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A bioinformatic approach to check the spatial epitope structure of an immunogenic protein coded by DNA vaccine plasmids

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HIGHLIGHTS

- Manipulation of an immunogenic gene to use as a DNA vaccine is a routine.
- Structures of DNA vaccine coded proteins were modeled and docked with antibodies.
- The effect of H9 gene fusion to gene segments on H9 spatial epitopes was evaluated.
- 3D structure of DNA vaccine immunogenic protein must be considered by researchers.

ARTICLE INFO

ABSTRACT

In this study, we used an approach to check the Hemagglutinin antigen–antibodies interactions after fusion of one or two gene segments to Hemagglutinin gene in some influenza DNA vaccines. We designed different DNA vaccine constructs containing Hemagglutinin 9 (H9) gene fused to four or eight 29 amino acids of C3d (4/8P29C3d) and/or 3, 4 domains of the Fc part of IgY (FcIgY) coding sequences. As there are receptors for P29C3d and FcIgY on the immune cells, fused H9 are targeted to these cells. Three dimensional (3D) structures of the DNA vaccine coded proteins were modeled and docked with two antibodies (1KEN, 1QFU) to evaluate the effect of the H9 gene fusion to the other gene segments (4, 8 P29C3d and FcIgY) on the interaction of two H9 spatial epitopes. Also, we docked DNA vaccine proteins containing Fc IgY to its receptor (CHIR AB1) and compare interaction affinity of Fc IgY alone with affinity of DNA vaccines containing Fc IgY. The average of 1KEN and 1QFU interface scores were 94.89 and 93.09% of H9 DNA vaccine–antibodies interface scores, respectively. These percentages showed a little change in the H9 immunogenic parts. Also, because of spatial freedom of H9 part in all DNA vaccine proteins, added parts may not interfere with antibody–antigen interactions. Once, H9+FcIgY and CHIR AB1 affinity decreased in comparison with affinity of Fc IgY alone and CHIR AB1, affinity of H9+8P29C3d+FcIgY and CHIR AB1 increased to 132%. So, this would be expectable that despite of loss of affinity in H9 and its antibodies in the H9+8P29C3d+FcIgY, dramatic increase of Fc IgY and CHIR AB1 affinity in this group, could repair the loss of H9 affinity and may lead to a better immunogenicity.

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1. Introduction

DNA vaccine is a plasmid DNA which encodes one or more genes of immunogenic proteins of infectious agent and is directly administered to a human or an animal. The advantages of the DNA vaccines are their action in the presence of maternal antibodies, its stability, save of the cost and the non-requirement of cold chain (Stenler et al., 2014). Because hemagglutinin of influenza virus is

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highly variable, it is desirable to develop vaccines that can be easily adapted to the new circulating strains and the DNA vaccine is good candidate for achieving this goal (Yan et al., 2014).

We have designed some targeted hemagglutinin 9 Influenza DNA vaccines for chickens which are supposed to be more effective than a simple plasmid encoding wild type hemagglutinin. Because there are receptors for C3d complement component (Li et al., 2009; Zhang et al., 2010, 2011) and domains 3 and 4 of Fc IgY (Taylor et al., 2008; Purzel et al., 2009) on dendritic cells, B lymphocytes and macrophages (antigen presenting cells (APC)), different gene segments such as four or eight repeats of 29 amino acids of C3d complement component (P29C3d) and domains 3 and 4 of the Fc part of IgY (FcIgY) were fused to hemagglutinin 9 (H9) gene in this study. Proteins encoded by DNA vaccines are targeted to the surface of APCs providing better importation into the APCs following likely better processing and more efficiency. Therefore, the DNA vaccine of Influenza may lead to a better immunogenicity than that of a DNA vaccine encoding H9 alone. Codon optimized (Jiang et al., 2007) and secretory form (Kim et al., 2003) of H9 gene was used to have better DNA vaccines. To the best of our knowledge, this study is the novel use of Fc IgY as an adjuvant in DNA vaccines.

The knowledge of protein 3D (three-dimensional) structures is important. Although X-ray crystallography is a powerful tool in determining protein 3D structures, it is time-consuming and expensive, and not all proteins can be successfully crystallized. Also, NMR is a very powerful tool in determining the 3D structures of membrane proteins (Berardi et al., 2011; OuYang et al., 2013), but it is also time-consuming and costly. To acquire the structural information in a timely manner, one has to resort to various computational or structural bioinformatics methods (Chou et al., 2003; Chou, 2004).

