Validation Tools for Predicted Linear B-Epitopes: Beta Turns

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ABSTRACT: It is claimed that amino acid replacements in surface loops usually do not perturb the three-dimensional structure of the protein, since surface loops are relatively flexible (Saunders and Baker 2002). Thus, the conservation variability of epitopes might be biased by the abundance of loops in epitopes. These results imply that epitopes do not tend to overlap functional regions, but rather cover separate regions. Pellequera, et. al., (1993), developed new turn scales based on the occurrence of amino acids at each of the four positions of a turn using a structural database comprised of 87 proteins. It is found that the scales correctly predicted a fraction of the turn regions in proteins with approximately 80% confidence. They used the turn scales for predicting the location of antigenic sites in proteins. The method was developed with the specific aim of predicting only a few peaks for each protein. They found that it leads to a high level of accurate prediction around 70% of correct prediction of known epitopes. In this article we update turn scales using large numbers of proteins and epitopes. Improved method will be more helpful in selecting protein regions to be synthesized in order to produce anti-peptide antibodies cross-reacting with the parent protein.

1. INTRODUCTION

Antigenic sites are residues of a native protein that are those recognized by antibodies. It is most probably that these sites are accessible or on the surface of antigenic proteins. Furthermore, these regions are possibly more mobile than interior regions. Since these sites are on the surface, they are probably hydrophilic. Indeed, scores for hydrophilicity, flexibility, and accessibility have been used to predict antigenicity.

At least for the past three decades, new tools are emerged to predict the Antigenic sites, which are called epitopes of antigenic proteins. Prediction of immunogenic epitopes using bioinformatics tools is a challenging task because of the inherent complexity of antigen recognition (Flower,
2003). At the beginning, to predict continuous B-cell epitopes, a number of algorithms have been developed based on physico-chemical properties of amino acids (Pellequer, et. Al. 1991), but their accuracies were very low. The commonly used properties are hydrophilicity (Parker, et. Al., 1986) flexibility (Karplus, and Schulz1985), accessibility (Emini, et. al 1985), and beta turns (Pellequer, et.al., 1993).

2. CHOU FASMAN SCORE TABLES TO PREDICT BETA TURNS

The identification of B cell epitopes on protein antigens has attracted the attention of many scientists. This would be useful for diagnostic purposes and also in the development of peptide vaccines. To save time and money in wet labs experiments, Levitt, (Levitt, 1976) started a tradition to create score tables to predict antigenic determinants. Hopp and Woods (1981) followed.

Parker et al., (1986) modified the approach of Hopp and Woods taking into account that antigenic sites are on the surface of the protein. They used three parameters i.e., hydrophilicity, accessibility and flexibility simultaneously. On the other hand, Welling et al. (1985) calculated the antigenicity value for each amino acid from its frequency of occurrence in antigenic regions in 20 proteins, with that of 314 proteins, and used these values to predict epitopes. Then in this article similar approach is used database used by these workers is very small and consists of only 606 amino acids from 20 proteins. In this article the same approach is used for a relatively big data of 80,592 non-redundant proteins of PDB, and 344,121 linear b-epitopes of iedb database. Kolaskar, and Tongaonkar derived a score table using experimental antigenic determinant data and physicochemical properties of amino acids.

Table 4. Modified scales of Vhinen et. al for average Bnorm-values for residueswith no rigid neighbors inD1column, residues with one rigid neighbor inD2, and residues for which both neighbors are rigid inD3.

| No | A Acid | C&F | No | A Acid | C&F |
|----|--------|-----|----|--------|-----|
| 1  | A      | 0.66| 11 | L      | 0.59|
| 2  | R      | 0.95| 12 | K      | 1.01|
| 3  | N      | 1.56| 13 | M      | 0.60|
| 4  | D      | 1.46| 14 | F      | 0.60|
| 5  | C      | 1.19| 15 | P      | 1.52|
| 6  | E      | 0.74| 16 | S      | 1.43|
| 7  | Q      | 0.98| 17 | T      | 0.96|
| 8  | G      | 1.56| 18 | W      | 0.96|
| 9  | H      | 0.95| 19 | Y      | 1.14|
| 10 | I      | 0.47| 20 | V      | 0.50|

