Computational design of multi epitope peptide based vaccine against SARS-COV-2

S. Sugunakala

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Abstract

COVID - 19 is a contagious disease caused by SARS – CoV2 (Severe Acute Respiratory Syndrome Coronavirus-2) virus which belongs to the family Coronaviridae. Its impact is ranging from uncontrolled mortality, fear, obsession and economic downturn and many countries joined together to take possible therapeutic measures. A slide of measures and promising ways to design vaccines for prophylactic purpose are being considered. In this work, peptides prediction and structure based approach are employed to design multiple subunit vaccine with respect to S1 domain of spike glycoprotein. A stable vaccine construct (consists of 12 B - cell and T – cell epitopes) was developed by using suitable chimeric adjuvant and the potential peptides were identified by their antigenic and non allergenic nature. Further, to know the binding energy requirement, docking analysis for individual peptides along with the target receptor TLR – 4 was carried out. The 2D and 3D structural analysis were done by using PSI Pred and Raptor – X servers. Restriction cloning of the designed peptides into pBluescript (+SK) vector was done by using SnapGene software. Finding from this work provides multi epitope based vaccine fragment that can be a potential candidate for the development of vaccine for SARS-CoV-2.

Key words: vaccine design, SARS-CoV-2, epitope prediction, subunit vaccine, adjuvant and linkers, restriction cloning.

INTRODUCTION

Prevailing of a virulent pathogenic virus that transmits rapidly across humans and potentially causes variety of symptoms related with respiratory problems was experienced in December 2019, at the town of Wuhan, China (Mitra, *et al.,* 2020). After a month of the outbreak, the disease has spread across the world. On 12th March 2020, the WHO acknowledged it as pandemic, advising countries all over the world to take suitable measures and to defend themselves to prevent disease spread. Research community concentrates on developing rapid diagnostic methods, isolation of affected individuals, providing substantial treatment with repurposed drugs etc., as prophylactic measures of the first line protection against the disease. More than a decade ago, manifestation of similar homologs of this disease caused by structurally and functionally similar virus (SARS-CoV) will help the researchers to understand the process of SARS-CoV-2 infection. Similar structural counterparts consist of spike glycoprotein, membrane glycoprotein, envelope protein and nucleocapsid protein (Morse, *et al.,* 2020; Wan *et al.,* 2020). The complete genome sequence of SARS-CoV-2 was made publically available in nucleotide sequence database (Lu, *et al.,* 2020; Wu, *et al.,* 2020) and protein 3D structural data as well as their interactions with human receptors are all available in RCSB (Shang, *et al.,* 2020; Walls, *et al.,* 2020; Wrapp, *et al.,* 2020; Yan *et al.,* 2020). These publically available data allowing the scientists to carry on the development of possible therapeutics like repurposing of drugs (Li and De Clercq, 2020), developing small molecule inhibitors, finding of unique targets, elucidating the molecular mechanism of entry and multiplications of pathogenic virus in to humans (O'Meara, *et al.,* 2020) etc., The SARS-CoV-2 genome consists of single stranded positive sense RNA which is capped and poly adenylated at 5′ and 3′ end respectively (Shereen, *et al.,* 2020). The major Open Reading frames (ORFs) are ORF 1a/b region coding replicase and other enzymes, the spike / S protein ORF, M / membrane glycoprotein ORF, E / small membrane protein ORF and the N / nucleocapsid ORF were found in SARS-CoV-2 (Walls, *et al.,* 2020). The process of viral entry into humans is initiated by the spike glycoprotein which interacts with the human ACE-2 receptor. Spike protein consists of two subunits S1 and

S. Sugunakala

email: *suguna_murali@hotmail.com*
Department of Bioinformatics, A.V.c. College (Autonomous) Mannampandal - 609 305, Mayiladuthurai, Tamil Nadur, South India

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S2 domains. S1 domain residues are responsible for binding with human ACE – 2 receptor and S2 is responsible for fusion of viral and host cellular membrane protein. Prior to the formation of stable viral protein and host receptor cleavage of S1 domain residues followed by priming of TMPRSS2 was observed (Hoffmann, *et al.,* 2020). Currently, there are no potential therapeutics are available to treat and cure the disease. Significant prophylactic and therapeutic measure includes development of potential vaccines are in demand. To date, numbers of several candidate vaccines are available. However, each one is having their own advantages and disadvantages and there is a need of developing prospective candidate vaccines. In this work, efforts were made to design and to develop multi epitope vaccine subunit by using immunogens of SARS-CoV-2 spike glycoprotein. The selected spike glycoprotein immunogens are computationally evidenced to elicit neutralizing antibodies and they may lead to acts as a prophylactic and to the establishment of immune response to prevent the viral entry.

