The Proteins and RNAs of St. Abb's Head Virus, a Scottish Uukuvirus

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SUMMARY

The proteins and RNAs of St. Abb's Head virus have been analysed, and were found to be characteristic of the Uukuvirus genus of the family Bunyaviridae. Two glycoproteins, G1 and G2 (mol. wt. 62K and 75K), and a nucleocapsid protein, N (mol. wt. 25K), were detected in infected cells by immunoprecipitation; the synthesis of N preceded the synthesis of G1 and G2. The glycoproteins were relatively cysteine-rich compared to the N protein, and the unglycosylated forms of G1 and G2 (using the inhibitor tunicamycin) had a mol. wt. of about 58K. Translation in vitro of mRNA from infected cells gave two immunoprecipitable products which are thought to be equivalent to N and G1/G2. Three RNA species were found in St. Abb's Head virus nucleocapsids, and were estimated to be 8500 bases (L), 3600 bases (M) and 1900 bases (S) in length. At least one additional virus-specific RNA species was detected in infected cells. The similarity between the proteins and RNAs of St. Abb's Head virus and Uukuniemi virus (the prototype of the genus) is discussed.

INTRODUCTION

Viruses of the family Bunyaviridae share the property of a tripartite, single-stranded RNA genome of negative polarity, and the family is classified into four genera: Bunyavirus, Nairovirus, Phlebovirus and Uukuvirus. The Uukuvirus genus comprises a single serogroup containing seven viruses, and in nature uukuviruses have been isolated from ticks, birds and certain rodents (Bishop & Shope, 1979; Bishop et al., 1980). The prototype of the genus is Uukuniemi virus (Oker-Blom et al., 1964) which has been the subject of many biochemical analyses (Gahmberg, 1984; Kuismanen, 1984; Kuismanen et al., 1982, 1984; Parker & Hewlett, 1981; Pesonen et al., 1982; Pettersson & Kääriäinen, 1973; Pettersson et al., 1971, 1977; von Bonsdorff & Pettersson, 1975; Ulmanen et al., 1981). The other six recognized members of the genus, Grand Arbaud, Manawa, Oceanside, Ponteves, Zaliv-Terpeniya and EgAn1825-61, are serologically related to Uukuniemi virus (Bishop et al., 1980) but no structural or biochemical analyses of these viruses have been reported. None of the uukuviruses has been associated with disease in man, though antibodies to Uukuniemi virus have been demonstrated in man and domestic animals (Molnar et al., 1973; Saikku, 1973; Sekeyova et al., 1970; Traavik & Mehl, 1977).

St. Abb's Head virus was isolated from a pool (M349) of Ixodes uriae ticks collected at a seabird colony in Berwickshire, Scotland, U.K. (Nuttall et al., 1981); electron microscopy and complement fixation tests indicated that the virus was related to Uukuniemi virus. Subsequently, we reported preliminary analyses of the proteins synthesized in St. Abb's Head virus-infected XTC-2 cells, which provided biochemical evidence to support the inclusion of St. Abb's Head virus in the Uukuvirus genus (Watret et al., 1985). In this paper we provide further data on the proteins of St. Abb's Head virus and describe the RNAs extracted from viral nucleocapsids and from infected cells.

METHODS

Cells and virus. Chick embryo (CE) cells were prepared from 12-day-old fertilized hens' eggs and maintained in Glasgow modified Eagle's MEM. XTC-2 cells were grown in L-15 medium as previously described (Watret et al., 1985). St. Abb's Head virus was originally obtained from Dr P. A. Nuttall, NERC Institute of Virology, Oxford, U.K. and subsequently triple plaque-purified in CE cells (Watret et al., 1985).
Immunofluorescence. Infected CE cells, grown on coverslips, were examined by the indirect immunofluorescent antibody method. After fixation in acetone at −20 °C, the cells were incubated with a rabbit anti-virus serum (Watret et al., 1985) for 45 min at 37 °C. The coverslips were then washed in phosphate-buffered saline (PBS) before staining with fluorescein isothiocyanate-conjugated sheep anti-rabbit serum (Wellcome Laboratories) for 45 min at 37 °C. After washing in PBS and distilled water, the cells were examined under u.v. illumination in a Leitz Orthoplan microscope.

