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ORIGINAL ARTICLE

Researching Correlated Mutations in M2 Proteins of Swine and Avian Influenza a virus

Rafał Filip*, Jacek Leluk, Gabriela Żaroffe
Department of Molecular Biology, Faculty of Biological Sciences, University of Zielona Góra, Zielona Góra, Poland
Corresponding author email: rafalfilip1988@o2.pl

ABSTRACT
The M2 protein plays a key role in the replicative cycle of the influenza A virus. Four identical M2 proteins form the M2 proton channel, which can be inhibited by the antiviral drugs amantadine and rimantadine. The drugs bind to the transmembrane region, sterically blocking the channel and preventing protons from entering the virion. Some mutation points and mutation clusters occurring in M2 proteins causing resistance to amantadine and rimantadine have been reported. In this paper, for different groups (avian, swine or both) of M2 protein sequences were found in new clusters of correlated mutations not previously reported, which may have potential importance for the occurrence of drug resistance. The sequences used in this work have been found in the UniProt database. The Corm program was used to find clusters of correlated mutations.
Keywords: *M2 protein, influenza A virus, correlated mutations, avian (birds), swine, drug resistance*

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INTRODUCTION

The influenza A virus is a pathogen that causes flu. One of the elements involved in the replication cycle of virus is the transmembrane matrix protein (M2). M2 is a small transmembrane protein containing approximately 97 amino acid residues [1]. Four identical M2 proteins form the ph-gated proton channel (M2 channel). The role of the M2 channel is to acidify the virus interior and host cells in which the virus replicates [2,3]. Acidification of host cells and virions enable the replication of a virus and it is a reason for disease [4]. The M2 protein is a target for drugs, specifically inhibitors like amantadine and rimantadine [5]. These two drugs block the channel preventing replication of the virus cycle. These medicaments are not very specific since they block not only virus channels but also host channels. Most strains of the current influenza virus are resistant to the old generation of antiflu medicines and effort is needed to develop new ones. In our previous study the M2 channel is correlated with mutational analysis, in which several potentially important mutations were detected. In this study, we extend our investigation to the correlated mutations of amino acid positions, which are very important in the function of the M2 channel in pathogenic avian H5N1 and swine H1N1 the influenza A viruses.

MATERIALS AND METHODS

M2 proteins amino acids sequences

Sequences were collected from the UniProt Knowledgebase [6] by using the BLAST search [7,8,9]. In this research, 359 M2 protein sequences were collected. Sequences have been arranged in three groups: a group containing sequences of viruses whose hosts are birds (group 1), a group containing sequences of viruses whose hosts are swine (group 2) and a group containing sequences of viruses whose hosts are both birds, swine (group 3) or an unidentified (group 4).

Bioinformatics software used in this publication

Consensus Constructor program – This was used to generate a consensus sequence [10]. Program parameters used in analysis: identity 69.62% (55), significance 29.11% (23), gaps 49.37% (39).

ClustalX and ClustalW – This software was used to do a multiple sequence alignment [11,12,13].
Corm – The Corm program was used to find correlated mutations within multiple sequence alignment data [14]. Program parameters were used to calculate minimum counts of amino acids: 3; maximum identity threshold: 98%.

RESULTS AND DISCUSSION

Consensus sequences

Figure 1. Multiple sequence alignment for four groups: sequence group belonging to viruses whose hosts are birds (Con.2), sequence group belonging to viruses whose hosts are swine (Con.3), sequence group belonging to viruses whose hosts are both birds and swine or whose host is unidentified (Con.1), and a group created from all sequences (Con.4).

Consensus sequences differ from each other and depend on the hosts. Most of the differences occur between avian and swine consensus sequences. The most important mutation seems to be Val27Ile, which resists amantadine and rimantadine [15]. In the sequence group belonging to viruses whose hosts are swine 73 to 132 (55.3%) at this position are isoleucine. In other groups, this mutation is also present, but occasionally, consensus sequence alignment (Figure 1) shows the most differences between the sequence group belonging to viruses whose hosts are birds and the sequence group belonging to viruses whose hosts are swine. Nine amino acid differences between them were found. There is no information in the literature on which features in the protein may present in amino acid mutations. However, these differences between groups may cause an evolutionary adaptation of viruses to their hosts.

Correlated mutations

The Corm program found various clusters of correlated mutations in three groups' multiple sequence alignments: sequences of viruses whose hosts are birds, sequences of viruses whose hosts are swine, and sequences of viruses whose hosts are both birds and swine or whose host is unidentified. Finally, there is the consensus sequence for all sequences. Results are shown below.

Table 1. Correlated mutations in sequence group belong to viruses whose hosts are birds.