Three-dimensional (3D) structure of proteins encoded from different constructs of DNA vaccines were modeled and docked with two antibodies to evaluate the effect of fusion of H9 gene to the other gene segments (4, 8 P29C3d and FcIgY) on the interaction of two H9 spatial epitopes(Du et al., 2007). Also, we docked DNA vaccine proteins containing Fc IgY to its receptor CHIR AB1 (Purzel et al., 2009) and compare interaction affinity of Fc IgY alone with affinity of DNA vaccines containing Fc IgY.

The information of a binding pocket for its ligand is very important (Chou, 2004). In the literature, the binding pocket of a protein receptor to a ligand is usually defined by those residues that have at least one heavy atom (*i.e.*, an atom other than
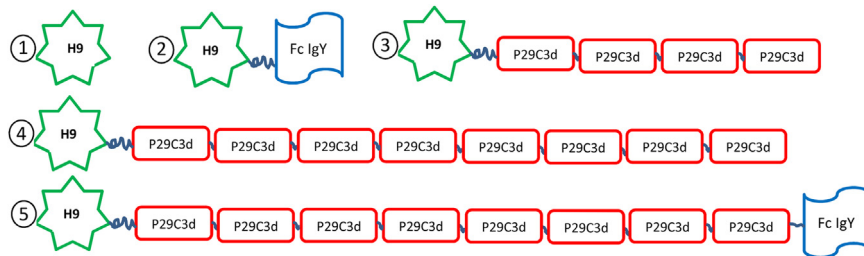
Fig. 1. Schematic figures of designed DNA vaccine coded proteins: (1) H9, (2) H9+FcIgY, (3) H9+4P29C3d, (4) H9+8P29C3d and (5) H9+8P29C3d+FcIgY. As the number of P29C3d repetition may effect on the immunogenicity of DNA vaccine, two DNA constructs were designed with four and eight repeats of P29C3d. There is DNA linkers between two gene segments providing spatial freedom of protein domains and contain some restriction sites to be used in the subcloning of DNA constructs.
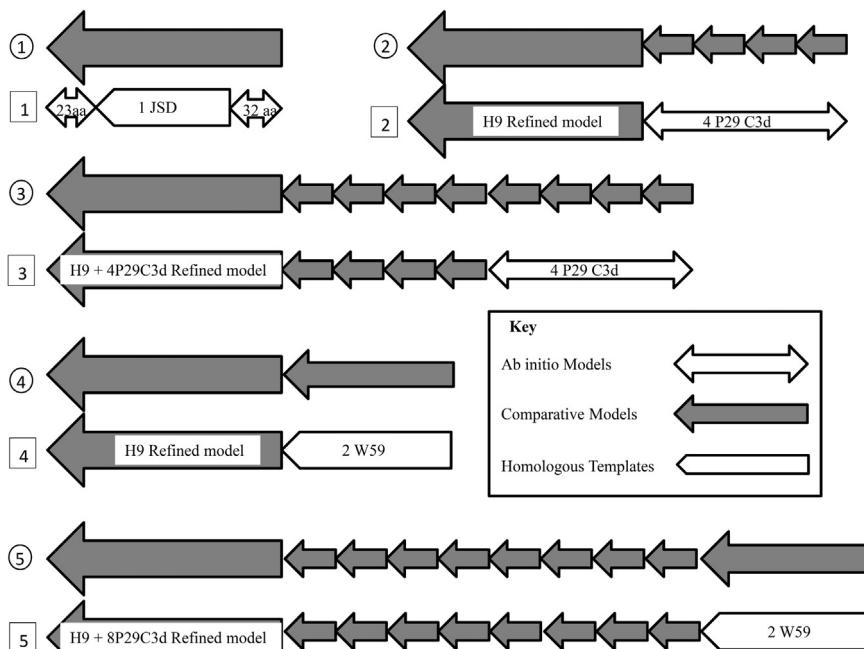
Fig. 2. Methods used in the DNA vaccine protein modeling: schematic figures of models (numbered in circles) and used templates (numbered in squares): (1) H9, (2) H9+4P29C3d, (3) H9+8P29C3d, (4) H9+FcIgY and (5) H9+8P29C3d+FcIgY. 23 aa and 32 aa indicates the number of amino acids of H9 DNA vaccine amino acids that did not align with H9 homolog, so their templates created by *ab-initio* modeling. Each model was used as template in next modelling. 1JSD: PDB ID of H9 homolog, 2W59: PDB ID of Fc IgY homologue.

