Structural Polypeptides of Hazara Virus

(Accepted 6 October 1980)

SUMMARY

Four structural polypeptides of Hazara virus, an agent closely related to the Crimean–Congo haemorrhagic fever (C-CHF) viruses, were resolved by SDS-polyacrylamide gel electrophoresis. Three glycoproteins were identified (mol. wt. 84000, 45000 and 30000) and were found to be associated with the virion envelope. A fourth polypeptide (mol. wt. 52000) was non-glycosylated and associated with the nucleocapsid. The structural proteins of Hazara virus differ markedly from those reported for other bunyaviruses.

Hazara virus was isolated in 1964 from ticks collected in the Hazara district of West Pakistan (Begum et al., 1970a, b) and has aroused interest in recent years due to its close serological relationship with the Crimean–Congo haemorrhagic fever (C-CHF) viruses (Casals & Tignor, 1974). Both Hazara and C-CHF viruses are classified as members of the family Bunyaviridae, primarily on the basis of their morphological appearance by electron microscopy (Murphy et al., 1973; Jelifiková et al., 1975; Korolev et al., 1976; Smirnova et al., 1977) and, together with others, comprise one of the many unique serogroups within the family (Porterfield et al., 1975/76).

Studies of the molecular structure of these viruses have been stimulated by the desire for an effective vaccine against C-CHF. Efforts have focused on Hazara virus since this agent elicits cross-protection in mice against C-CHF virus challenge, may be safely handled in the laboratory and replicates to 10-fold higher titres in cell culture than C-CHF strains (R. S. Foulke et al., unpublished observations).

Hazara virus, strain JC280, in the 8th suckling mouse brain (smb) passage was obtained from J. Casals (YARU, New Haven, Conn., U.S.A.), passaged in suckling mice and cloned from the 11th smb passage by three terminal dilution passes in BHK-21 cells. Virus was propagated by inoculation of BHK-21 cell monolayers (5 × 10^8 cells) with virus (0.1 p.f.u./cell) and incubation under medium 199 (Earle's) containing 1/40 normal amino acids (Gibco), 5% dialysed foetal calf serum (FCS), 0.01 M-Hepes buffer and antibiotics. Radiolabelled metabolites (New England Nuclear) were added 4 h post-infection (³H-labelled amino acids, glucosamine or uridine, 10 µCi/ml; ¹⁴C-labelled amino acids, 4 µCi/ml) and infected supernatants were harvested 24 h post-infection, briefly centrifuged (380 g, 10 min) and then clarified (8000 g, 30 min). Virus samples were concentrated by direct pelleting (SW27, 116000 g, 60 min) or by (NH₄)₂SO₄ precipitation (Rosato et al., 1974); each procedure yielded similar amounts of virus. Concentrates were resuspended in TNE (0.01 M-tris-HCl, 0.1 M-NaCl, 0.001 M-EDTA) and purified by equilibrium centrifugation [SW50.1, 250000 g, 60 min for pellets; SW27, 116000 g, 4 h for (NH₄)₂SO₄ precipitation] on two successive continuous gradients of 20 to 50% (w/v) sucrose in TNE. Gradient fractions were assayed for radioactivity by addition of Scintilute containing 10% (v/v) Scintisol (Isolab, Akron, Ohio, U.S.A.) before using a Beckman LS8000 beta counter. Infectivity titres were determined by plaque assay with SW-13 cell monolayers (ATCC CCL105) under medium 199 overlays containing 5% FCS and 0.6% agarose. Plaques were counted after addition of neutral red at 3 days post-infection.

A representative purification gradient of Hazara virus is shown in Fig. 1(a). Infectivity titres closely paralleled a single radioactive peak with a mean density of 1.16 g/ml. Purified
virus from the appropriate fractions of similar gradients were used to characterize the viral components and identify the structural proteins by SDS–polyacrylamide gel electrophoresis (SDS–PAGE).

Purified virus labelled with $^3$H-uridine and $^{14}$C-amino acids was disrupted at 4 °C for 1 h in 2% (v/v) Nonidet P-40 (NP-40) detergent (Shell Oil); degraded virions were separated into components on 20 to 60% (w/v) sucrose gradients (SW27.1, 116 000 g, 18 h) (Fig. 1b). Three radioactive peaks were observed: a high density (1.26 g/ml) uridine-rich peak presumably representing viral nucleocapsid; a small, moderately dense peak (1.16 g/ml) probably of residual intact virus; and a protein-rich band remaining near the top of the gradient.

