Comparision of Structural Polypeptides of Two field Isolates of IBH Virus

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Abstract

Inclusion Body Hepatitis (IBH) caused by adenovirus is a reemerging diseaseof mainly young broiler chicks of 3-5 weeks age. The polypeptide pattern of a local isolate of virus causing Inclusion Body Hepatitis was analyzed by sodium dodecyl slulphate-polyacrylamide gel electrophoresis. A total of 9 polypeptides ranging in molecular weight between 35.2 and 110.0 kDa were observed. Western blot analysis of structural polypeptides also revealed that all 9 polypeptides were immunogenic. Out of these 9 polypeptides, 2 viz 97.4 kDa and 53.9 kDa were found to be major immunogenic polypeptide based on intense staining.

Keywords : Inclusion Body Hepatitis, Immunogenic polypeptides, Structural polypeptides;

Introduction

Avian adenoviruses are known to cause many disease conditions of all age groups among poultry. Inclusion Body Hepatitis (IBH) is frequently encountered in young broiler chicks of 3-5 weeks age (Kharole *et al.*, 1991 and Shandhu *et al.*, 1994) in many parts of the world including India (Kumar, 2003). All serotypes of Fowl adenovirus has been established as the causative agent of IBH. The disease is characterized by hepatitis, a swollen, discolored and friable liver with basophilic or eosinophilic inclusion body, necrotic & congested kidneys (Sandhu *et al*., 1994; and Kumar *et al*., 2003). So as a prerequisite to investigate the possible variation in polypeptide composition and to study their immunogenic property, the present investigation was designed to characterize structural proteins of IBH isolates recovered from tarai region of Uttrakhand, India, which may in turn help in the development of suitable immunodiagnostic and immunoprophylaxis.

Materials and Methods

Virus Isolation : Two local isolates of IBH virus, recovered from IBH outbreak in Bazpur (Uttrakhand, India) in year 2004 and 2005 were assigned names as IBH B and IBH B1 respectively for identification. All the virus isolates were typed as FAV-4 and for bulk production, two separate groups of 25 broilers, each were injected with 20% liver homogenate @ 1ml each bird by intramuscular route.

Preparation of Antiserum : Antiserum against the local isolates was raised in 3 week old broilers as per the method of Kumar *et al*. (1997). Broiler birds of 3 week age were initially infected with 0.5 ml of heat inactivated liver homogenate extract (prepared from liver of experimentally infected birds) by sub-cutaneous route. After one week, 0.5ml of liver homogenate blended with equal volume of Freunds Incomplete Adjuvant (FIA) was injected sub-cutaneously followed by weekly injection of 0.75, 1.0 and 1.25 ml of same preparation mixed with equal volume of Freunds Complete Adjuvant. In last 1.5 ml of liver homogenate mixed with equal volume of FIA was injected. Ten days after last injection blood was collected from immunized chicken, serum was separated, pooled and stored at -20^{0}C until used. Before use, serum was tested against liver homogenate from control birds.

Purification of IBH virus: A 20% (w/v) homogenate of infected liver tissue in PBS was clarified and filtered through 0.2 µM membrane filters (Millipore, Bradford, MA, USA). Virus purification was carried out by the method of Ganesh *et al.* (2002) with a few modifications. The filtrate was pelleted by centrifugation through a 35% sucrose cushion in TBS (10 mM Tris-HCl and 150 mM, NaCl, pH 7.4) at 90000g for 2 hr at 4^0 C. Pellet obtained was dissolved in TBS and layered over cesium chloride (Sigma, USA) gradient (1.5 ml of density 1.45g/ml and 1.5 ml of 1.33 g/ml in TBS) and centrifuged at 90000 g for 2 hr in swing out rotor (Beckman, USA). The opalescent viral band at interface were collected and pelleted at 1,00,000 g for 2 hr at 4^{0}C and final pellet obtained were dissolved in

700 µl of TE buffer.

Protein Estimation: Protein concentration of purified virus preparation was estimated by spectrophotometric method as described by Warberg and Christian (1941). The optical density of purified virus suspension was determined at 260 and 280, and protein concentration was calculated by the formula:

Protein(mg/ml) = 1.56 OD 280nm -0.76 OD 260nm.