hydrogen) within a distance of 5 Å from a heavy atom of the ligand (Chou et al., 1999; Zhang et al., 2002). In order to really understand the action mechanism of receptor-binding, we should consider not only the static structures concerned but also the dynamical information obtained by simulating their internal motions or dynamic process. To realize this, the flexible docking is one of the feasible tools (Chou, 1988).

Addition or deletion of gene segment(s) to an immunogenic gene to use as a DNA vaccine is a routine in the field of DNA vaccine research and 3D structure of coded immunogenic protein have to be considered by researchers.

2. Methodology

2.1. DNA constructs

Five DNA constructs were designed by Clone Manager (v7.01) base on the H9 gene followed by four or eight repeats of 29 amino acids of C3d coding sequences and/or domains 3, 4 of Fc IgY coding sequences. The first DNA construct had H9 gene alone (H9), the second and the third constructs have H9 gene fused to four (H9+4P29C3d) and eight repeats of 29 amino acids of C3d coding sequences (H9+8P29C3d), the fourth construct contains H9 gene fused to domains 3, 4 of Fc IgY coding sequences (H9+FcIgY) and the fifth one has H9 gene fused to eight repeats of 29 amino acids of C3d and domains 3, 4 of Fc IgY coding sequences (H9+8P29C3d+Fc IgY) (Fig. 1).

2.2. Modeling

2.2.1. Comparative modeling

The process of comparative modeling of protein structure usually needs first an existing template structure or a template created for some parts which did not have any homologous structure, secondly sequence-structure alignments (PIR format) and finally building and evaluating models. The modeling process is divided into three steps. (1) Initial construction of comparative models achieved by MODELLER (v9.9). (2) Energy minimization of models facilitated by HyperChem Professional software (v7). (3) Models were evaluated by ERRAT (Colovos and Yeates, 1993), VERIFY3D (Luthy et al., 1992), PROCHECK (Laskowski et al., 1993) and WHAT IF(Hooft et al., 1996) on the web and were visualized by Swiss PdbViewer (v4).

2.2.2. Ab-initio modeling

Since for some parts of the sequence a homolog was not found, *ab-initio* modeling was used. In *ab-initio* methods, an initial effort to elucidate secondary structures (alpha helix, beta sheet, beta turn, *etc.*) from primary structure is made by utilization of physicochemical parameters and neural net algorithms. From that point, algorithms predict tertiary folding. Robetta server (http://robetta.backerlab.org) was used for *ab-initio* modeling (Simons et al., 1997).

2.2.3. Modeling of DNA vaccines proteins

Both *ab-initio* and comparative modeling were used for the modeling of DNA vaccines proteins. Proper homologous sequences were found for H9 and Fc IgY but not for four or eight repeats of C3d P29. For modeling of H9, amino acid sequence of H9 homolog (PDB ID: 1JSD) was aligned with DNA vaccine coded H9 sequence. 23 and 32 amino acids of the beginning and the end of our H9 protein sequence were not aligned with H9 homolog. Therefore, *ab-initio* modeling was used to construct the 3D structure of these parts (Fig. 2). Finally, using the result of the *ab-initio* modeling an alignment file (PIR) for the whole H9 homolog was obtained.

In order to model the complex of H9+4P29C3d protein, the previous refined H9 model and four repeats of P29C3d model obtained by *ab-initio* modeling were utilized. The complex of H9+8P29C3d protein was modeled by the use of the H9+4P29C3d refined model and another 4P29C3d *ab-initio* model and finally, H9+8P29C3d refined model and Fc IgY pdb file (PDB ID: 2W59) were used for modeling of H9+8P29C3d+FcIgY (Fig. 2). The crystal structure of CHIR AB1 (PDB ID: 2VSD) was retrieved from PDB database (www.rcsb.org/).