3. MATERIALS AND METHODS

To have an idea about the success of using several antigenicity tables to predict the linear B-epitopes of antigenic peptides, a sample of five antigens, Plasmodium Falciparum, Human Polio Virus Sabin Strain, Meningitis, Plasmodium Vivax and Mycobacterium Tuberculosis are considered.

3.1 Plasmodium Falciparum:
Plasmodium falciparum is a protozoan parasite that causes an infectious disease known as malaria. P. falciparum is the most severe strain of the malaria species correlated with almost every malarial death. The other 3 species that causes malaria include: P. vivax, P. ovale, and P. malariae. Humans become infected by a female Anopheles mosquito which, transfers a parasitic vector through its saliva into the blood stream. The 26 wet lab reported linear B Cell epitopes of Plasmodium Falciparum are given in Isea, R. (2017), andAbidi, and Can (2017).

3.2 Human Polio Virus
Poliovirus, the causative agent of paralytic poliomyelitis, is an enterovirus spread by the oral route. The principal infection associated with the poliovirus is enteritis with the prodromal illness of fever, headache, arthralgia, vomiting, and diarrhea lasting 3–4 days. About half of the patients do not develop paralytic manifestations. In the remaining, a biphasic course evolves. As the initial enteritis subsides, the paralysis begins. Severe back and limb pain, headache, and meningismus develop, accompanied by severe and disabling muscle spasms. Paralysis tends to occur in a patchy, multifocal distribution. Weakness of individual muscles comes on rapidly over days and typically reaches a maximum within 1 week. The virus has a specific tropism for the motor neurons, resulting in motor neuron death. Virtually any of the skeletal muscles, including bulbar, limb, and respiratory muscles, can be affected. The time from being infected with the virus to developing symptoms of disease (incubation) ranges from 5 - 35 days (average 7 - 14 days).

FASTA OF Human Polio Virus Sabin strain is down loaded from GenBank with identification number AAN85444.1 polyprotein[Human poliovirus 3]. 64 linear B-epitopes are reported (Nomoto, et. al., 1982; Kanduc, et. al., 2015;Abidi, and Can 2017).

3.3 Mycobacterium Tuberculosis
Members of the genus Mycobacterium are characterized by a verycomplex cell wall envelope that is responsible for the remarkable low permeability of their cells as well as the characteristic differential staining procedure (known as Zhiel-Neelsen acid-fast stain), which specifically stains all members of the genera. Both features are due to the presence of long chain a-alkyl, β-hydroxyfatty acids in their cell wall. The Mycobacterium genus is
usually separated into two major groups on the basis of their growth rate. Tuberculosis remains the most devastating bacterial cause of human mortality (1). Despite improved diagnosis, surveillance, and treatment regimens, the incidence of TB increases annually (2). For Mycobacterium Tuberculosis, 13 linear B-epitopes are reported (Young et al., 2013) and Abidi, and Can (2017).

3.4 Meningitis
Viral meningitis is contagious and infectious disease in which there is an inflammation of the meninges of cerebrospinal fluid (CSF). The membranes and cerebrospinal fluid (CSF) encase and bath the brain and spinal cord. Viral meningitis is the most common type of meningitis. Bacterial meningitis is less common. Viral meningitis is also sometimes called aseptic meningitis. Meningitis is by far the most common neurological manifestation of mumps virus infection. Before widespread immunization, mumps was a common cause of meningitis, which occurred in 15% of patients with mumps. Mumps meningitis can precede or follow the parotid swelling, and 50% of cases occur in the absence of parotitis. Meningitis is more common in males than in females. Diagnostic tests include a lumbar puncture, also called a spinal tap. For meningitis, 9 linear B-epitopes are reported (Chandra, and Singh (2012)), Abidi, and Can (2017).