Analyses of viral proteins. The procedures to radiolabel intracellular viral proteins, of radioimmunoprecipitation and of polyacrylamide gel electrophoresis have been detailed previously (Watret et al., 1985). In the experiments described in this paper 15% polyacrylamide gels were used throughout. St. Abb’s Head virus was purified by polyethylene glycol precipitation and sucrose gradient centrifugation (Obijeski et al., 1976; Pettersson & Kääriäinen, 1973).

Analyses of viral RNAs. St. Abb’s Head virus nucleocapsids were isolated from infected cell lysates by CsCl gradient centrifugation, and nucleocapsid RNAs were purified by phenol extraction as detailed by Leppert (1973). RNA preparations were treated with glyoxal, separated by agarose gel electrophoresis and stained with ethidium bromide as described by McMaster & Carmichael (1977).

Two procedures were used to examine intracellular RNAs. (i) Infected XTC-2 cells were labelled with 60 μCi/ml 5,6-[3H]uridine in the presence of 2 μg/ml actinomycin D from 17.5 to 22.5 h post-infection, and the labelled RNA was purified as described by Pringle et al. (1984). The RNA was analysed by formaldehyde–agarose gel electrophoresis (Barrett & Mahy, 1984). (ii) Infected CE cells were labelled with 250 μCi/ml [32P]orthophosphate in the presence of actinomycin D from 6 to 24 h post-infection, and the cellular RNA was purified as described by Collins et al. (1978). Radiolabelled RNAs were analysed on 2.6% polyacrylamide gels containing 7 M-urea in Tris-borate-EDTA buffer (Sleigh et al., 1979).

St. Abb’s Head virus RNA destined for translation in vitro was isolated from unlabelled infected cells, and fractionated by centrifugation on 15 to 30% sucrose gradients (Ulmanen et al., 1981). Fractions (600 μl) of the gradients were collected and the RNAs were recovered by ethanol precipitation and dissolved in distilled water.

Translation in vitro. RNAs were translated in a message-dependent rabbit reticulocyte system (New England Nuclear). Reactions (15 μl) contained 2 μg of RNA and, where appropriate, 2 μl of a dog pancreas microsomal membrane preparation (the New England Nuclear membrane preparation was diluted 1:5 for these experiments). Following incubation at 37 °C for 1 h, reactions were terminated by boiling with an equal volume of protein dissociation buffer (Watret et al., 1985) or viral proteins were immunoprecipitated with a specific antiserum; after incubation with 3 μl antiserum for 3 h at 4 °C, immune complexes were collected with Staphylococcus aureus Protein A and washed with lithium chloride buffer (Watret et al., 1985).

RESULTS

Growth and cytoplasmic replication of St. Abb’s Head virus

St. Abb’s Head virus replicates and produces plaques in both CE cells and in an amphibian cell line, XTC-2 (Pudney et al., 1973), derived from Xenopus laevis (Watret et al., 1985). Fig. 1 compares the growth curves of St. Abb’s Head virus in these two cell types, infected at the same multiplicity (5 p.f.u./cell), and shows that the kinetics of growth were similar. After a lag phase of about 4 to 5 h, the maximum yields of released virus were achieved by about 24 h post-infection.

Immunofluorescence experiments (data not shown; G. E. Watret, unpublished results) confirmed that St. Abb’s Head virus replicated in the cytoplasm of infected cells, a characteristic of the Bunyaviridae. Furthermore, intense areas of fluorescence were seen in the perinuclear region of the cell. Similar observations have been reported for Uukuniemi virus-infected cells, and these have been shown to reflect the maturation of the virus in the Golgi complex (Kuismanen et al., 1982).

