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Biochemical Characterization of Rift Valley Fever Virus[1]

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Rift Valley fever virus (RVFV) polypeptides were shown to share similar biochemical properties with members of the family Bunyaviradae. Electrophoretic analysis of RVFV revealed one nonglycosylated and two glycosylated major proteins with molecular weights of 25,000, 56,000, and 65,000, respectively. In addition, a 100,000 MW glycoprotein was found. The 25,000 MW protein was identified as the major nucleocapsid protein. The virion density in CsCl was 1.21 g/ml, while that of the nucleocapsid was 1.29 g/ml. The number and molecular weights of major structural polypeptides of several diverse RVFV isolates were identical. The presence of three RNA segments, large, medium, and small, with molecular weights of 2.7, 1.7, and 0.6×10^6, was demonstrated. The close relationships of RVFV proteins and RNA with reported molecular weights of some members of the Phlebotomus fever (PHL) group viruses were compatible with the serological cross-reactivity among these viruses. These findings support the classification of RVFV with the PHL group viruses in the family Bunyaviradae.

Rift Valley fever (RVF) is an acute arthropod-borne viral disease of many species, primarily sheep, cattle, and man (*1–4*). In 1975, severe clinical disease with human fatalities was observed (*5*). Two years later extensive animal and human disease with numerous fatalities was reported in the Nile Delta (*6*). The causative agent of this epizootic, Rift Valley fever virus (RVFV) has been shown to be morphologically similar to the bunyaviruses (*7, 8*) and is classified as a member of the family Bunyaviradae (*9*). Biochemically, this family is characterized by single-stranded RNA composed of three unique segments, a nucleocapsid protein associated with the RNA and an evelope, which contains at least one virus-specific glycopeptide (*10*). Although previously reported to be serologically distinct from all other viruses (*4*), RVFV was recently found to cross-react by hemagglutination-inhibition tests with viruses of the Phlebotomus fever (PHL) group (*11*).

This report provides the initial biochemical and biophysical characterization of RVFV and supports the classification of this virus as a member of the family of Bunyaviridae.

Radiolabeled virus preparations were obtained in the following manner. Viruses were adsorbed at a multiplicity of 0.001 plaque-forming unit (PFU) per cell to BHK-21 cells for 1 hr. RVFV was labeled by growing the virus in the presence of Earle's Medium 199 (GIBCO, Grand Island, N. Y.) with 2% heat-inactivated and dialyzed fetal bovine serum containing one or a combination of the following: ^{3}H-amino acids, [^{3}H]uridine, L-[^{35}S]methionine, [^{3}H]glucosamine, or [^{32}P]orthophosphate. Each radioisotope (New England Nuclear, Boston, Mass.) was added at a concentration of 10 μCi/ml. Following maximal cytopathic effect (44–56 hr), supernatants were clarified by centrifugation at 10,000 *g* for 20 min at 4° and virus was pelleted in a Beckman 21 fixed-angle rotor at 59,000 *g* for 2 hr at

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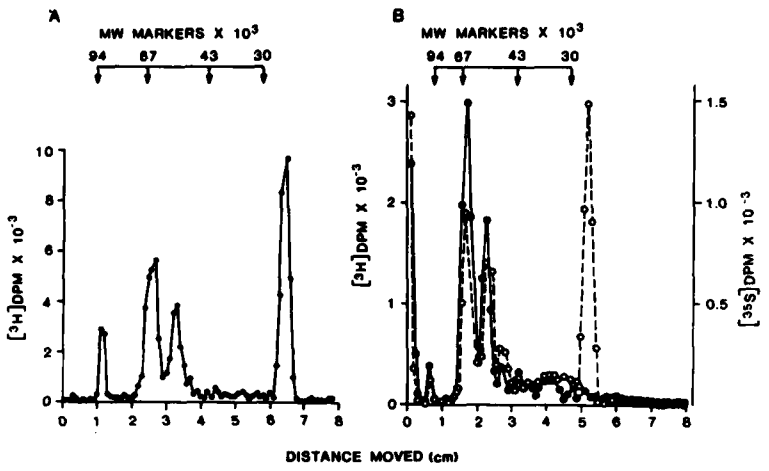

FIG. 1. Analysis of RVFV polypeptides by 12% SDS–PAGE. Labeled SA-51 strain was prepared and analyzed by methods described in the text. Presented are profiles of (A) ^{3}H-amino acid-labeled and (B) [^{3}H]glucosamine- (●) and L-[^{35}S]methionine-labeled (○) virion polypeptides. Unlabeled reference proteins, phosphorylase B, bovine serum albumin, ovalbumin, and carbonic anhydrase having molecular weights of 94,000, 67,000, 43,000, and 30,000, respectively, are located in the positions indicated. Molecular weights of viral polypeptides were determined from reference proteins electrophoresed in parallel gels. Positions of reference proteins were detected by Coomassie blue staining (*15*).