MATERIALS AND METHODS

Target selection

The surface spike glycoprotein consists of two subunits (S1 & S2) in which the S1 subunit consists of receptor binding domain (RBD) which plays a vital role in binding with ACE-2 receptor with high affinity. The crystal structure of SARS-CoV-2 spike receptor binding domain bound with ACE2 (PDB ID: 6M0J) was selected and the sequence for S1 subunit was retrieved (Lan *et al.,* 2020).

Prediction and validation of T and B cell Epitopes

By using resources of IEDB server, the T & B – cell epitopes were predicted (Astuti, 2020) for S1 subunit of SARS-CoV-2 spike glycoprotein. The T cell epitope – MHC class I and class II molecules were predicted by using Tepitool (Paul, *et al.,* 2016). The prediction and selection of MHC class I peptides was carried out by screening the query sequence against 27 most frequent class – I HLA of A & B alleles namely (A*01:01, A*02:01, A*02:03, A*02:06, A*03:01, A*11:01, A*23:01, A*24:02, A*26:01, A*30:01, A*30:02, A*31:01, A*32:01, A*33:01, A*68:01, A*68:02, B*07:02, B*08:01, B*15:01, B*35:01, B*40:01, B*44:02, B*44:03, B*51:01, B*53:01, B*57:01, B*58:01). The parameters were adapted for the length of 9-mer oligopeptide and to remove the duplicate peptides. Peptides were selected based on predicted IC_{50} (Nielsen *et al.,* 2007; Hoof *et al.,* 2009) of <500 nm. Similarly, the MHC class – II peptides were predicted by screening against 22 most frequent class – II HLA alleles of DP, DQ and DR namely HLA-DPA1*01/DPB1*04:01, HLA-DPA1*01:03/DPB1*02:01, HLA-DPA1*02:01/DPB1*01:01, HLA-DPA1*02:01/DPB1*05:01, HLA-DPA1*03:01/DPB1*04:02, DQA1*01:01/DQB1*05:01, DQA1*01:02/DQB1*06:02, DQA1*03:01/DQB1*03:02, DQA1*04:01/DQB1*04:02, DQA1*05:01/DQB1*02:01, DQA1*05:01/DQB1*03:01, HLA-DRB1*01:01, HLA-DRB1*03:01, HLA-DRB1*04:01, HLA-DRB1*04:05, HLA-DRB1*07:01, HLA-DRB1*08:02, HLA-DRB1*09:01, HLA-DRB1*11:01, HLA-DRB1*12:01, HLA-DRB1*13:01, HLA-DRB1*15:01. The parameters were tailored for the length of 15-mer peptides and then the duplicate peptides were also removed. Based on the

Table 1. T – Cell Epitopes of class – I HLA molecules

S. No.	Alleles Code	Peptide sequence	Start	End	Antigenicity Score	IC_{50}	Allergen/ Non Allergen
1	HLA-A*31:01	KSNLKPFER	140	148	0.9490	14.33	Non Allergen
2	HLA-A*02:03	VLSFELLHA	194	202	1.0776	29.35	Non Allergen
3	HLA-A*23:01	PYRVVVLSF	189	197	1.0281	66.3	Non Allergen
4	HLA-A*30:01	KSTNLVKNK	211	219	0.8596	100.89	Non Allergen

Table 2. T – Cell Epitopes of class – II HLA molecules

S. No.	Alleles Code	Peptide sequence	Start	End	Antigenicity Score	Percentile rank	Allergen/ Non Allergen
1	HLA-DPA1*01/DPB1*04:01	GYQPYRVVVLSFELL	186	200	1.0740	0.49	Non allergen
2	HLA-DPA1*01:03/DPB1*02:01	FNCYFPLQSYGFQPT	168	182	0.9170	9.9	Non allergen
3	HLA-DQA1*01:01/DQB1*05:01	YRVVVLSFELLHAPA	190	204	0.7072	5.6	Non allergen
4	HLA-DRB1*01:01	LSFELLHAPATVCGP	195	209	0.5062	0.03	Non allergen
5	HLA-DRB1*04:05	QPYRVVVLSFELLHA	188	202	0.9109	3.5	Non allergen

