Trends in Global Warming and Evolution of Matrix Protein 2 Family from Influenza A Virus

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Abstract: The global warming is an important factor affecting the biological evolution, and the influenza is an important disease that threatens humans with possible epidemics or pandemics. In this study, we attempted to analyze the trends in global warming and evolution of matrix protein 2 family from influenza A virus, because this protein is a target of anti-flu drug, and its mutation would have significant effect on the resistance to anti-flu drugs. The evolution of matrix protein 2 of influenza A virus from 1959 to 2008 was defined using the unpredictable portion of amino-acid pair predictability. Then the trend in this evolution was compared with the trend in the global temperature, the temperature in north and south hemispheres, and the temperature in influenza A virus sampling site, and species carrying influenza A virus. The results showed the similar trends in global warming and in evolution of M2 proteins although we could not correlate them at this stage of study. The study suggested the potential impact of global warming on the evolution of proteins from influenza A virus.

Key words: evolution, global warming, influenza A virus, matrix protein 2.

1 Introduction

The global warming imposes the new danger not only on environments, but also on humans and various species (Thomas *et al.*, 2004). In other words, we would say that the global warming would have effects on the evolution of various species, as a result we would also expect to see the composition of species, for example, proteins, under the influence of global warming although some proteins could be hidden deeply inside cells. Thus, it is important to compare the trends in global warming and protein evolution of interest family in order to see if there are similar trends in both.

It is well known that the evolution of protein family is a process of mutations, therefore we could represent this evolution if we could represent mutated proteins along the time course. We need to do so because the global warming is the change in temperature over time. However, a mutation in protein is an event of changing one letter to another because amino acids in protein are presented as 20 letters, which are neither scalar data nor vectors, whereas the temperature is a scalar datum.

This means that we need to convert the letter-based proteins into scalar data in order to plot them along the time course to see their evolutionary trend. Actu-

ally there are quite a few methods, which can transfer a protein sequence into a series of numerical codes or numeric sequence for predicting its various attributes (Chou, 2001, 2005c; Xiao *et al.*, 2005, 2006, 2007, 2008, 2009a,b; Xiao and Chou, 2007; Ding *et al.*, 2007; Zhang *et al.*, 2008). Since 1999, our group has developed three approaches to convert either a single amino acid or a protein into a scalar datum based on random principle (Wu and Yan, 2002, 2006a,b, 2008a). Using our approaches, we can effectively represent a protein family over time, which provides the basis for conducting the study on analyzing the trends in global warming and evolution of proteins of interest.

At this moment, we are particularly interest in the matrix protein 2 (M2) from influenza A virus, because the influenza is threatening the world with possible pandemics. Among various studies on influenza A virus (Gong *et al.*, 2009; Wei *et al.*, 2006; Wang *et al.*, 2007, 2009; Du *et al.*, 2007, 2009; Guo *et al.*, 2008; Huang *et al.*, 2008), much more progress has been made recently on M2 protein (Schnell and Chou, 2008; Pielak *et al.*, 2009). The M2 protein of influenza A virus forms a proton channel in the virion and is essential for infection (Betakova, 2007; Pinto and Lamb, 2007). The M2 ion channel blockers approved to treat influenza virus infections (Beigel and Bray, 2008; Hayden, 2006; Kelly *et al.*, 2003). A proof-of-concept study shows that the

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endocytosis of virion into host cells can be a valid drug target because M2 protein is involved in the endocytosis process (Hsieh and Hsu, 2007). However, the use of M2 inhibitors is limited by high frequencies of their resistance among currently circulating strains (Basler, 2007; Hayden, 2009). Also, it is promising that a vaccine is based on the conserved ectodomain of M2 protein from influenza A virus (Schotsaert *et al.*, 2009).

