Biochemical Characterization of the Virus Causing Leukoencephalitis and Arthritis in Goats

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SUMMARY

Goat leukoencephalitis–arthritis virus (GLV) has the density of a retrovirus in sucrose and contains an endogenous RNA-dependent DNA polymerase (reverse transcriptase). The virion reverse transcriptase utilizes the synthetic RNA template poly(rA).(dT)_{12} but not the synthetic DNA template poly(dA).(dT)_{12}. A high mol. wt. RNA similar in size to visna virus RNA was isolated from $^3$H-uridine-labelled virions. The major structural protein of GLV has the same mol. wt. as that of visna virus. From these data the GLV appears to be a retrovirus.

Goat leukoencephalitis virus (GLV) has recently been identified as the agent which causes leukoencephalitis, arthritis and interstitial pneumonia in goats (Cork et al. 1974b). This multifaceted disease complex of goats has been identified in herds of goats in several areas of the United States (Cork et al. 1974a). It may also be related to agents causing a similar disease complex in other parts of the world (Stavrou et al. 1969; Weinhold, 1974; O'Sullivan et al. 1978). The disease evolves slowly leading to paralysis and lameness and/or dyspnea. The pathological lesions in the central nervous system (CNS) and lungs of infected goats closely resemble those observed in visna/maedi of sheep. Visna is a slowly progressing disease of the CNS characterized by inflammatory and demyelinating lesions. Maedi is characterized by chronic interstitial pneumonia. Both diseases are classic examples of slow virus disease and the goat disease appears to fit into the same classification. The biological characterization of GLV has recently shown that the virus replicates and causes c.p.e. in goat cells in culture and has the morphology of a retrovirus (Narayan et al. 1980). In addition, the major structural protein of GLV cross-reacts in immunodiffusion tests with the major structural protein of visna virus (Narayan et al. 1980).

The GLV strain used in this study was derived in our laboratory from a thymus explant culture (Narayan et al. 1980) of a goat which had been inoculated 6 weeks previously with an infectious brain suspension from a naturally affected animal (Cork et al. 1974b). GLV was grown in goat synovial membrane cells (Narayan et al. 1980) in the presence of $^3$H-uridine (20 μCi/ml of culture media). The $^3$H-uridine-labelled virus supernatants were made 20 mM with Hapes (pH 7-4) buffer; ammonium sulphate (35 g/100 ml) was added and the fluid stirred for 1 h at 4°C. The mixture was centrifuged for 1 h at 10000 rev/min in a Sorvall GSA rotor (at 4°C) and the pellets resuspended in 1% of the original volume of virus in 10 mM-tris (pH 7-4), 100 mM-NaCl and 5 mM-Na₂EDTA (TNE). The virus was purified on a step gradient of 5 ml of 60% (w/w) sucrose and 10 ml of 25% (w/w) sucrose which was centrifuged for 2 h at 20000 rev/min in a Sorvall SS-90 (at 4°C). The virus band was at the interface of the two sucrose solutions. The banded virus was diluted 1:1 with 5.3 M-NaCl (Traul et al. 1975) and incubated at 37°C for 30 min and re-banded on a gradient of 60% (w/w) sucrose and 25% (w/w) sucrose. This double-banded virus was used for all subsequent studies. The purified GLV banded in sucrose at a density of 1.155 g/ml and had a co-sedimenting RNA-dependent DNA polymerase activity. This has recently been reported by Crawford et al. (1980).
Fig. 1. (a) Isolation of GLV RNA. GLV RNA was isolated from $^3$H-uridine-labelled virus in a linear gradient of 15 to 30% (w/w) sucrose in TNE containing 0.1% SDS. The gradient was centrifuged at 27,000 rev/min in a SW27 rotor (Beckman) for 4.5 h at 20°C. Aliquots (20 µl) from each fraction were tested for acid-insoluble material before (▲) and after (▲) digestion with 100 µg/ml of RNase A. Sedimentation is from right to left. The position of visna virus RNA is indicated by the arrow. (b) The high mol. wt. $^3$H-uridine-labelled RNA from GLV was heated for 3 min at 80°C and sedimented on a linear gradient of 15 to 30% (w/w) sucrose in TNE containing 0.1% SDS. The gradient was centrifuged at 20,000 rev/min in a SW27 rotor (Beckman) for 16 h at 20°C. The amount of $^3$H-uridine in 20 µl amounts of each fraction was determined by liquid scintillation counting. Mol. wt. markers of 28, 18 and 4S RNA were run on a parallel gradient. Sedimentation is from right to left.