Table 3. Selected B – Cell Epitopes

S.No	Peptides	Start	End	Propensity Score	No. of Residues	Antigenicity Score	Allergen / Non Allergen
1	NNLDSKVGGNYN	439	450	0.713	12	0.7538	Non allergen
2	YGFQPTNGVGYQ	495	506	0.66	12	0.7136	Non allergen
3	YGVSPTKLNDLCFTN	380	394	0.642	15	2.0602	Non allergen

percentile ranking, the peptides were selected. By using Ellipro tool from IEDB server, B – Cell epitopes were predicted. (Ponomarenko, *et al.,* 2008). In this method, based on the solvent accessibility and flexibility, peptides were predicted. Finally, all the obtained epitopes were subjected for their antigenicity and allergenicity by using Vaxigen and Allergen FP server respectively (Doytchinova and Flower, 2007; Dimitrov *et al.,* 2014). Only the sequences having antigenic and non allergenic peptides were selected for further analysis and non antigenic sequences were eliminated. To prevent antibody reaction against self antigen in humans, the selected peptides were validated by searching against complete proteome of Homo sapiens by using Multiple Peptide Match Tool from PIR (Chen *et al.,* 2013).

Molecular docking of epitopes and Human TRL – 4

The main reason for choosing this TRL – 4 (Toll like receptor) is the fact that it is involved in defending action against infection and is an inducer of the innate immune response. It has been experimentally evidenced with number of RNA based viruses like SARS – CoV, HIV, Influenza etc., (Akira *et al.,* 2006) for their anti infective property. Hence, the protein structure (PDB ID: 3FXI_A) was downloaded from PDB and considered as target protein. It was prepared by removing all water molecules and bound ligands, addition of polar hydrogen and optimization at physiological pH using UCSF Chimera. For docking analysis the designed multi epitopes were used as ligands and the target protein is used as receptor. Docking analysis was carried out by using HPEPDOCK server (http://huanglab.phys.hust.edu.cn/hpepdock/) (Zhou, *et al.,* 2018). Docking of designed peptides with target receptor was done by fast modeling of peptide conformations and global sampling of binding orientations.

Construction of Sub unit Vaccine Model

Subunit vaccine was designed by using 03 - linear B – cell epitopes, 05 class –I HLA molecules and 04 class – II HLA molecules, linked by GPGPG and AAY linkers. Rearrangement of epitopes fragment offers different conformers of peptide subunit model. Their 2D and 3D structures were predicted and validated by using PSI Pred (Jones, 1999; Buchan and Jones, 2019) (URL: http://bioinf.cs.ucl.ac.uk/psipred/), Raptor X (Wang, *et al.,* 2017; Xu, *et al.,* 2021) (URL: http://raptorx.uchicago.edu) and SAVS server (Laskowski *et al.,* 1993; Laskowski, *et al.,* 1996) (URL: https://saves.mbi.ucla.edu) and ProSA web server (Wiederstein, *et al.,* 2007) (https://prosa.services.came.sbg.ac.at/prosa.php).

Selection of Adjuvants

Immune adjuvants are pre requisite for vaccine formulation and they are unique in their activity. Alhydrogel (Adjuvant I.d – VO-0001241) i.e. a classic formulation of Aluminium hydroxide (Al(OH)3) of immunological investigation (Agarwal, *et al.,* 2020) was retrieved from the Vaxjo server which was used as an adjuvant. Provocation of IL-4 & Th cell with enhanced IgG1 and IgE formation was proved by the use of Aluminium adjuvants (Eisenbarth *et al.,* 2008). Significant safety with other recombinant proteins, human tolerance, generation of significant amount of immune response are all considered as potential benefits of this aluminium as an adjuvant (Munks *et al.,* 2010). Another adjuvant Matrix – M, synthesized by saponin, phospholipid and synthetic cholesterol elicit strong immune responses by stimulating both Th and Tc cell types with minimum doses and have lesser risk for allergenic response. Evidences showed that it is well tolerated by humans and has been used to cure Ebola viral disease (Cox *et al.,* 2015; Bengtsson *et al.,* 2016; Suschak and Schmaljohn., 2019).