Many studies from various research laboratories around the world have indicated that mathematical analysis, computational modeling, and introducing novel physical concept to solve important problems in biology and medicine, such as modeling important 3D protein structures for drug design (Chou, 2004a-d, 2005a) molecular docking (Huang *et al.*, 2008, Du *et al.*, 2009; Chou *et al.*, 2003; Zhou *et al.*, 2004; Zhou and Troy, 2003, 2005a,b; Zhang *et al.*, 2006; Gao *et al.*, 2007), molecular packing (Chou *et al.*, 1984, 1988), pharmacophore modelling (Sirois *et al.*, 2004; Chou *et al.*, 2006), Mote Carlo simulated annealing approach (Chou, 1992; Wu, 1998a,b; Wu and Yan, 2001), diffusion-controlled reaction simulation (Chou and Jiang, 1974; Li and Chou, 1976; Chou and Zhou, 1982), graph/diagram approach (Chou *et al.*, 1979; Chou and Forsen, 1980; Chou and Liu, 1981; Chou, 1981, 1989a, 1990; Zhou and Deng, 1984; Myers and Palmer, 1985; Kuzmic *et al.*, 1992; Althaus *et al.*, 1993, 1993a,b; Andraos, 2008), bio-macromolecular internal collective motion simulation (Chou and Chen, 1977; Chou, 1983a,b, 1984a-c, 1987, 1988, 1989b; Zhou, 1989), QSAR (Prado-Prado *et al.*, 2008; Gonzalez-Diaz *et al.*, 2006, 2008; Dea-Ayuela *et al.*, 2008), protein subcellular location prediction (Chou and Shen, 2006, 2007b,c, 2008, Zhou and Doctor, 2002), identification of GPCR and their types (Xiao *et al.*, 2009a; Chou and Elrod, 2002; Chou, 2005b), identification of proteases and their types (Chou and Shen, 2008a,b; Shen and Chou, 2009; Zhou and Cai, 2006), protein cleavage site prediction (Chou, 1993, 1996; Shen and Chou, 2008), and signal peptide prediction (Chou and Shen, 2007a; Shen and Chou, 2007) can timely provide very useful information and insights for both basic research and drug design and hence are widely welcome by science community. The present study is attempted to analyze the trends in global warming and evolution of matrix protein 2 family from influenza A virus in hope to provide useful insights on the potential impact of global warming on the evolution of proteins from influenza A virus.

2 Materials and Methods

2.1 Temperature data

The global, north and south hemispheric temperature anomalies from 1850 to 2007, whose anomaly is based on the period 1961-1990, were obtained from

HadCRUT3v (Rayner *et al.*, 2006; Climatic Research Unit, 2008). The local temperature from 1959 to 1998 based on 0.5 by 0.5° latitude and longitude grid-box basis cross globe was obtained from New *et al.* (2000).

2.2 M2 data

A total of 5926 full-length M2 sequences of influenza A virus sampled from 1959-2008 was obtained from the influenza virus resources (Influenza virus resources, 2009). After excluded identical sequences, 1084 M2 proteins were used in this study.

2.3 Converting M2 proteins into scalar data

For presenting M2 protein family along the time course, we need to convert each M2 protein into a scalar datum that must differ for different M2 proteins. Among our three random approaches (Wu and Yan, 2002, 2006a, b, 2008a), the simplest one is the amino-acid pair predictability, by which we view if the combination of two adjacent amino acids can be explained by the permutation. For a whole protein, we can determine the percentage of how many amino-acid pairs can be predicted according to the permutation. We have used this method in many studies (Wu and Yan, 2008a-d).

For an M2 protein, we counted the first and second amino acids as a pair, the second and third amino acids as another pair, until the next to terminal and the terminal amino acids as the last pair. Then, we determined whether an amino-acid pair could be explained by permutation, or predicted by random mechanism in other words. Finally we calculated the percentage of how many amino-acid pairs in an M2 protein were predictable and unpredictable.

For example, an M2 protein of swine H1N1 influenza A virus isolated from USA in 1976 (accession number ABQ45416) was composed of 97 amino acids. This M2 protein had 8 serines “S” and 10 leucines “L”. If the appearance of amino-acid pair SL could be explained by permutation, it would appear once in this M2 protein ($8/97 \times 10/96 \times 96 = 0.82$). Actually there was only one SL in this M2 protein, so the appearance of SL was predictable. By clear contrast, there were 4 alanines “A” in this M2 protein. According to the permutation of amino-acid pairs, AA would not appear ($4/97 \times 3/96 \times 96 = 0.12$) in this M2 protein. However, it appeared twice in reality and was unpredictable. In this way, we classified all of the amino-acid pairs in this M2 protein as predictable and unpredictable.

It is absolutely necessary that the predictable/unpredictable portion is subject to a tiny difference between two M2 proteins, thus different M2 proteins should have different values to be distinguishable. In the past, we have tested many proteins to verify this request and got the positive answer (for review, see Wu and Yan, 2002, 2006a,b, 2008a). For instance, the predictable and unpredictable portions were 25% and 75% for ABQ45416 M2 protein. For

another swine H1N1 influenza A viruses isolated from USA in 1976 (accession number ABQ45438), its M2 protein has only one amino acid at position 18 different from that of ABQ45416 M2 protein. However, its predictable and unpredictable portions were 24.72% and 75.28%.

In this manner, we converted 1084 letter-symbolized M2 proteins into 1084 scalar data (Amino-acid pair predictability, 2009). As each M2 protein had its sampling year, we thus had two scalar datasets, the temperature recorded each year and the unpredictable portion of M2 protein sampled each year. Hence we could plot both datasets along the time course to observe their trends.