2.2.4. Evaluating the DNA vaccine protein models

Initial models were subjected to evaluation, mainly by visual examination of structural consistency in Swiss-PdbViewer (v4), Viewer Lite (v4.2) and structure matching with the secondary structure prediction using Jpred3 server (http://www.compbio.dundee.ac.uk/www-jpred/) (Cole et al., 2008). Two types of evaluation were performed: stereo chemical quality and side chain environment based on web services such as PROCHECK, WHAT IF, VERIFY3D and ERRAT (http://nihserver.mbi.ucla.edu/SAVES/). PROCHECK test was employed to assess the quality of the conformation of the polypeptide backbone and side chains using a Ramachandran plot. The steric overlap of atoms (bad clashes) was checked using bump check within WHAT IF.

VERIFY3D and ERRAT were used to assess the compatibility between the amino acid sequence and the environment of the amino acid side chains in the model. The result of ERRAT program was the main factor for examining of the progress in comparative modeling of target protein. Also, there were logical agreement between the position of helices and sheets in each generated model and those in the secondary structure prediction by Jpred3 server. This agreement approved the accuracy of modeling procedure for target protein. Also, the changes of H9 3D structure in different models were determined by Swiss-PdbViewer (v4) and reported as Root Mean Square (RMS).

2.2.5. Model regeneration and refinement

If the modeling was unsuccessful (bad points and scores in evaluation), we stepped backward and realigned or regenerated

Table 2
Root Mean Square (RMS) of H9 in the DNA vaccine proteins.

RMS	H9+4C3d	H9+8C3d	H9+FcIgY	H9+8P29C3d+FcIgY
H9	1.76	2.1	1.23	1.83

Table 1
Different checking results of the DNA vaccine protein models. Pre- and post-refinement results are shown and the best model used in the docking step (gray columns).

HyperChem refinement	H9		H9+4P29C3d		H9+8P29C3d		H9+FcIgY		H9+8P29C3d+FcIgY	
	Before	After	Before	After	Before	After	Before	After	Before	After
Procheck (Ramachandran plot, core %)	84.9	72.5	86.3	73.7	86.1	70.4	90.2	76.7	86.9	68.5
Errat	65.9	71.82	64.8	76.9	60.4	76.8	68.6	78.6	57.9	70.3
Verify 3D (%)	72	76.77	78.4	81.6	74.4	76.2	76.3	78.3	59.2	0

models sometimes with totally different alignment or template till an acceptable model was created. To improve the models, we used HyperChem Professional software (v7) to minimize the free energy of models and provide more scores in rechecking the models in evaluation sites and programs.

In the Modeller software (v9.9) CHARMM force filed has been used. The constructed models were optimized with the variable target function method with conjugate gradient and were refined using molecular dynamics with simulated annealing. Then the models were subjected to molecular mechanics optimization with Polak–Ribiere algorithm using HyperChem Professional software.

2.3. Docking

Each modeled DNA vaccine protein was docked with two hemagglutinin antibodies (PDB ID: 1QFU and 1KEN) by ROSIE server (http://antibody.graylab.jhu.edu/) and The ROSIE server performs a local docking search which means that the algorithm searches a set of conformations near the given starting conformation for the optimal fit between two partners (Lyskov and Gray, 2008; Lyskov et al., 2013). In order to have the best starting conformation, H9 of modeled DNA vaccines were replaced hemagglutinin of the original antibody-hemagglutinin PDB taken from RCSB site by Swiss-Pdb Viewer (v4). Finally, the least interface score of each docking which is associated with binding affinity of that partner was recorded and the least interface scores of H9 DNA vaccine with that of other DNA vaccines was compared.

3. Results

3.1. Modeling of different DNA vaccine coded proteins

Models were assessed by WHAT IF, PROCHECK, ERRAT and Verify3D and the selected results of pre and post refinement section

are shown in Table 1. The amount of changes in the 3D structure of H9 was different in the DNA vaccine coded proteins (Table 2). 3D structures of three modelled proteins are shown in Fig. 3.