To analyse the virus RNA the $^3$H-uridine-labelled GLV was lysed with SDS and centrifuged through a sucrose gradient containing SDS (Fig. 1a). Similarly labelled and purified visna virus (Narayan et al., 1977) was extracted and run on a parallel gradient. The radioactivity was measured in a Beckman liquid scintillation counter. The rapid sedimenting RNA of both viruses co-sedimented at 64S as shown in Fig. 1(a). A slower sedimenting peak of RNA was present in both viruses which was approx. 4S and smaller. The sensitivity of the GLV RNA to RNase was tested (Fig. 1a). The rapidly sedimenting peak of RNA was completely susceptible to digestion with RNase. Thus, GLV contains a high mol. wt. RNA, the structure of which is mainly single-stranded, as well as low mol. wt. RNA, both of which are characteristic of other retroviruses (Vogt & Hu, 1977). When the high mol. wt. RNA of other retroviruses is denatured by heat it sediments in sucrose gradients at approx. 35S (Vogt & Hu, 1977). To determine if the 64S RNA of GLV contained such a subunit structure, the 64S RNA from a gradient was heated to 80°C for 3 min and sedimented on a sucrose gradient (Fig. 1b). A discrete peak of $^3$H-labelled RNA migrated at 33S. However, much of the RNA migrated more slowly in a broad peak at the top of the gradient. This large amount of degraded RNA at the top of the gradient may be due to long periods between virus harvest. In addition, high levels of RNase may be present in the virus supernatants since the virus causes cell lysis.

The RNA-dependent DNA polymerase activity of GLV was measured in a reaction mixture which contained a final concentration of 0.25% (v/v) NP40, 50 mM-tris-HCl (pH 8.0), 7.5 mM-MgCl₂, 20 mM-dithiothreitol, 5 μCi $^{32}$P-TTP (20 μCi/mmol) and 2 mM each of dATP, dCTP, dGTP. Zero time samples were withdrawn from the reaction mixtures; the reaction was run at 37°C. Samples were withdrawn at timed intervals, the acid-insoluble product was measured by precipitation with trichloroacetic acid and the radioactivity was quantified in a Beckman liquid scintillation counter.

GLV, when disrupted with NP40, polymerized deoxynucleotide triphosphates into an acid-insoluble product. The synthetic template poly(rA).oligo(dT) (5 µg/ml) served as a
Fig. 2. (a) SDS-polyacrylamide gel electrophoresis (PAGE) analysis of GLV. SDS-PAGE was performed as described in Methods. GLV labelled with $^{35}$S-methionine (△—△) and $^3$H-glucosamine (▲—▲) were run on parallel lanes of a slab gel. The gels were dried as described, 2 mm slices were cut, solubilized and counted in a liquid scintillation counter. (b) Autoradiographs of SDS–PAGE of GLV and visna virus. GLV labelled with $^3$H-amino acids (lane A), $^3$H-glucosamine (lane B), $^{35}$S-methionine (lane C) and visna virus labelled with $^{35}$S-methionine (lane D) were run in parallel lanes on a gradient slab gel. The mol. wt. markers were β-galactosidase (135,000), carbonic anhydrase (32,000) and lysozyme (14,880).