Characterization of St. Abb’s Head virus proteins

The synthesis of proteins in St. Abb’s Head virus-infected CE cells was monitored by pulse-labelling with [35S]methionine. Cells were infected at 5 p.f.u./cell, i.e. one-step growth cycle conditions. The spectrum of virus-induced proteins was similar to that observed in infected XTC-2 cells (Fig. 5 in Watret et al., 1985): two glycoproteins G1 and G2 (mol. wt. 62K and 75K), a nucleocapsid protein N (mol. wt. 25K), and two non-structural proteins, p58 and p30 were detected (gels not shown). A protein equivalent to the L protein of other Bunyaviridae (Bishop & Shope, 1979; Bishop et al., 1980) was not reproducibly identified. Because little shut-off of host protein synthesis occurred, radioimmunoprecipitation experiments were performed using a
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Fig. 1. Growth curves of St. Abb's Head virus in XTC-2 cells (○) and CE cells (●). The virus released into the supernatant fluid at various times after infection was titrated in XTC-2 cells (Watret et al., 1985).

rabbit antiserum prepared against purified virus (Watret et al., 1985) (Fig. 2). This technique clearly demonstrated the temporal regulation of virus-specific protein synthesis. The N protein was detected from 4 h post-infection whereas G1 and G2 were not detected before 8 h post-infection. The non-structural proteins, p58 and p30, were not precipitated using this antiserum. No additional virus-specific proteins were detected if cells were 14C-labelled with a mixture of amino acids, none of the viral proteins was phosphorylated, and G1 and G2 were the only viral proteins which incorporated [3H]mannose (data not shown).

The proteins of purified St. Abb’s Head virus labelled with [35S]methionine are shown in Fig. 3(a). The N protein and a doublet of G1/G2 were identified but no L protein was evident. When run under non-reducing conditions (lane 2), G1 and G2 were more clearly separated [a similar observation was reported by Pesonen et al. (1982) for Uukuniemi virus G1 and G2, and Robeson et al. (1979) detected two distinct glycoproteins only when Karimabad virus (Phlebovirus genus) was analysed in the absence of a reducing agent]. Also evident in Fig. 3(a) lane 2 is an aggregate of material above G1. This possibly reflects the relatively high cysteine content of the glycoproteins, as shown in Fig. 3(b). For this experiment, infected cells were labelled with either [35S]methionine or [35S]cysteine, and the viral proteins immunoprecipitated. The results indicated that G1/G2 were relatively cysteine-rich and methionine-deficient compared to the N protein, whereas the N protein was relatively methionine-rich and cysteine-deficient. Fig. 3(c) shows the effect of the glycosylation inhibitor, tunicamycin, on the synthesis of viral proteins. Infected cells were labelled with [35S]methionine in the presence or absence of tunicamycin, and the viral proteins immunoprecipitated. Tunicamycin had no effect on the N protein, but unglycosylated G1/G2 (G10/G20) had a considerably faster electrophoretic migration than the glycosylated species. The molecular weight of the unglycosylated forms was estimated to be about 58K. Tunicamycin also inhibited the amount of infectious virus present in the tissue culture supernatant fluid: when cells were infected at a multiplicity of 5 p.f.u./cell and the released virus was assayed 16 h post-infection, the titre of virus from the tunicamycin-treated cells was 1% of that from the control culture (6-8 × 10^4 compared to 6-8 × 10^6 p.f.u./ml).

Analysis of RNAs in infected cells

Actinomycin D does not significantly inhibit the replication of bunyaviruses (Bishop & Shope, 1979; Kascak & Lyons, 1977). Therefore, intracellular viral RNA species can be radio-labelled under conditions in which the synthesis of host cell RNAs is minimal. Infected XTC-2 cells were labelled with [3H]uridine in the presence of 2 μg/ml actinomycin D, and the labelled
Fig. 2. Time course of the synthesis of St. Abb's Head virus proteins in infected CE cells. Cells were labelled for 1 h with $[^{35}S]$methionine at the indicated times post-infection, and then immunoprecipitated. c, Whole cell lysate; p, precipitation with preimmune rabbit serum; i, precipitation with hyperimmune rabbit serum; Mock, mock-infected.