3.2. Docking of different DNA vaccine coded proteins with antibodies

In order to check the DNA vaccine antigen–antibody interaction affinity, we docked each DNA vaccine protein with two anti-hemagglutinin antibodies, available on the RCSB server. For each docked partner, there were a huge number of docking results that each one had scores related to the positions of two proteins. For example, five docking results for H9-1KEN partner are shown in Fig. 4. All DNA vaccine proteins had lesser interface scores than H9 vaccine protein (Table 3)

As indicated in Fig. 5, the least interface scores of 1QFU antibody with H9 in the different DNA vaccine proteins were decreased by the addition of different components to the H9 protein at most %16.5 while the decline average was 5.11%. The least interface scores of 1KEN antibody with H9 in the different vaccine proteins were 85.67% and the average was 93.9%. The reference (100%) was H9 group–antibody interface score in this regard (Fig. 5).

3.3. Docking of CHIR AB1 with DNA vaccine proteins containing Fc IgY

In order to check the effect of Fc IgY fusion to the other component on the interaction of Fc IgY with its receptor (CHIR AB1), CHIR AB1 with H9+FcIgY and H9+8P29C3d+FcIgY were docked and the least interface scores were compared with that of truncated Fc IgY-CHIR AB1 docking. The interface score decreased by %10 and increased by %32 in H9+FcIgY and H9+8P29C3d+FcIgY respectively in comparison with Fc IgY alone (%100) (Table 4).
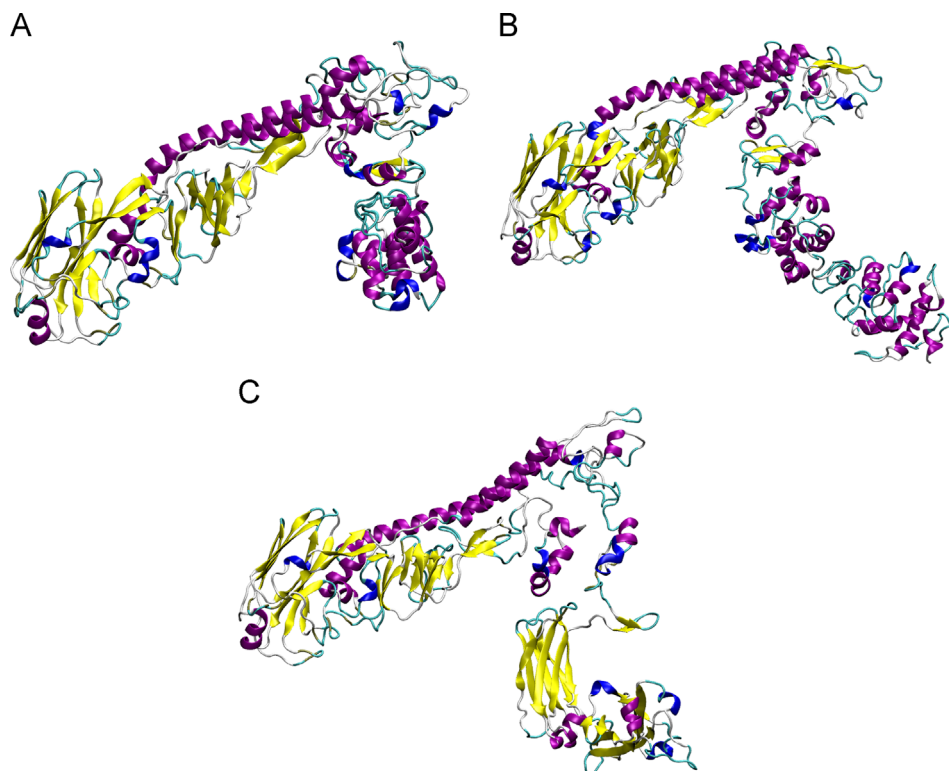
Fig. 3. Figures of three modeled proteins: (A) H9+4P29C3d, (B) H9+8P29C3d and (C) H9+FcIgY. Structure of hemagglutinin9 contained two long helices in the stalk and some sheets in the globular head. Four or eight P29C3d generally formed helices but FcIgY had sheets.
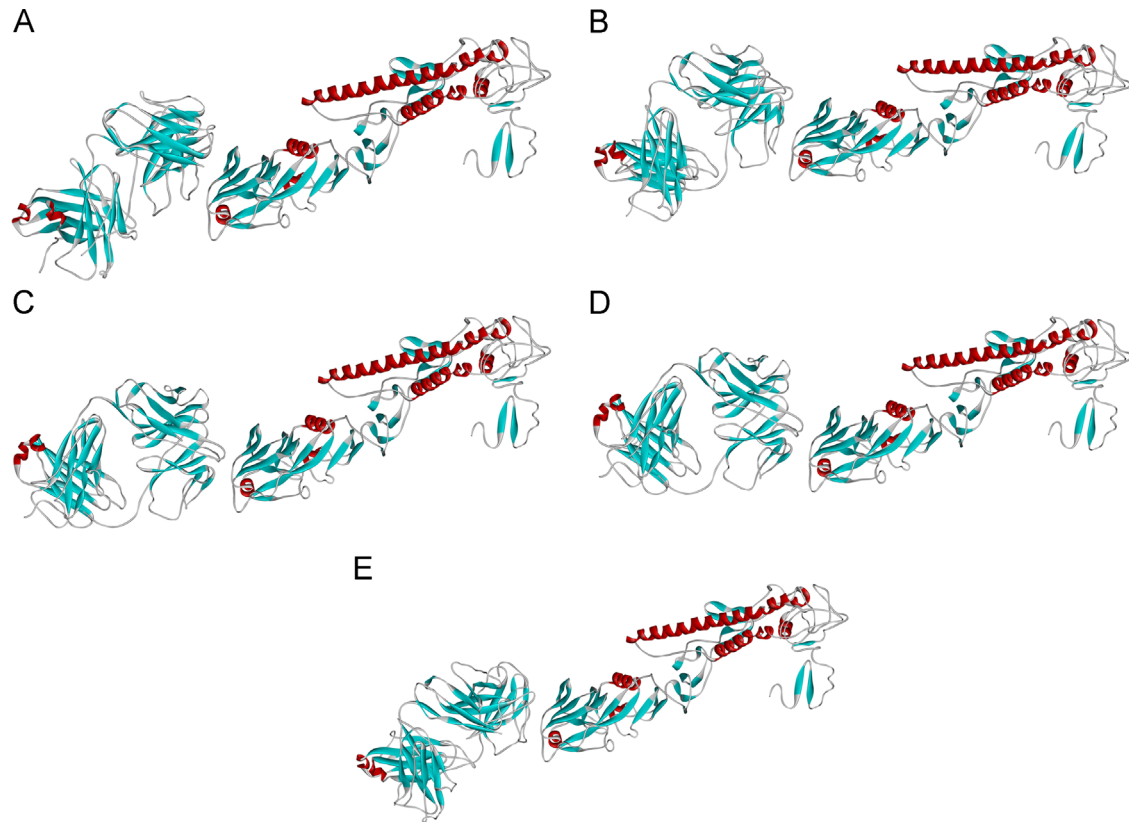
Fig. 4. Figures of five lowest "total score" docking results for H9 DNA vaccine and 1KEN antibody: (A) proteins.ppk_0188 with interface score (I_sc) of −4.857, (B) proteins. ppk_0400 with I_sc of −3.745, (C) proteins.ppk_0207 with I_sc of −5.573, (D) proteins.ppk_0201 with I_sc of −4.097 and (E) proteins.ppk_0048 with I_sc of −3.606. Proteins.ppk_0207, shown in figure C, had the lowest interface score between all of the H9-1KEN docking results. Comparison of figures reveals that the positions of two proteins have been changed.

Table 3
The least Interface scores of docking of different DNA vaccine proteins with two hemagglutinin antibodies.