Amino acid position	Number of sequences	Correlated positions
20(K)	4	10(L), 32(V)
20(N)	22	10(HP), 32(I)
20(S)	120	10(HLP), 32(FIV)
28(A)	3	20(N)
28(F)	4	20(S)
28(I)	56	20(DKNS)
28(V)	83	20(KNRS)
54(F)	7	78(KN), 86(AT), 93(S)
54(R)	140	78(Q), 86(V), 93(N)
78(K)	6	54(F), 86(AT), 93(S)
78(Q)	141	54(LR), 86(V), 93(N)
79(E)	145	95(-EKQ)
79(K)	3	95(V)
86(A)	6	54(F), 78(KN), 93(S)
86(V)	141	54(LR), 78(Q), 93(N)
89(G)	138	10(HLP)
89(S)	7	10(P)
89(V)	3	10(L)
93(N)	141	54(LR), 78(Q), 86(V)
93(S)	7	54(F), 78(KN), 86(AT)
95(E)	142	79(E)
95(V)	3	79(K)

Table 2. Correlated mutations in sequence group belong to viruses whose hosts are swine.

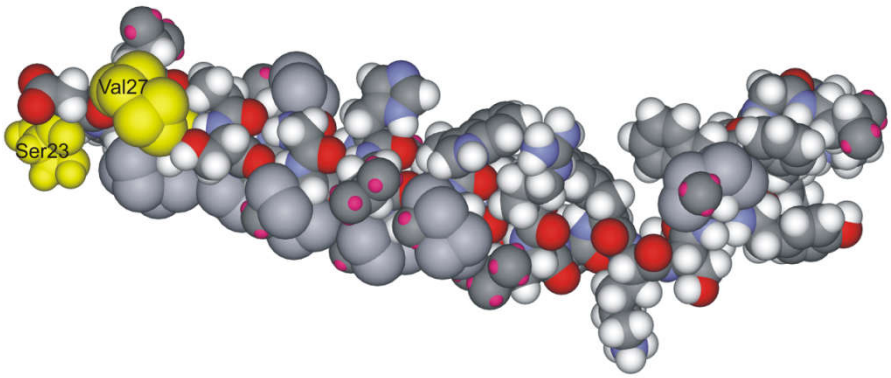
Amino acid position	Number of sequences	Correlated positions
23(G)	6	27(V)
23(N)	7	27(IT)
23(S)	119	27(FILSTV)
27(I)	73	28(ACDGTV), 60(KQ)
27(T)	4	28(D), 60(Q)
27(V)	51	28(AFITV), 60(K)
28(A)	66	27(FILV), 60(KQ)
28(D)	37	27(IST), 60(Q)
28(I)	12	27(V), 60(K)
28(V)	11	27(IV), 60(K)
95(-)	3	96(-), 97(-)
95(A)	13	96(L), 97(E)
95(E)	20	96(L), 97(EK)
95(V)	92	96(LS), 97(-ET)
96(-)	3	95(-), 97(-)
96(L)	124	95(AELMSV), 97(-EK)
96(S)	5	95(V), 97(T)
97(-)	5	96(-L)
97(E)	120	96(L)
97(T)	5	96(S)

Table 3. Correlated mutations in sequence group belong to viruses whose hosts are both birds, swine or host is unidentified.

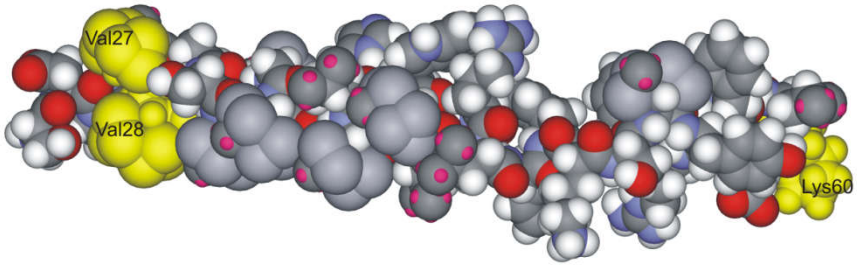
Amino acid position	Number of sequences	Correlated positions
1(-)	3	2(-)
1(M)	75	2(S)
2(-)	3	1(-)
2(S)	75	1(M)
27(I)	4	31(S), 70(E)
27(T)	3	31(KN), 70(DK)
27(V)	70	31(NS), 70(EK)
28(A)	9	79(K)
28(I)	18	79(EK)
28(V)	51	79(E)
36(L)	72	54(CFLRS)
36(V)	6	54(I)
48(F)	72	50(C)
48(S)	5	50(S)
50(C)	73	48(FL)
50(S)	5	48(S)
54(F)	14	36(L), 43(L), 57(H), 78(KN)
54(I)	6	36(V), 43(I), 57(H), 78(E)
54(L)	12	36(L), 43(L), 57(HY), 78(EKQ)
54(R)	44	36(L), 43(ILT), 57(Y), 78(KNQ)

M2 proteins are highly conservative but some clusters are still possible to mutate. In the results for individual groups, multiple sequence alignments are different but some positions in the clusters are the same. Some clusters and positions responsible for drug resistance and important ant for the functioning of the channel have been reported in publications [16]. These include the following mutations: L26F,

