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Biochemical characterization of foot-and-mouth disease virus*

RITA NATH[1], RAM LAKHAN PRASAD[2] and SANKAR SARMA[3]

Birsa Agricultural University, Ranchi, Jharkhand 834 006 India

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Foot-and-mouth disease virus (FMDV) is the first animal virus to be identified in 1898 by Loeffler and Frosch. One of the most fearful viral pathogens of animals since it is highly contagious among all cloven-hoofed animals including cattle, sheep, goats and swine (Carrillo *et al*. 2005, Klein 2007). It is caused by a small single-stranded, positive-sense RNA virus of about 8500 nucleotides. It belongs to genus *aphthovirus*, family Picornaviridae. The present study was carried out to determine the molecular weight and mobility of the proteins of FMD virus. The epidemiology and etiology of FMD have been extensively investigated (Yang *et al.* 1993) but, a few published reports on the biochemical characterization of FMD virus are available (Gokce *et al.* 2004). Keeping these in view, the work was designed to run in cattle so that FMD in Indian origin is better understood.

The study was performed with two groups of animals — group 1 consisted of 10 crossbred cattle from farm of Ranchi Veterinary College and group 2 consisted of 30 crossbred cattle having clinical signs of FMD. The control animals were screened on the basis that they were clinically healthy and free of disease since last 6 months and were non pregnant. A complete case history and owner complain were recorded for each animal under group 2(test). They were clinically examined and there was presence of excessive secretion of stringy or foamy saliva, fever as well as blisters on the feet that had ruptured and caused lameness. Presence of vesicles in the mouth and on the muzzle and teats were also noticed in the animals. Animals without characteristic lesions of FMD were not considered for the study. All the animals under study were not vaccinated against FMD. Blood samples were collected, taking all aseptic precautions, from the animal by jugular vein puncture. The samples collected in sterile test tubes were left undisturbed for 4–6 h to separate the serum. The separated serum samples were cleared by centrifugation at 3000 rpm for 5 min. Characterization of FMD virus was carried out using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Walker (1985) with slight modification by Prasad (1983) using vertical slab gel electrophoresis. The mobilities (Rf) of unknown proteins were calculated and molecular weight (MW) were determined according to the method of Weber and Osborn (1969). After electrophoresis, the length of each slab gel samples and distance of the marker dye from starting point were noted before staining. After destaining, the gel length and bands were measured again. Molecular weight of unknown proteins was calculated from semi-log graph\ plotting using standard SDS-6H of known proteins. The FMD trivalent vaccine of strain A, O and Asia-1 and serum of FMD affected cattle were used for the characterization of FMD virus.

The standard SDS-6H of known proteins was used for calculating the molecular weight of different proteins. Iqbal *et al*. (1972) used 4 protein markers ie. bovine serum albumin, chicken egg albumin, pepsin and trypsin having 66, 000, 45, 000, 34, 500 and 23, 500 Da molecular weight, respectively. They were also loaded in the same gel along with the test

Table 1. Molecular weight and mobilities of protein

Protein	Mobility	Mol. wt. (K Da)
ß- glycosidase	0.205	116
Phosphorylase b of rabbit muscle	0.236	97
Albumin bovine	0.505	66
Albumin egg	0.678	45
Carbonic anhydrase	0.868	29
FMD viral protein 1	0.268	101
FMD viral protein 2	0.521	62
γ – globulin	0.094	150
ß-globulin	0.126	140
Unidentified protein	0.186	120
Heptoglobin	0.268	100
Albumin	0.536	60
α_2 globulin	0.631	48
Viral protein	0.710	40
Unidentified protein	0.805	33

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Present address: [1] Assistant Professor (ritasankr @rediffmail.com), Department of Veterinary Biochemistry, College of Veterinary Science, Khanapara, Guwahati, Asom.

[2] Chairman and Head (rlpprd@gmail.com), Department of Veterinary Biochemistry, Ranchi Veterinary College.