	H9	H9+4P29C3d	H9+8P29C3d	H9+FcIgY	H9+8P29C3d+FcIgY
1KEN antibody	−4.649	−4.593	−4.533	−4.538	−3.983
1QFU antibody	−5.456	−5.246	−5.348	−5.166	−4.557
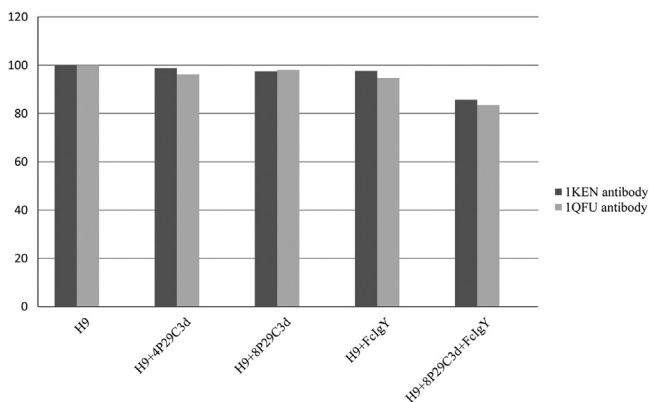
Fig. 5. The percentage of the least interface score of the different DNA vaccine proteins with two antibodies (H9=%100).

Table 4
The least interface score of CHIR AB1 with DNA vaccines proteins containing Fc IgY.

	FcIgY	H9+FcIgY	H9+8P29C3d+FcIgY
CHIR AB1	−5.949 (%100)	−5.412 (%90.97)	−7.897 (%132.74)

HyperChem Professional software (v7) and their 3D structures were checked over the web. Finally, the models were docked with two anti-hemagglutinin antibodies, to check the interaction of antibodies with immunogenic parts of the DNA vaccine proteins.

In the most cases, model refinement by HyperChem led to a better quality in the Errat test but a worst stereochemistry quality in the Ramachandran plot and often did not change verify 3D percent dramatically. But in the case of H9+8P29C3d+FcIgY model, the refinement failed and so, pre-refined model was applied in the docking step (Table 1).

As the least interface score of the ROSIE docking result is associated with the binding affinity of docking partner, we used this score to compare the effect of the addition of some P29C3ds and Fc IgY to the basic H9 DNA vaccine protein on two immuno-genic spatial epitopes of hemagglutinin. Docking results of dif-ferent DNA vaccines showed that the addition of 4 and/or

4. Discussions

Different DNA vaccines were designed and translated *in silico*. Their coded proteins were modeled by MODELLER (v9), refined using

8 P29C3d and Fc IgY to the H9 decreased the least Interface score (Table 3) showing decrease of the binding affinity. All DNA vaccine proteins interacted weaker with both antibodies than H9 DNA vaccine protein. The superimposition of the pair of proteins in Swiss-PdbViewer confirmed the conformational changes (Table 2) which might explain the reduction of the affinity.

As fusion of unrelated genes to an immunogenic gene in a DNA vaccine would normally leads to antibody affinity decrease, the amount of affinity decline may be informative. Since all DNA vaccine proteins were docked with 1KEN and 1QFU, we compared the average of interface scores of the "proteins–antibody" with "H9–antibody" and found that the average of "proteins–antibody" interface scores (1KEN: 94.89% and 1QFU: 93.09%) were lesser than that of "H9–antibody" (100%). These percentages showed a little decline; therefore changes in the H9 immunogenic epitopes were not a lot in different DNA vaccine proteins in comparison with those of H9 alone. Based on the docking partner views, we could also assume that the 4 and/or 8 P29C3D and Fc IgY of the DNA vaccine complexes may not interfere with antibody and H9 antigen interactions. The interface scores of H9+8P29C3d+Fc IgY DNA vaccine decreased to 85% of H9 DNA vaccine–antibodies interface scores. This decline could be due to the bigger size of added parts and the fact that we used an unrefined model in the docking step.

The binding affinity of Fc IgY with CHIR AB1 in the H9+FcIgY and the H9+8P29C3d+FcIgY DNA vaccines was compared. While, H9+FcIgY showed lesser affinity (90%), H9+8P29C3d+FcIgY affinity increased to 132%. We might assume that the dramatic increase of Fc IgY and CHIR AB1 affinity in this group might compensate decrease of affinity in H9 and its antibodies in the H9+8P29C3d+FcIgY and may lead to a better immunogenicity.In this paper, we presented a bioinformatic approach for checking of the immunogenic structure of a manipulated gene coded protein in a DNA vaccine. Addition or deletion of gene segment(s) to an immunogenic gene to use as a DNA vaccine is a routine in the field of DNA vaccine research (Zhu et al., 2013; Luo et al., 2014; Sjatha et al., 2014) and 3D structure of coded immunogenic protein have to be considered by researchers.

Acknowledgments

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