V27A, A30T, S31N, G34E, L38F. In this study, there are also clusters containing some of the above positions: 23-27, 27-28-60 in the group belonging to viruses whose hosts are swine. 27-31-70 are in the group belonging to viruses whose hosts are both birds and swine or whose host is unidentified. Position 27 plays an important role in the function of the channel. It is a place to interact with the channel inhibitors, the antiviral drugs amantadine and rimantadine [17,18,16]. Position 27 is located at the narrowest region of the channel pore. Drugs blocking the pore of the channel interact with the 27th amino acid. Amantadine inside the duct is surrounded by the amino acids, in which mutations can lead to resistance to this drug. Such may be the amino acid occurring in position 31, which does not interact directly to the drug. Position 27 is functionally related to the position 31 occupied primarily by serine.
In the sequence group belonging to viruses whose hosts are birds, the position 27 is occupied firstly by valine but also isoleucine. The position 31 is occupied by serine and there are only a few sequences with asparagine. Presented here, bird sequences with point mutation Ser31Asn belong to only two strains: H5N1 and H9N2. The samples of H5N1 come from North China (years 2001 - 2005). 83.3% of this H5N1 sample are resistant for amantadine, and 80% from this 83,3 have point mutation Ser31Asn [19].
The third group identified is the following cluster: 36-43-54-57-78. In the model M2 (18-60), the amine group of rimantadine is at a distant hydrogen bond from the carboxyl group of Asp44. The adamantyl group interacts with the hydrophobic parts of the chain formed by Leu40, Ile42, and Leu43 [20]. At position 43 is leucine; in the case of seven sequences, isoleucine appears there. Beyond that in this cluster is position 36, which is close to His37, which functions as a pH sensor [21,22,23]. There is no information about the exact share of these positions in the functioning of the channel.
The most interesting clusters were shown below in the M2 protein molecule fragment.

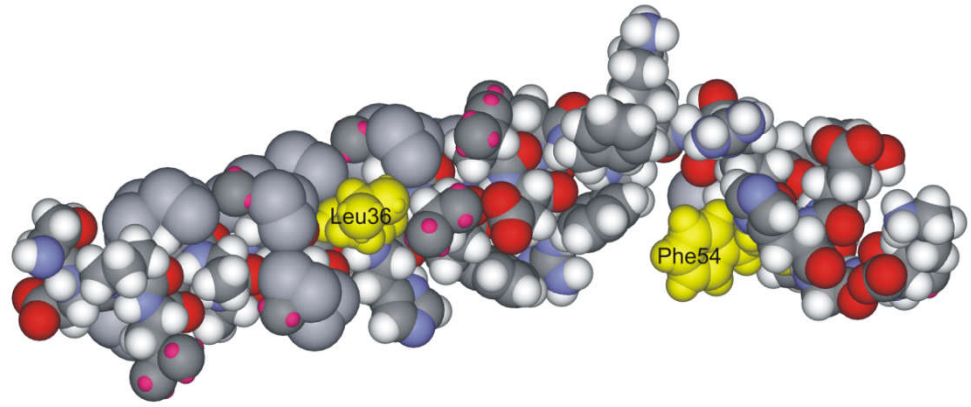


[a]

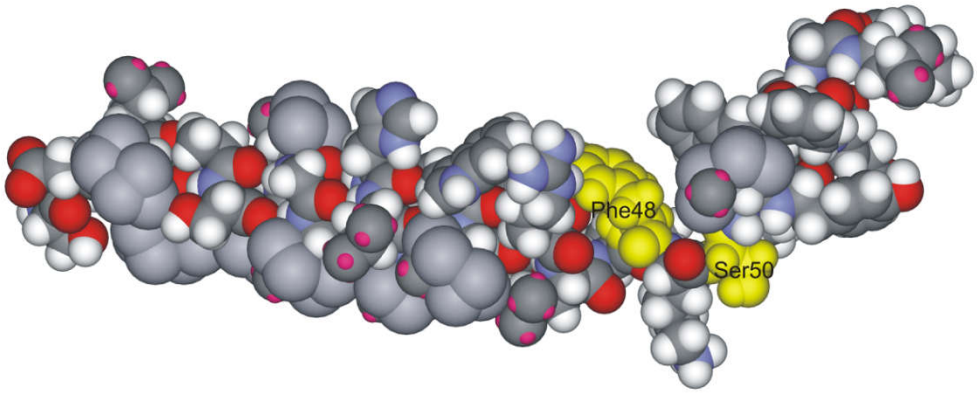


[b]

Figure 2a and 2b. Selected correlated mutations in the sequence group belonging to viruses whose hosts are swine shown in a molecule fragment.



[a]



[b]

Figure 3a and 3b. Selected correlated mutations in a sequence group belonging to viruses whose hosts are both birds and swine or whose host is unidentified shown in a molecule fragment.

CONCLUSION

Most clusters of correlated mutations shown by Corm are not reported in literature as essential to the functioning of the M2 proton channel. These are new results (clusters, single positions), whose function is unknown—but these results may show the direction in finding a mutation responsible for the resistance of some virus strains, and new drugs.

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