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CHARACTERISATION OF IBD VIRAL PROTEINS IN SDS POLYACRYLAMIDE GEL ELECTROPHORESIS

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Four structural polypeptides of IBDV strain Cu-1 with molecular weights of 90 K (VP_1), 41 K (VP_2), 35 K (VP_3) and 28 K (VP_4) and the additional polypeptide VP_x (47 K) precursor of VP_2 were determined in SDS-PAGE (Dobos, 1979; Dobos *et al.*, 1979). Hirai *et al* (1979) reported that seven structural polypeptides of IBDV strain GBF-1, consisted of two major and five minor polypeptides. Azad *et al.* (1987) reported that an Australian isolates type I IBDV was composed of two major structural proteins of molecular weight 37 K (VP_2b) and 32 K (VP_3) and three other proteins of molecular weight 92 K (VP_1), 42 K (VP_2a) and 27.5 K (VP_4). Das and Ram (1994) showed that five different structural polypeptides of IBDV strain AP/ 81 with molecular weights of 75.8 K, 45 K, 40.7 K, 31.1 K and 27 K were detected in SDS-PAGE. Praveen *et al.* (1995) concluded that all the field isolates contained similar molecular weight polypeptides such as 97 K, 56 K, 53 K, 50 K, 45 K, 29 K and 24 K. In the present experiment, local isolates of IBD virus were characterised in SDS-PAGE analysis to study the protein profile for strain differentiation.

Materials and methods

Isolation of IBD virus in chicks

IBD suspected samples collected from different outbreak areas of Tamil Nadu during the period of 1995-97 were used for virus isolation and the samples were processed as per the method of Hirai *et al.* (1972). The bursal homogenates were extracted with equal volume of Freon (Sigma). The aqueous phase was harvested and tested for the presence of antigen using Agar gel precipitation test (AGPT). Six positive samples were inoculated into embryonated eggs and titrated at 5th passage as per the method described by

Nakamura *et al.* (1993). EID_{50} was calculated as per the method of Reed and Muench (1938). The six isolates were inoculated into five weeks old susceptible seronegative chicks with 0.1 ml of 100 EID_{50}.

Concentration and purification of IBDV

The concentration and purification of six IBDV isolates were carried out as per the method of Dobos *et al.* (1979) with few modifications. The clarified bursal homogenate for each isolates was treated with 2.2 per cent (w/v) sodium chloride and 5 per cent polyethylene glycol (mol. wt. 20000) and stirred for 4 hrs at 4°C. The precipitated material was extracted with freon and aqueous phase was layered onto two step caesium chloride (CsCl) gradient (1.2 g and 1.4 g/ml density). This preparation was centrifuged at 1,32,000 g for 4 hrs at 4°C using Beckman T_1 60 rotor. The ultracentrifuge tubes were illuminated in a dark room and the viral band located between two caesium chloride gradient was collected.

Polyacrylamide gel electrophoresis (PAGE)

The SDS - PAGE was done as per the method described by Laemmli (1970). Fifty microlitre of each purified virus isolate was mixed with 20 ml of sample dye containing 1% SDS and 0.005% Bromophenel blue and heated in a boiling water bath for 30 min. Electrophoresis was carried out at 50 volt until the dye front migrate to the bottom of the gel. The gel was stained with 0.025 per cent coomassie brilliant blue stain. the molecular weight of the different viral proteins were determined as per the method described by Shapiro *et al.* (1967).

Results and discussion

The virus was concentrated and purified by PEG precipitation and freon extraction followed by discontinuous CsCl gradient centrifugation. In this method, the clear distinct viral band was observed. The total protein content of the purified virus isolates ranged from 4.2 to 4.5 mg/ml. PEG was found to be highly effective in precipitating the viral particles and freon was useful in releasing the intact viral particles from the bursal homogenate.

After staining the gel with coomassie brilliant blue, the viral protein bands were identified. The relative mobilities of the marker protein were plotted against the logarithm of their molecular weight and the molecular weight of viral polypeptides were determined by reading from the graph.

In the present study, all the field isolates possessed five viral polypeptides with molecular weights of 91 K (VP_1), 39 K (VP_2), 32 K (VP_3), 27.5 K (VP_4) and a precursor polypeptide 49 K (VP_x) were detected in SDS-PAGE (Fig 1). Similar results were reported by Dobos (1979) and Fahey *et al.* (1985). In addition to five structural polypeptides, one faint polypeptide above VP_1 was obtained as reported by Jackwood *et al.* (1984). Molecular weight difference among the structural proteins of serotype 1 strains were minor (Jackwood *et al.* 1984; Praveen *et al.* 1995) and similar migration pattern observed between variant and classic IBDV strains (Ture and saif, 1992). In this study, the migration pattern of structural polypeptides of all the field isolates were almost identical. This findings are in agreement with previous research works.

Hence, the molecular weight of the structural proteins of serotype 1 strains were almost identical and protein profile study probably of no value in differentiating these viruses.

Summary

The field isolates of IBD virus was characterised using protein profile study. Four structural polypeptides of IBD virus with molecular weights of 91 K (VP_1), 39 K (VP_2), 32 K (VP_3), and 28 K (VP_4) and the additional polypeptide 47 K (VP_x) preceusor of VP_2 were detected in SDS-PAGE.

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