not shown). However, RNA comprising just stem-loop II (BUNSS5SL2 RNA) competed similarly to yeast RNA and is therefore not the sole contributor of the high degree of competition seen with BUNSS5(65). In addition, Mfold does not predict that BUNSS5(135) contains stem-loop II, although stem loop I is still predicted (data not shown). BUNSS3(33/22), an RNA possessing the 3’ terminus but lacking the 5’ terminus, did not demonstrate a greater degree of competition than yeast RNA, which suggests that the 3’ terminus does not contribute significantly to the selectivity observed for BUNSS5(32)/3’(33). The preference of N for the 5’ terminus is unlikely to be due to N binding dsRNA nonspecifically, since N did not possess a high preference for ORF dsRNA. Stem-loop I in the 5’ terminus might also have implications for the structure of the putative panhandle involving the ends of the RNA segment, particularly if this structure was retained after N binds, because loop I resembles the hook structures that have been proposed in the 5’ termini of influenza virus and Thogoto virus RNAs (10, 17, 31).

Normally only full-length genome and antigenome segments are encapsidated in bunyavirus-infected cells, and hence a mechanism must exist to distinguish between these and viral mRNAs (or indeed cellular RNAs); it has been proposed that the presence of the additional primer-derived bases at the 5’ termini of viral mRNAs plays a role (25). We suggest that such additional bases may affect the secondary structure in the 5’ terminus, and experiments to test this hypothesis are under way. La Crosse bunyavirus N protein is reported to bind S segment-derived mRNA when N is present at high concentrations in infected cells, providing a feedback mechanism to regulate its own concentration (12), and this suggests that although authentic N is capable of binding RNAs other than genomes and antigenomes, it can only do so when present at high concentration. Competitive-binding experiments reported above suggest that BUN N is also capable of binding nonviral RNAs since yeast RNA could compete to a low but reproducible degree (Fig. 8), which is presumably not sequence specific. Together, our observations may indicate two types of binding by N protein to RNA, selective or preferential binding to the 5’-terminal region and nonspecific binding. Other proteins have exhibited characteristics similar to those observed with BUN N. Heterogeneous nuclear ribonucleoproteins are capable of both preferential binding to certain sequences and less sequence-specific binding at high concentrations, probably serving to hinder the formation of secondary structure in RNA (5, 6). Many positive-sense RNA virus core or coat proteins often possess the ability to selectively bind specific sequences (13, 15, 32, 34), sometimes binding hairpins in the 3’ terminus, as well as binding RNA nonspecifically. Preferential binding to an encapsidation signal has also been observed for rabies virus (33) and human immunodeficiency virus (1).

FIG. 9. Predicted secondary structure of the 5’ terminus of BUN S segment RNA. Secondary structures of BUNSS5(65) (a) and BUNSS5(32) RNA (b) were predicted using Mfold (18, 35). The most energetically favorable structures are shown, and stem-loops I and II are indicated.

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