RNA analysed on a formaldehyde-containing agarose gel; labelled RNA from mock-infected and actinomycin D-treated mock-infected cells was also run on the gel (Fig. 4a). Three distinct bands were seen in the virus-infected cell RNA preparation (lane 3) which were not present in mock-infected cells labelled under the same conditions (lane 2). These bands are thought to be equivalent to the viral L, M and S RNAs. A faint band was observed above the L band: this may correspond to the band observed in Fig. 4(b). Infected CE cells were incubated in the presence of actinomycin D and $^{32}$P, from 6 to 24 h post-infection, and cytoplasmic RNA was prepared by a rapid extraction method (Collins et al., 1978). The radiolabelled RNA was analysed on a denaturing polyacrylamide gel (Fig. 4b). Five virus-specific bands were located and have been designated L, L', M, S and S': 28S and 18S ribosomal RNAs were also identified. In uninfected CE cells, treated and labelled under the same conditions, only the 28S and 18S RNAs were seen (data not shown). The sizes of the L, M and S RNA species correspond to the sizes of the genomic RNAs (see below); the band designated S' is presumed to correspond to a shorter transcript from the S RNA segment, as has previously been reported for other members of the Bunyaviridae (Abraham & Pattnaik, 1983; Bouloy et al., 1984; Patterson et al., 1983; Ulmanen et al., 1981).
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Fig. 3. (a) Polyacrylamide gel electrophoretic analysis of purified [35S]methionine-labelled St. Abb's Head virus proteins under reducing (lane 1) and non-reducing (lane 2) conditions; Agg, aggregate. (b) Comparison of the labelling of St. Abb's Head virus with [35S]methionine (lane 1) or [35S]cysteine (lane 2). Infected cells were labelled with one of the radioactive precursors and the viral proteins were immunoprecipitated. (c) Effect of tunicamycin on St. Abb's Head virus protein synthesis. Cells were labelled with [35S]methionine in the absence (lanes 1 and 5) or presence of tunicamycin at 5 μg/ml (lanes 2, 4 and 6) or 10 μg/ml (lanes 3 and 7). After labelling, cell lysates were analysed by polyacrylamide gel electrophoresis either directly (lanes 1 to 3), or after precipitation with preimmune serum (lane 4) or with hyperimmune serum (lanes 5 to 7).

The band designated L' runs slightly ahead of L; whether this indicates a shorter mRNA transcript from the L RNA segment or an electrophoresis artefact has not yet been established.