3 Results and Discussion

Figure 1 showed the trends in both global warming and evolution of M2 proteins, where both trends revealed quite similar as indicated by their regressed lines. The unpredictable portion of M2 proteins increased over time, which was similar to that the global temperature increased along the time course.

Fig. 1 Global temperature anomaly ($^{\circ}\text{C}$) and evolution of M2 proteins from influenza A viruses. The dotted lines and points were regressed lines and the mean of all M2 proteins at a given year ($n=1084$ from 1959 to 2008).

Moreover, the global temperature was generally divided into north and south hemisphere, so we could group M2 proteins accordingly to see if the trend still held on in such circumstance. As shown in Fig. 2, the similar trend was much clearer in north hemisphere than in south hemisphere, which could be explained by the fact that most of M2 proteins were sampled in north hemisphere.

Actually the data of M2 proteins in Fig. 1 and Fig. 2 were averaged in each year, for example, there was only a single sample in 1959, but 58 M2 proteins were sampled in 2007. Another way to analyze the trends is to apply the point-to-point method, that is, we coupled each M2 protein with temperature according to

Fig. 2 Trends in temperature and M2 evolution grouped according to north ($n=987$) and south ($n=72$) hemispheres. The dotted lines were regressed lines.

the sampling place and year. In other word, we took the temperature measured at each geographical latitude and longitude of place where an M2 protein was sampled at the same year to make the comparison.

For example, there was a chicken H5N1 virus (accession number ABI85108) sampled in 1959 at Scotland, whose latitude and longitude were 56.49 and -4.20 according to Get Lat Lon (Get Lat Lon, 2009). Its average yearly temperature was 7.5°C in 1959 according to the 0.5° by 0.5° latitude and longitude grid-box basis cross globe obtained from New *et al.* (2000).

Figure 3 displayed 279 point-to-point relationships between temperature and unpredictable portion of M2 proteins from 1959 to 1998, and their regression indicated the similar trends. The results in Fig. 3 were in consistent with what we found in Figs. 1 and 2, that is, there were similar trends between global warming and evolution of M2 proteins.

Because influenza viruses are hosted in different species, we could advance our analysis by the point-to-point relationship between temperature and species, from which the M2 proteins were sampled. Figure 4 demonstrated the trends of evolution of M2 proteins with respect to temperature in three major host species. The results suggested that the trends were parallel in human, similar in swine, but different in avian.

In this study, we found the similar trends in global warming and evolution of M2 proteins from influenza A virus. This is very suggestive, because this founding indicates that the effect of global warming on many dif-

Fig. 3 Point-to-point temperature versus M2 proteins ($n = 279$) from 1959 to 1998. Each point presented a local temperature ($^{\circ}\text{C}$) at the given year (upper panel), corresponding to the place where an M2 protein was sampled (lower panel). The dotted lines were regressed lines.

ferent levels on biological evolution, even the proteins hidden inside cell could be subject to the global warming. This is understandable because all the biological functions are interconnected from macro level to micro level.

However, can we correlate both trends in this study statistically? At this stage, it would be difficult to determine such correlation because (i) to the best of our knowledge there is no statistical method available to determine the correlation between two lines including a discontinued one in this study; and (ii) many statistical books tell that the correlation does not mean the cause-consequence relationship, that is, even we would find the so-called correlation between two trends, we still need to determine if there is any direct or indirect cause-consequence relationship.

On the other hand, we cannot ignore these trends because we cannot create another earth without global warming but with active influenza virus for comparison over the same time scale. As the validation of global warming is done through the comparison along the time course, we would argue that the validation of M2 protein evolution should also be done along the time course, i.e. the comparison between any two different time points.

This study demonstrated the changes in the unpredictable portions of M2 proteins were different in different species. In human and swine, the trends of evolution of M2 proteins were similar to that of temperature, but not in avian (Fig. 4). This difference could be due to the fact that the place where avian was sampled would not be the place where the mutation occurred, because migratory birds are common reservoirs responsible for spreading avian influenza viruses (Krauss *et al.*, 2004; Garamszegi and Møller, 2007; Weber and Stilianakis, 2007; Jahangir *et al.*, 2008), on the other hand the human and swine were generally localized.

Fig. 4 Global temperature anomaly ($^{\circ}\text{C}$) and M2 evolution sampled from different species.

Although the trends in global warming and M2 evolution are similar in this study, they only indicate the direction for studies in future, because we would not expect to determine such an important issue within a few studies, and we still have much to discuss.

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