3.5 Plasmodium Vivax
Plasmodium vivax is a protozoal parasite and a human pathogen. The most frequent and widely distributed cause of recurring (Benign tertian) malaria. P. vivax is one of the six species of malaria parasites that commonly infect humans. It is less virulent than Plasmodium falciparum, the deadliest of the six, but vivax malaria can lead to severe disease and death due to splenomegaly (a pathologically enlarged spleen). P. vivax is carried by the female Anopheles mosquito, since it is only the female of the species that bite. Plasmodium vivax malaria is prevalent in many regions of the world. It accounts for more than half of all malaria cases in Asia and Latin America. Despite of the high prevalence of disease caused by this parasite, research regarding its effects has lagged disproportionately. Organ dysfunction which is seen in P. falciparum malaria, is not seen in P. vivax infections. Thus, severe malaria is reported with P. falciparum but not with P. vivax infection. 26 linear B cell epitopes are reported (Caro-Aguilar, et. al., 2002), and Abidi, and Can (2017).

4. PREDICTING ANTIGENIC DETERMINANTS USING SCORE TABLES
In this section as demonstration, the antigenic residues of Meningitis are found. The fasta of the antigen consists of 361 residues:

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MKKTLAALIVGAFASAAANAAVYNNENGTNVELG
GRSLISAEQNSTVDNQKQHQALRNGGGSFHIKAT
HNFGDGFLYAQQILETRFVTKASENGSDNFGDITSKY
AYVTLENGAFVEKLGRGKTAIDGTSAEDEKEYGV
LNNSDYIPSNTGVTYFQIDGLVLGANYLLAQKR
EGAKGENKRPNKAGEVRIEINNGIQVGAKYAND
DIVAKIAYGRTNYKYNESDEHKQQLNGVLATLGYR
FSDLLGVLDSGYAKTKNYKIKHEKRYFVSPGFQY
ELMEDTNVYGNUFKEYRTSVDQGETREQAVLFVGD
HKLHKQLLTYEGAYARTRTETGKVKEKSV
GVGLRIVYF
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Using the formula

\[ a_i = \frac{1}{7} \sum_{j=1}^{7} c_{i+j-k} \quad i = 4, \ldots, 358 \]

Where \( c_k \), \( k = 1, \ldots, 361 \) is the betaturn score from Table 2. of the amino acid at the position \( k \) of the sequence.

When the local average antigenicity scores of residues are plotted, we get an antigenicity profile of the antigenic protein as in Figure 1.

![Figure 1. Beta turn profile of the antigenic protein.](image)

The average antigenicity of the antigenic protein is found to be 0.039, and as seen as the boundary of the shaded regions. We claim that the projections of shaded regions upper side of the average on the horizontal coordinate axis are antigenic regions on the protein. The starting and ending addresses of these regions, when regions shorter than 6 residues are eliminated are

\[
\{\{1, 13\}, \{16, 22\}, \{31, 38\}, \{81, 87\}, \{101, 108\}, \{141, 147\}, \{1, 61, 172\}, \{235, 257\}, \{269, 281\}, \{308, 315\}, \{320, 329\}\}
\]