Translation in vitro of RNA from St. Abb's Head virus-infected cells

To characterize further the proteins of St. Abb's Head virus, the RNA extracted from infected cells was translated in vitro in a rabbit reticulocyte translation system. No viral proteins were produced in vitro if oligo(dT)-cellulose-selected RNA (i.e. polyadenylated RNA) was translated (data not shown). Therefore, total infected cell RNA was fractionated on a sucrose gradient, and fractions from the gradient which were enriched for viral mRNAs were used to detect virus-specific proteins; the results are shown in Fig. 5. A protein of about 25K was observed to be a specific translation product of the infected cell RNA (lane 3). Immunoprecipitation of the translation products (lane 4) suggested that this was the N protein. A high molecular weight protein (approx. 200K) was also immunoprecipitated but this is thought to be non-specific precipitation as a similar protein was precipitated from the translation products of uninfected cell RNA (lane 1). Proteins equivalent to p58 or p30 could not be reliably detected. In order to detect the viral glycoproteins, translation was performed in vitro in the presence of dog pancreas microsomal membranes (Blobel & Dobberstein, 1975). The inclusion of the microsomal membrane preparation resulted in the reduction of many proteins, presumably because of contaminating ribonucleases which degraded the template mRNA (lane 6). However, the 25K
Fig. 4. Gel electrophoresis of radiolabelled RNAs from St. Abb's Head virus-infected cells. (a) XTC-2 cells were labelled with [3H]uridine from 17.5 to 22.5 h post-infection in the presence or absence of actinomycin D, and the RNA was analysed by formaldehyde-agarose gel electrophoresis. Lane 1, RNA from mock-infected cells; lane 2, RNA from actinomycin D-treated mock-infected cells; lane 3, RNA from infected cells treated with actinomycin D. (b) Infected CE cells were labelled with 32P in the presence of actinomycin D from 6 to 24 h post-infection, the cytoplasmic RNA was extracted and analysed on a 2.6% gel containing 7 M-urea. Lane 1, virus-specific RNAs, designated L, L', M, S and S'; lane 2, 32P-labelled, HindIII-digested bacteriophage λ DNA. Sizes of the DNA fragments are given in kilobase pairs at the right of the figure.
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Fig. 5. Translation in vitro of RNA extracted from uninfected CE cells (lanes 1 and 2) or St. Abb's Head virus-infected cells (lanes 3 to 6). Following translation, the products were analysed directly (lanes 2, 3 and 6) or after precipitation with St. Abb's Head virus antiserum (lanes 1, 4 and 5). Microsomal membranes were added to the translation reactions analysed in lanes 5 and 6. Proteins specific for the infected cell RNA translations are indicated.

protein was detectable as well as a protein of about 68K. The 68K protein was also immunoprecipitated (lane 5) and is presumed to represent the virion glycoproteins. The 68K protein synthesized in vitro was not precipitated as efficiently as the 25K protein compared to the situation in vivo (Fig. 2, 3b, c); we believe this is because G1 and G2 are not completely processed in vitro (see Discussion) and therefore are not as readily recognized by the antibody. Neither the 25K nor the 68K protein were produced when similar experiments were performed using uninfected cell RNA (data not shown).

Analysis of nucleocapsid RNAs

St. Abb's Head virus nucleocapsids were extracted from infected cells and purified by centrifugation in CsCl gradients (Leppert et al., 1979). Only genomic (negative sense) and full-length complementary (positive sense) RNAs are found in nucleocapsids and thus nucleocapsids provide a source of genomic RNA for analysis. The RNA was extracted from the nucleocapsids, denatured with glyoxal (McMaster & Carmichael, 1977) and analysed by agarose gel
Fig. 6. Agarose gel analysis of glyoxylated RNA extracted from St. Abb's Head virus intracellular nucleocapsids. The RNAs from five different nucleocapsid preparations are shown in lanes 2 to 6. Lane 1, 28S and 18S ribosomal RNAs; lane 7, purified tobacco mosaic virus RNA, 6395 bases in length (Goelet et al., 1982).

electrophoresis. Fig. 6 shows an ethidium bromide-stained gel of five different preparations of St. Abb's Head virus nucleocapsid RNAs, together with ribosomal RNAs and tobacco mosaic virus RNA as markers. Three species of viral RNA were observed in all five samples, and their sizes were estimated to be 8500 bases (L), 3600 bases (M) and 1900 bases (S).

DISCUSSION

Seven viruses are currently classified in the Uukuvirus genus of the family Bunyaviridae, although only one of these, Uukuniemi virus, has been the subject of biochemical analyses (see Introduction). The recently isolated St. Abb’s Head virus is a candidate for inclusion in the Uukuvirus genus on the basis of serological and biochemical criteria (Nuttall et al., 1981; Watret et al., 1985). The results presented in this paper indicate that Uukuniemi virus and St. Abb’s Head virus are very similar with regard to the sizes of the virion proteins and RNAs; these data are summarized in Table 1. The unglycosylated forms of the glycoproteins (Glo and G2o) apparently have close if not identical molecular weights and could not be separated electrophoretically. This suggests that the difference in size of the mature glycoproteins is due to differences in the degree of glycosylation. A similar observation was reported by Kuismanen (1984) for the unglycosylated forms of G1 and G2 of Uukuniemi virus. However, it should be noted that we have not formally proven that G1 and G2 are different proteins, e.g., by peptide mapping. The glycoproteins of St. Abb’s Head virus were relatively cysteine-rich compared to the N protein (Fig. 3b), a feature common also to viruses of the Bunyamwera serogroup (Bunyavirus genus; Elliott, 1985).