4°. Pellets were resuspended in TNE (0.01 *M* Tris, 0.15 *M* NaCl, and 0.002 *M* EDTA, pH 7.4) and layered onto a continuous 15–40% (w/v) Renografin (E. R. Squibb and Sons, Princeton, N. J.)–TNE gradient and centrifuged at 280,000 *g* in a SW 41 rotor for 4 hr at 4°. Virus was harvested, diluted in TNE, and pelleted at 380,000 *g* in a SW 50.1 rotor for 45 min at 4°. Pellets were resuspended in 100 μl of TNE. Viral polypeptides were dissociated and resolved by discontinuous SDS–polyacrylamide gel electrophoresis (PAGE) on 12% (w/v) gels at 3 mA per gel, as described by Laemmli (*12*). Protein molecular weight markers and gel electrophoresis reagents were obtained from Pharmacia, Inc. (Piscataway, N. J.) and Bio-Rad Laboratories (Richmond, Calif.), respectively. Following electrophoresis radioactivity distribution was determined (*13*).

To obtain the number and apparent molecular weights of structural polypeptides of RVFV, the isolate SA-51 was initially chosen for analysis. This isolate was obtained from sheep during a South African epizootic of RVF in 1951. The third sheep passage (*14*) was obtained from J. S. Walker, Plum Island Animal Research Laboratory, and passed two times in fetal rhesus lung cells (FRhL-103). This working stock was kept at −70° and had a titer of 10^8 PFU/ml. As seen shown in Fig. 1A, SDS–PAGE analysis on 12% gels shows three major polypeptide peaks with apparent molecular weights of 65,000, 56,000, and 25,000. In addition, a 100,000 MW protein has been observed in varying amounts in different preparations of the virus. In order to obtain complete electrophoretic separation of the 65,000 and 56,000 MW species, 12% gels were required. Coelectrophoretic studies comparing SA-51 with several RVFV isolates separated by time, geographical location of origin, nature of host, and animal pathogenicity revealed that the number and molecular weights of the major polypeptides of all isolates were identical (data not shown). These results are not surprising in the light of recent evidence showing that all RVFV isolates tested to date are indistinguishable by cross-neutralization

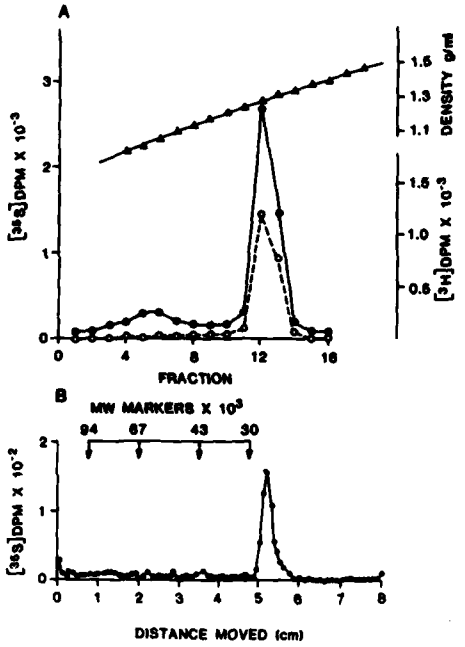

FIG. 2. Identification of RVFV nucleocapsid. Purified [^{3}H]uridine- and L-[^{35}S]methionine-labeled SA-51 incubated with an equal volume of 2% NP-40 (v/v) (Accurate Chemicals and Science Co., New York) for 1 hr at 20° was layered onto a linear preformed 1.14 to 1.40 g/ml CsCl gradient. Following centrifugation at 300,000 *g* in a Beckman SW 50.1 rotor for 18 hr, the gradient was fractionated. Radioactivity of each fraction (0.3 ml) was determined by methods previously described (*13*) and density was obtained by measurement of refractive index (*20*). (A) Top of gradient is to the left, L-[^{35}S]methionine (●) and [^{3}H]uridine (○). Density (▲) is in g/ml. Peak fractions (12 and 13) from the CsCl gradient were pooled and dialyzed against 8000 vol of TNE. (B) Samples were analyzed by 12% SDS–PAGE and L-[^{35}S]methionine activity was determined by methods described in the text. Molecular weights were obtained as shown in Fig. 1.

tests (*16*). Although these isolates are structurally and serologically indistinguishable, differences in pathogenicity for certain inbred strains of rats (*16*) and possibly man (*17*) have been reported.