Four structural polypeptides of Hazara virus having mol. wt. of 84 000, 52 000, 45 000 and 30 000 were resolved using SDS–PAGE as described by Laemmli (1970) (Fig. 2a) from the single peak shown in Fig. 1(a). Mol. wt. were estimated by co-electrophoresis (3 mA/gel for 2 h) of $^{14}$C-labelled Hazara virus proteins with $^3$H-labelled Venezuelan equine encephalitis (VEE) and Oriboca virus standards. When virus labelled with $^3$H-glucosamine and $^{14}$C-amino acids was similarly electrophoresed, only one polypeptide species (mol. wt. 52 000) failed to incorporate radioactive glucosamine (Fig. 2b); the three glycoproteins were designated GP1, GP2 and GP3 in order of decreasing mol. wt. It was possible that the 45 000 mol. wt. protein was actin, derived from the cell, since cellular actin from BHK-21 cells (mol. wt. 43 000) has been co-purified with rabies virus (Naito & Matsumoto, 1979). However, cellular actin is non-glycosylated and GP2 incorporated large amounts of radioactive glucosamine. We therefore concluded that this was a viral protein.
Fig. 2. Polyacrylamide gels (8%) of Hazara virus, sliced and counted by liquid scintillation. (a) 
\(^{14}\text{C}-\text{amino acid}-\text{labelled Hazara virus} (\bullet - \bullet) \text{ was mixed with } ^{3}\text{H}-\text{labelled virus standards} (\bigcirc - \bigcirc), \text{ VEE (mol. wt. 59000, 53000, 32000; Pedersen & Eddy, 1975) and Oriboca (mol. wt. 119000, 32000, 23000; Obijeski & Murphy, 1977). VEE and Oriboca virus standards were calibrated} 
\text{using Coomassie brilliant blue-stained protein standards (LMW calibration kit, Pharmacia) (Weber & Osborn, 1969). Four polypeptides were resolved and designated GP1, N, GP2 and GP3 in order of}
\text{decreasing mol. wt.} (b) \text{Hazara virus labelled with } ^{3}\text{H}-\text{glucosamine} (\bigcirc - \bigcirc) \text{ and } ^{14}\text{C}-\text{amino acids} 
(\bigcirc - \bigcirc).

Gel profiles from the nucleocapsid band and soluble protein fraction of the NP-40-treated 
sample suggest structural locations of each polypeptide within the virion. The nucleocapsid 
fraction contained large amounts of the non-glycosylated protein (mol. wt. 52000), referred 
to as nucleoprotein N, and small amounts of GP1 (mol. wt. 84000). Conversely, soluble 
fractions from the top of the gradient contained very little N protein while all three 
glycoproteins were present in approximately normal molar ratios. Extrinsic iodination of 
intact virus by the glucosoxidase–lactoperoxidase technique (Hubbard & Cohn, 1972) 
supported these findings in that GP1, GP2 and GP3 were heavily labelled and the N protein 
was labelled to a much lesser extent (data not shown). Data from both labelling techniques 
strongly suggest that the virion envelope is composed of three glycoproteins, while the 
nucleocapsid contains a single, non-glycosylated polypeptide.

The N protein of Hazara virus (mol. wt. 52000) differs markedly in size from nucleocapsid 
proteins reported for other bunyaviruses (mol. wt. 19000 to 25000). In addition, the 
glycoprotein profile is dissimilar to patterns found among other bunyaviruses. However,
glycoprotein size varies considerably among *Bunyaviridae* genera, while nucleocapsid proteins remain in a narrow mol. wt. range (Obijeski & Murphy, 1977). Recently, reorganization of the family *Bunyaviridae* has been proposed to classify this large group of viruses on the basis of serological cross-reactivity and molecular structure. Due to a slight, but detectable, cross-reaction between C-CHF and Nairobi sheep disease viruses (Casals & Tignor, 1980), the C-CHF group has been assigned to the Nairovirus genus in this new system. A polypeptide composition similar to that of Hazara virus has been observed in all the members of the Nairovirus genus so far examined (Bishop et al., 1980).

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**REFERENCES**


*(Received 8 September 1980)*

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