Abstract

In this study, the full-length M2 gene of the avian influenza virus (H9N2) was isolated, analyzed, and studied in detail. Total RNA was extracted and cDNA of the M2 mRNA was obtained by reverse transcriptase polymerase chain reaction (RT-PCR) using random hexamer oligoenes; specific primers were used for amplification of the M2 open reading frame (ORF) region. PCR was able to amplify the desirable fragment (294-bp) of the spliced M2 gene. The nucleotide sequence homology between the Iranian isolate and other H9 and H5 subtypes of influenza A from different hosts and geographical areas deposited in GenBank ranged from 92 to 98% and the amino acid sequence homology ranged from 97 to 100%.

Keywords: M2 gene; Sequence; RT-PCR; H9N2

Influenza A virus expresses two highly immunogenic, but variable, transmembrane proteins; hemagglutinin (HA) and Neuraminidase (NA) which constantly evolve by the mechanisms of antigenic drift and antigenic shift (Webster et al., 1992). Another transmembrane protein of the influenza A virus is an M2 integral membrane protein. A large number of M2 molecules are expressed at the plasma membrane of the influenza virus-infected cell surface, with a ratio of approximately two M2 molecules per HA (Zebedee et al., 1988). The 97-amino acid M2 protein is a homotetrameric integral membrane protein that exhibits ion channel activity and is composed of 24 extracellular amino acids, 19 transmembrane amino acids, and 54 cytoplasmic residues (Bauer et al., 1999; Holsinger et al., 1991; Lamb et al., 1985). Disulfide bonds link the protein through cysteines located in the extracellular region (Holsinger et al., 1991); the protein (ion channel) is the target of antiviral drugs like amantadine and rimantadine. The ion channel activity of M2 is important both during virion uncoating and during viral budding. Certain mutations in the M2 gene lead to viruses that are resistant to antiviral drugs (Pinto et al., 1992; Hay et al., 1985).

In recent years scientists have focused on the influenza M2 protein as a candidate for a universal vaccine. The present study involved the analysis of the M2 open reading frame (ORF) of the A/chicken/Iran/101/1998 avian influenza (H9N2) isolate using as an inactivated vaccine seed from the Razi Vaccine and Serum Research Institute in order to evaluate the homology level between this isolate and others deposited in GenBank.

For this purpose the avian influenza A/chicken/Iran/101/1998 (H9N2) used as a vaccinal seed in the Marand branch of Iran’s Razi Institute was grown in primary chicken embryo cell cultures and cells were maintained in a humidified air-5% CO₂ atmosphere at 37°C. Then the Influenza virus infected cells were then collected after 18 h of incubation by centrifugation at 3000 ×g for 10 min following cell culture tripinsization.

Total RNA was extracted by the guanidinium isoth-
The M2 gene ORF containing restriction enzymes site sequences and the fragment (1000 pb) of the segment 7 gene belonging to the A/chicken/Iran/101/1998 (H9N2) (Fig. 1). The nucleotide sequence homology between the Iranian isolate and the other GenBank deposited isolates of influenza A viruses from different hosts and geographical areas ranged from 87% to 98% (data not shown). The maximum M2 gene homology was observed between the Iranian isolate and the H9 and H5 isolates from Dubai, Pakistan, and Hong Kong states (Fig. 2).

To perform a direct comparison between the Iranian M2 extracellular domain and those of the other isolates deposited in GenBank, the amino acid sequences of all isolates were compared with that of the A/chicken/Iran/101/1998 (H9N2) representing as a baseline sequence. The amino acid sequence homologies ranged from 92% to 100% among all H5 and H9 isolates of influenza A obtained from different hosts and geographical areas (Fig. 3).

The nucleotide sequence of the Iranian M2 gene isolate diverged by 2% from the most closely related viruses in GenBank. At the nucleotide level based on the M2 gene, the Iranian isolate was more closely related to its neighboring states, such as Dubai and Pakistan. A similar relationship could be seen between the Iranian isolate and the Hong Kong H9 and H5 isolates, exhibiting nucleotide sequence differences of approximately 2% and amino acid sequence homology of 100%. Nucleotides similarities observed between

Figure 1. Analysis of the PCR product on 1.2 % (w/v) agarose gel. Lane 1, contains a 100-bp DNA ladder (Fermentas, Germany); lane 2 and 3, contain positive samples with the upper bands representing segment 7 (26-1027 nt) and the lower bands representing the M2 gene (ORF); lane 4, contains a negative control; lane 5, contains a 1kb DNA ladder (Fermentas, Germany).
the H5 and H9 isolates were more than other isolates that were derived from hosts located in different areas. The N-terminal domain of the influenza A M2 protein is the major part of this immunogenic protein (Neirynck et al., 1999). Hence, in this study the M2 open reading frame was translated and the region between amino acids position 1 and 24 of the M2 protein was analyzed. Sequence alignment based on the amino acid sequences of the M2 protein exhibited less diversity than the nucleotide-based sequence alignments. The 100% homology level between the N-terminal domain of the H5 and H9 isolates was considerable. Those which did not show 100% homology only possessed substitution of 1 and rarely 2 residues within residue 10 and 16 over a span of 24 residues. These changes are consistent with those described in previous reports (Widjaja et al., 2004).

Meanwhile, comparisons of the amino acid sequences of the M2 protein belonging to the Iranian H9N2 isolate with all other isolates of influenza A deposited in GenBank, indicated that the gene and amino acid sequences between all H9 and H5 isolates are more closely related than others. In this study, from the alignment of the M2 gene and comparison of amino acids, it was not possible to find an M2 gene or amino acid substitution that was a host or region-related substitution. They tend to vary more between species than within them and do not appear to change progressively over time, such as the surface glycoproteins.

![Alignment of the M2 gene sequences. Nucleotides matching those of the A/chicken/Iran/101/1998(H9N2) are shown as dashes.](www.SID.ir)
Acknowledgments

This project was financially supported by a grant from the Razi Vaccine and Serum Research Institute (No. 2-18-18-86007). The authors wish to thank Dr. H. Paykari, Dr. M. Esmailzade, Dr. A. Mirjalili and Dr. S.M. Mirafzali, faculty members at the Razi Vaccine and Serum Research Institute, for their helpful contributions to this project.

References