primer for the enzyme while no activity was found using poly(dA).oligo(dT) (5 μg/ml), a template specific for a DNA-directed DNA polymerase. Both the endogenous and primed reverse transcriptase reactions require a divalent cation. Either magnesium or manganese was utilized by the enzyme. The optimal concentration of manganese (0.2 mM) was far lower than for magnesium (7.5 mM) and the magnesium stimulated the reaction to a greater extent.
To study the polypeptide composition of GLV the virus was labelled with $^{35}$S-methionine (20 $\mu$Ci/ml) in methionine-free minimum essential medium (MEM) supplemented with 10% MEM, 0.5% foetal calf serum (FCS) and 5% glutamine. Labelling of virus with $^3$H-amino acid mixture to obtain uniformly labelled virus polypeptides was done in amino acid-free MEM supplemented with 10% MEM, 0.5% FCS and 1% glutamine. $^3$H-glucosamine (20 $\mu$Ci/ml) in MEM supplemented with 0.5% FCS and 5% glutamine was used to identify glycosylated polypeptides of the virus. The labelled virus preparations were disrupted in 1% SDS and 0.1 M-2-mercaptoethanol and the proteins were separated on slab gels (12 $\times$ 16 cm) of 5 to 20% SDS–polyacrylamide (Laemmli, 1970) which were subjected to electrophoresis for 0.5 h at 140 V; 1.5 h at 120 V; and 1 h at 200 V. The proteins of GLV resolved into multiple bands with a mol. wt. range of 140000 to 13500 (Fig. 2a).

There was no difference in the labelled polypeptide patterns obtained with the $^3$H-amino acid or $^{35}$S-methionine, indicating that the major structural polypeptides contained sufficient methionine residues to utilize this label for subsequent protein analyses. The major protein migrated with an apparent mol. wt. of 25000 which is very similar to the major structural protein of visna virus (Fig. 2b). A large protein of approx. 140000 mol. wt. was also observed. This protein is similar in size to the glycoprotein of visna virus. Two other small proteins of 16500 and 13500 appeared to be structural components of the virus. These polypeptides were major bands when the gels were stained with Coomassie blue or the radioactivity quantified by fluorography or liquid scintillation counting. When the polypeptides of visna virus and GLV were analysed in parallel lanes in a gel, all the major polypeptides except for the 16500 mol. wt. protein of GLV migrated with identical mol. wt. (Fig. 2b).

When virus labelled with $^3$H-glucosamine was analysed by electrophoresis in SDS–polyacrylamide gels, a single band contained the radioactive label. The gel was fluorographed (Bonner & Laskey, 1974) as well as cut and quantified by liquid scintillation counting (Fig. 2a). The single $^3$H-glucosamine-containing polypeptide migrated with an apparent mol. wt. of 140000. This polypeptide migrated with the same apparent mol. wt. as the glycoprotein of visna virus (Fig. 2b).

The major structural protein of GLV has an apparent mol. wt. of 25000 in denaturing gels, being identical in migration to the major structural protein of visna virus. These proteins share a common antigenic determinant, since a protein from GLV cross-reacts in immunodiffusion studies with visna anti-p25 (Narayan et al. 1980). The glycoprotein of GLV migrates in gels with the same apparent mol. wt. as that of visna virus. The striking similarity between these two major virus proteins establishes the structural relationship of GLV to the sheep retroviruses which cause visna, maedi and progressive pneumonia (Haase & Baringer, 1974; Haase, 1975). Since antibody to the glycoprotein of visna virus is responsible for virus neutralization (Scott et al. 1979), the failure of anti-visna virus sera to neutralize GLV establishes an antigenic distinction between GLV and visna virus glycoprotein. This observation is not surprising since different strains of visna virus show varying degrees of neutralization with antisera to visna virus (Gudnadottir, 1974; Narayan et al. 1978). Furthermore, the differences in the small structural protein (16500) may be involved in either the altered host range of GLV or in the extended disease potential of GLV.
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