SDS-PAGE and WESTERN BLOT: Purified IBH virus isolates were subjected to discontinuous 12.5% SDS-PAGE as per the method of Laemmli (1970). The viral polypeptides of IBH viruses were identified and characterized by SDS-PAGE and were electropho-retically transferred to nitrocellulose membrane (0.22 µm) in transfer buffer (192 mM glycine, 20 % methanol, 0.025 M Tris and 20% Methanol) at 0.8 mA/cm^2 for 1 hr as per the method of Towin *et al*. (1979). On the basis of polypeptide composition of IBH virus examined with 12.5% SDS-PAGE and the resultant polypeptides were visualized on staining of gel by coomassie brilliant blue.

Results

The absorbance ratio of purified virus band at 260 and 280 nm was found to be 0.60. The protein content of purified virus was estimated and diluted to 0.8 µg/µl for SDS-PAGE analysis. The polypeptide pattern of both the isolates was similar and revealed 9 polypeptides of molecular weights 97.4, 84.7, 79.8, 74.5, 66.0, 61.2, 53.9, 40.0 and 35.2 kDa (Fig.2).The purified virus separated on 12.5% gel and transferred onto the nitrocellulose membrane (0.22 µm) by semi-dry system, revealed that all the 9 polypeptide are immunogenic in western blotting. Of these 9 polypeptides, two 97.4 and 53.9 kDa were found to be major immunonogenic polypeptides.(Fig.3).

Discussion

Liver tissue from HHS affected birds constituted the known source of virus and was used in virus purification as earlier workers have shown that hepatocytes contain the maximum viral load (Chandra *et al*., 1997 and Ganesh *et al*., 2002). Pelleting through sucrose cushion was done as per the method of Ganesh *et al.* (2002) followed by purification of virus isolates through CsCl density gradient (1.45g/ml and 1.33 g/ml). Purity of virus suspension was checked by absorbance ratio at 260 and 280, and the ratio obtained was 0.60, which indicates that virus is purified to a good extent. Hawk *et al*. (1947) also demonstrated purity of virus suspension on the basis of absorbance ratio. Ganesh *et al.* purified the HPS virus by 10-55% sucrose density gradient and obtained the virus containing band between 25 and 35% sucrose bands. Maiti and Sarkar reported purification of avian adenovirus type I using caesium chloride step gradient ultracentrifugation.

In the present investigation, purified IBH virus isolates were subjected to SDS-PAGE, which revealed 9 polypeptides ranging in molecular weight from 97.4 kDa to 35.2 kDa in 12.5% resolving gel. Ishar-ul-Haq *et al.* (1997) reported 8 polypeptides ranging in molecular weights from 119.0 kDa to 15.7 kDa in 10% resolving gel. This difference in number of polypeptides may be attributed to difference in concentration of resolving gel and various other physical conditions. Polypeptides having narrow difference in molecular weight may not be resolved in 10% resolving gel.

The western blot analysis of purified virus isolates separated on 12.5% gel showed 9 immunogenic proteins ranging in molecular weight from 97.4 kDa to 35.2 kDa. Of which two proteins, (97.4 kDa and 53.9 kDa), stained more intensely and were considered as major immunogenic proteins. Out of these major immunogenic proteins, 97.4 kDa protein yielded thick band indicating larger quantity of this protein, which could be eluted from gel and used as subunit vaccine. However, further studies are needed to establish its immunogenic nature and feasibility for use as vaccine. Kumar and Chandra (2004) reported 7 immunogenic polypeptides in a field isolate of HPS virus out of which 3 proteins were major immunogenic proteins (110 kDa, 50.1 kDa and 41.6 kDa). Sambrook *et al.* (1989) reported that western blotting is extremely useful for identification and quantitation of specific proteins in a complex mixture of protein that are not radiolabelled. The technique is almost as sensitive as solid radioimmunoassay. Furthermore, because electropho-retic separation of protein is always carried out under denaturing conditions, the problem of solublization, aggregation and coprecipitation of target proteins with adventitious proteins are eliminated.

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