[3] Veterinary Assistant Surgeon (sankritavet@rediffmail.com), Feed Analysis Laboratory, Guwahati.

samples and the technique of SDS-PAGE was used. In our study, the FMD trivalent vaccine segregated into 2 bands ie viral protein 1 and 2 after running SDS-PAGE. These results provide new evidence that FMD like other animal picorna viruses, contain different polypeptide chains (Laporte and Lenoir 1973). We also observed 7 distinct bands segregated in each control and FMD affected serum samples after running SDS-PAGE. The bands in the control sample from the top of the gel were identified as γ globuli, ß globulin, unidentified protein, heptoglobin, albumin, α_2 globulin and unidentified protein. In the FMD affected sample, γ –globulin was undetectable which may be because during FMD infection the immunity decreases, and a major portion of γ globulin may have been involved in defence mechanism and antigen-antibody complex formation. But an extra band was noticed in the infected serum sample, which was identified as the FMD viral protein as the FMD affected animals had FMD virus in the blood.

Iqbal *et al*. (1972) in their study used locally isolated vaccinal strains of FMD virus types A, O and Asia- 1 procured in the form of cell suspension from Veterinary Research Institute, Lahore. In their study, the results of SDS-PAGE revealed that cell culture suspension of FMD virus type O and A segregated into 7 bands while in the virus type Asia-I, the third band was missing. The position of the 3 structural (capsid) proteins i.e. VP2, VP3 and VP1 was indicated having molecular weights 38, 500, 33, 500, and 16, 000 Da respectively. The virus used by Laporte and Lenoir (1973) was a mutant of type O of FMD virus obtained by cloning and subjected to electrophoresis in sodium dodysel sulphate polyacrylamide gel. They observed it to contain equimolar proportions of 4 polypeptides with molecular weights of 34, 20, I7 and 14 K Da. Two minor components were also present with molecular weights of 51 and 25 K Da and in the molar ratio of 0.07 and 0.28. The differences between the results of ours and among the different workers maybe because of difference in the sample used.

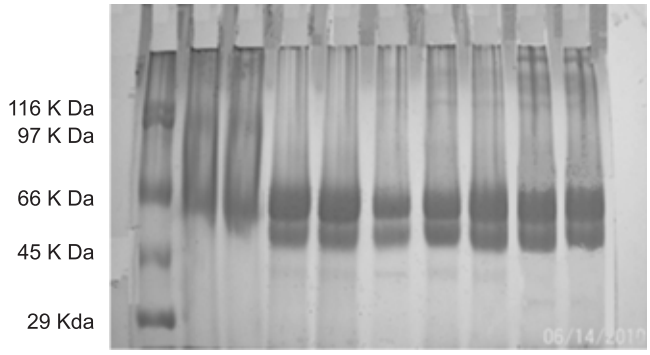

Fig. 1. Electrophoretic pattern of different protein samples.

Well no. 1, Marker proteins: From top-ß galactosidase, phosphorylase rabbit muscle, albumin bovine, albumin egg and carbonic anhydrase; 2 and 3, FMD trivalent vaccine: from top- viral protein 1, 2; no. 4, 5, 6, 7 and 8: Serum of FMD affected cattle: From top- ß globulin, unidentified protein, heptoglobin, albumin, a_2 globulin, FMD viral protein and unidentified protein; no. 9 and 10: serum of control cattle: From top- g globulin, ß globulin, unidentified protein, heptoglobin, albumin, a_2 globulin and unidentified protein.

Hence, it can be concluded that the FMD virus is made up of different proteins having different molecular weights and mobilities.

SUMMARY

The present study was carried out to determine the molecular weight and mobility of the proteins present in the FMD virus. FMD trivalent vaccine and FMD infected serum samples were used for the study. The standard SDS-6H of known proteins was used for calculating the molecular weight of different proteins.The molecular weight of the FMD viral proteins 1 and 2 were 101 and 62 K Da and mobilities were 0.268 and 0.521 respectively. Seven distinct bands segregrated in control and FMD affected serum samples. The bands in the control sample from the top of the gel were identified as γ globulin, ß globulin, unidentified protein, heptoglobin, albumin, α_2 globulin and unidentified protein. Their molecular weights were 150, 140, 120, 100, 60, 48 and 33 K Da and mobilities were 0.094, 0.126, 0.186, 0.268, 0.536, 0.631 and 0.805 respectively. In the FMD affected sample, γ –globulin was undetectable but an extra band was noticed in the infected serum sample which was identified as the FMD viral protein.

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