Translation of mRNA from St. Abb’s Head virus-infected cells in vitro in the presence of microsomal membranes produced an immunoprecipitable protein of mol. wt. 68K, which we suggest is an incompletely processed form of G1/G2. Ulmanen et al. (1981) described a similar translation product using Uukuniemi virus mRNA in vitro; these workers further demonstrated, in the absence of microsomal membranes, a precursor to the glycoproteins, designated p110. Surprisingly, we were unable to detect an equivalent protein in our system (Fig. 5, lanes 3 and 4), even though our antiserum efficiently precipitates the mature proteins. We also made extensive efforts to demonstrate a precursor in infected cells by experiments involving salt inhibition, protease inhibitors, amino acid analogues and pulse–chase, but could not detect such a precursor (data not shown; G. E. Watret, unpublished results). Our experiments suggest that the precursor (if it exists for St. Abb’s Head virus) must be co-translationally or rapidly post-translationally cleaved to the mature glycoproteins.
Table 1. Comparison of the proteins and RNAs of St. Abb’s Head virus and Uukuniemi virus

<table>
<thead>
<tr>
<th>Protein</th>
<th>St. Abb’s Head virus</th>
<th>Uukuniemi virus*</th>
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<tr>
<td>L</td>
<td>ND†</td>
<td>180–200‡</td>
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<tr>
<td>G1</td>
<td>75</td>
<td>75</td>
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<tr>
<td>G1,</td>
<td>58</td>
<td>54–57</td>
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<tr>
<td>G2</td>
<td>62</td>
<td>65</td>
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<tr>
<td>G2,</td>
<td>58</td>
<td>54–57</td>
</tr>
<tr>
<td>N</td>
<td>25</td>
<td>25</td>
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<tr>
<td>Non-structural</td>
<td>58, 30</td>
<td>30</td>
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<tr>
<td>RNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>8500§</td>
<td>8000</td>
</tr>
<tr>
<td>M</td>
<td>3600</td>
<td>3500</td>
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<tr>
<td>S</td>
<td>1900</td>
<td>1900</td>
</tr>
</tbody>
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* Data taken from Kuismanen (1984), Pettersson et al. (1977) and Ulmanen et al. (1981).
† ND, Not detected.
‡ Molecular weight × 10⁻³.
§ Number of bases.

The sizes of the three RNA species extracted from St. Abb’s Head virus nucleocapsids were similar to those of Uukuniemi virus (Pettersson et al., 1977), and these sizes are apparently distinctive for the Uukuvirus genus within the family Bunyaviridae (Bishop et al., 1980). Five RNA species were detected in infected cells (Fig. 4b), three corresponding in size to the genomic RNAs and the other two being respectively shorter than L and S RNA. We have not determined the origin of the latter transcript but by analogy with other bunyaviruses, we suggest that it is derived from the S RNA.

Biochemical analyses are now available for two viruses of the Uukuvirus genus which were isolated about 20 years apart in separate geographical locations. Based on the sizes of proteins and RNAs (with the proviso that these comparisons have not been made directly) the viruses are very similar, and show much less variation than do viruses within other genera of the Bunyaviridae, e.g. within the Bunyavirus genus (Elliott, 1985; Ushijima et al., 1980). In addition, St. Abb’s Head virus and Uukuniemi virus are indistinguishable by the complement fixation test (Nuttall et al., 1981). Whether the Uukuvirus genus exhibits less variation than occurs within the other genera awaits analyses of other uukuviruses. The exact relationship of St. Abb’s Head virus to Uukuniemi virus is also unclear and requires more detailed examination, for example with monoclonal antibodies or by comparative nucleotide sequencing.

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