which correspond to the strings

```
MKKTLAALIVGAFASAAANAAVYNNENGTNVELG
GRSLISAEQNSTVDNQKQHQALRNGGGSFHIKAT
HNFGDGFLYAQQILETRFVTKASENGSDNFGDITSKY
AYVTLENGAFVEKLGRGKTAIDGTSAEDEKEYGV
LNNSDYIPSNTGVTYFQIDGLVLGANYLLAQKR
EGAKGENKRPNKAGEVRIEINNGIQVGAKYAND
DIVAKIAYGRTNYKYNESDEHKQQLNGVLATLGYR
FSDLLGVLDSGYAKTKNYKIKHEKRYFVSPGFQY
ELMEDTNVYGNUFKEYRTSVDQGETREQAVLFVGD
HKLHKQLLTYEGAYARTRTETGKVKEKSV
GVGLRIVYF
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The wet lab reported antigenic sites of this antigenic protein are
The numbers of residues in red are 
{2,7,8,19,2,13,2}

The numbers of residues in web lab reported peptides are 
{28,29,28,22,26,16,25,27}

In three predicted regions, the number of correctly predicted residues are 
{19, 13}. These antigenic regions are accepted as correctly predicted, since more than half of residues are correctly anticipated.

This calculation is repeated for each of five antigens and six antigenicity scores.

5. CONCLUSION

When the calculation in Section 4. is repeated for each of five antigens and six antigenicity scores, we get the following Table 3.

Table 3. When calculation in Section 4. is repeated for each of five antigens and six antigenicity scores, we get the table of correctly predicted antigenic regions.

| Antigen | Lab | C&F |
|---------|-----|-----|
| Menin   | 9   | 2   |
| Falcip  | 26  | 7   |
| Polio   | 49  | 12  |
| Esat6   | 13  | 4   |
| Vivax   | 26  | 10  |
| Total   | 123 | 35  |

From Table 3. It is seen that the antigenicity scores of Welling et. al. (1985) performs best. Although antigenicity scores list is based on observations of the amino acid composition of known antigenic regions of 20 proteins and other 314 proteins.Almost 2/3 of the antigenic regions are correctly predicted. On the other hand, in this research a data of 80,592 non redundant proteins of PDB Database, and 344,121 linear b-epitopes of iedb database are used with the same technique, and the resulted antigenicity score list could predict only 1/3 of antigenic regions. The abundance of the information weakens the efficiency.

5. A THRESHOLD FOR B-EPI TOPE VALIDATIONS

There is a belief that antigenic peptides are more flexible than others. To support the hypothesis more strongly, 337,259 b-epitopes of lengths 7-25 amino acids are downloaded from iedb database, average flexibility scores of b-epitopes are computed according to the formula (2).

On the other hand 103,590 non epitopes of length 7-25 amino acids are constructed artificially. Their average flexibility scores are also computed by the same formula (2).The descriptive statistics of the results are shown in Table 4.

Table 4. The descriptive statistics of the average flexibility scores of epitopes, and non epitopes computed by Karplus, and Shults scales.

```
| Flexibility | Epitopes | Nonepitopes |
|-------------|----------|-------------|
| Mean        | 1.0091   | 0.9877      |
| S. Deviation| 0.1331   | 0.1098      |
| Skewness    | 0.2275   | 0.0823      |
| Kurtosis    | 2.9618   | 3.1126      |
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Figure 2 Average beta turn scores of epitopes, and non-epitopes distribute almost normally, with slightly different means 1.0091, and 0.9877 respectively.

4. CONCLUSION

Online Karplus & Schulz Flexibility Prediction tool in iedb Analysis Resource, yields flexibility scores of residues of peptides that are epitope candidates. It is possible also to compute average flexibility scores of these peptides. To derive a validation tool from this residue flexibility prediction tool in iedb to validate whether a given peptide is a linear b-epitope or not, one needs a threshold value.

\(^1\)http://www.iedb.org/database_export_v3.php
For this purpose b-epitopes from iedb database are downloaded. Also, large number of non-epitopes are created.

Using both Karplus, and Shults, and Vhinen et. al. flexibility scales, average flexibility scores of epitopes, and non-epitopes are computed. The percentage difference between average flexibility scores of epitopes and non-epitopes is found to be less than 0.03%

Therefore if average flexibility scores of a peptide is larger, the peptide is more likely is a b-epitope, and this property can be used as validation tool to eliminate some of the peptides with small average flexibility scores, while keeping others.

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