Analysis of [^{3}H]glucosamine-labeled RVFV strain SA-51 revealed two major glycopeptides with molecular weights of 65,000 and 56,000. These two glycoproteins appear to be similar in molecular weight to those of Uukuniemi virus (*18*) and some of the PHL group viruses (*19*). In addition, the 100,000 MW polypeptide was also glycosylated (Fig. 1B), but the 25,000 MW species was not. Because the 100,000 MW glycosylated protein was not found in constant amounts in all virus preparations, it is possible that this polypeptide could represent a precleavage viral protein or an aggregate of the two major glycoproteins. We are currently carrying out studies, including tryptic mapping, to further characterize the identity of this protein, as well as the other RVFV glycoproteins.

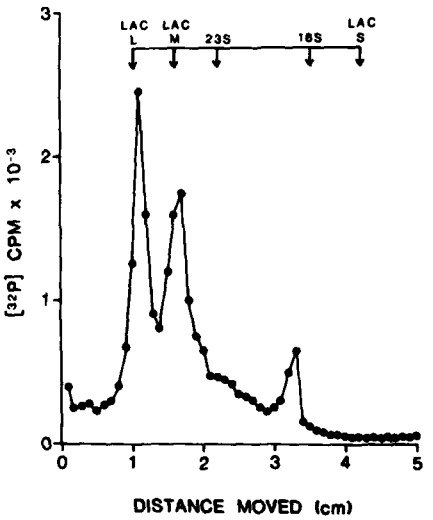

FIG. 3. Electrophoretic resolution of RVF viral RNA. The isolate SA-75 used for this study was obtained from an uncomplicated human febrile case of RVF in South Africa in 1975 courtesy of B. M. McIntosh, Department of Health, Republic of South Africa. The second passage, FRhL-103-propagated virus at a titer of 5×10^7 PFU/ml, was used as working stock. ^{32}P-Labeled viral RNA was extracted, purified, and analyzed on 2.4% PAGE by methods previously described (*21–23*). Tritiated 23 and 16 S ribosomal RNA (Miles Biochemicals, Elkhart, Ind.) and ^{32}P-labeled LaCrosse (LAC) (ATTC No. 744) RNA electrophoresed in parallel were used as molecular weight standards. The molecular weights of LAC L, M, and S RNA are 2.9, 1.8, and 0.4×10^6 (*22*) and 23 and 16 S ribosomal RNA are 1.1 and 0.56×10^6 (*21*), respectively.

Although the major glycopeptides of RVFV differed in molecular weights from many members of the family Bunyaviradae, the molecular weight of the nonglycosylated polypeptide was comparable (*11*). In order to ascertain if this species is a nucleocapsid protein (N), as is the case for other Bunyaviradae (*11*), dual-labeled viral nucleocapsids were isolated and examined. As shown in Fig. 2A, peak activities of both [^{3}H]uridine and L-[^{35}S]methionine appeared at a density of 1.29 g/ml. This density is indicative of bunyavirus nucleocapsids. Electrophoretic analysis of pooled peak fractions confirmed that the protein associated with RNA is the 25,000 MW N protein (Fig. 2B). Nondisrupted RVFV analyzed under identical conditions revealed a peak of radiolabel incorporation at a density of 1.21 g/ml. Subsequent SDS–PAGE analysis of peak fractions demonstrated the presence of intact virus (data not shown).

An important characteristic for classification in the family Bunyaviradae is a three-part segmented single-stranded RNA genome. These segments have been designated large (L), medium (M), and small (S) (*11*). Electrophoretic analysis of ^{32}P-labeled RVFV RNA shows a segmented genome (Fig. 3) with apparent molecular weights of 2.7, 1.7, and 0.6×10^6. These values are similar to those reported for other bunyaviruses (*18*, *19*, *22*). The segments of several bunyaviruses have been shown to be unique by oligonucleotide mapping (*18*, *19*, *24*). We are currently carrying out RNA T1 oligonucleotide mapping studies of RVFV in conjunction with D. H. L. Bishop and P. Cash of the University of Alabama, Birmingham. Evidence from these studies suggests that L and M RVFV RNA segments contain unique sequences (unpublished results).

In addition to this technique, we are currently using other methods, such as tryptic mapping and two-dimensional SDS–PAGE–isoelectric focusing to further characterize RVFV. These studies, in conjunction with data presented here, may help to relate molecular structure to the differences in pathogenicity of various isolates (*16*, *17*).

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