Reverse Transcription of Multi Epitope Peptides, Restriction cloning and expression analysis

To find the expression of designed multi epitope vaccine in an expression vector, the constructed epitope residues was reverse transcribed and codon optimization was also carried out by using Java Codon Adaptation Tool (JCat) server (http://www.jcat.de) (Grote, *et al.,* 2005). Then the optimized codon was used as an insert fragment to express the final vaccine construct in the *E.coli* K12 strains (Host). Additional options like avoiding rho – independent transcription termination, restriction enzyme cleavage sites and prokaryotic ribosome binding site were used for optimization of codons. Use of restriction enzymes to prepare an insert and vector for ligation is the basic principle of restriction cloning. *Insilico* analysis of the cloning and expression studies for the designed multi

epitope vaccine on E.coli K12 strain was performed by using SnapGene software (Kalita *et al.*, 2020).

RESULTS

The S1 subunit of spike glycoprotein of SARS-CoV-2 consists of 229 amino acids. Initial screening against T – cell epitopes for class – I HLA alleles resulted with 221 peptides. Based on the antigenic (Score > 0.8) and non allergenic properties, 04 peptides were selected for further analysis (Table – 1). Similarly, the class – II HLA alleles screening resulted with 44 peptides. Among them, 05 antigenic (score > 0.5) and non allergenic peptides were selected for further analysis (Table – 2). The B – cell epitopes screening results 10 peptides with varying length of residues (4-22 residues). Among them, 03 potential peptides were identified based on their antigenicity (score > 0.7) and non allergenicity (Table - 3). Validation of all the peptides results that the screened peptides were unique to virus, not matched with *Homo sapiens* proteomes and does not involved in self antigen – antibody response.

The designed vaccine consists of 197 amino acid residues (merging of 12 epitopes and linkers).

Multi epitope vaccine (Fig – 1) was designed by changing the epitopes arrangement and their 2D and 3D (Fig – 3) structures were analyzed for their structural stability. Secondary structure prediction showed that the region 19 – 26, 34 – 41, 115 – 121, 151 – 155, 185 – 194 favors strand structure and 122 -129, 135 -137, 156 -158, 161 – 165, 167 – 170 favors helical structure (Fig – 2). The structural stability analysis of predicted 3D structure (Fig – 3) was validated by Ramachandran plot using Procheck 3.5 results 78% amino acid fragments are in favored regions and 22% are in additionally allowed regions (Fig – 4) and by ProSA web server analysis resulted with Z- score of -3.37 (normal range 1.18 – 5.46) indicates that the predicted model was in good quality (Fig – 5).

Docking of individual antigenic peptides with TLR – 4 resulted the binding energy requirement of peptides (Table – 4).

Results from JCat tool showed that the codon adaptation index (CAI) for designed multi epitope vaccine was 0.95 (0.8 to 1.0 indicates good score (Shankar, *et al.*, 2021) and the GC content percentage was 56.34. The expression level of protein was

NNLDSKVGGNYNGPGPGYGFQPTNGVGYQGPGPGYGVSPTKLNDLCFTNGPGPGKSNL
KPFERGPGPGVLSFELLHAGPGPGPYRVVVLSFGPGPGKSTNLVKNKGPGPGGYQPYRV
VVLSFELLAAYFNCYFPLQSYGFQPTAAYYRVVVLSFELLHAPAAAYLSFELLHAPATV
CGPAAYQPYRVVVLSFELLHA

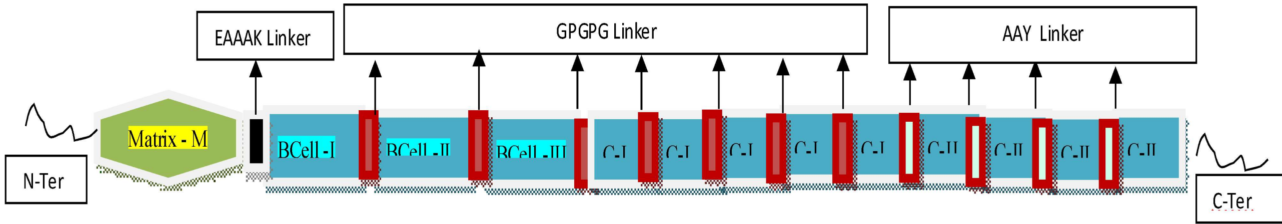

Fig. 1. Schematic representation of the designed multi – epitope vaccine The 197 residues long peptide sequence containing an adjuvant (green) at N- terminal linked with epitopes EAAAK linker. The B cell and T cell class - I epitopes are linked by GPGPG linker and class –I and class – II epitopes are linked by using AAY linker.

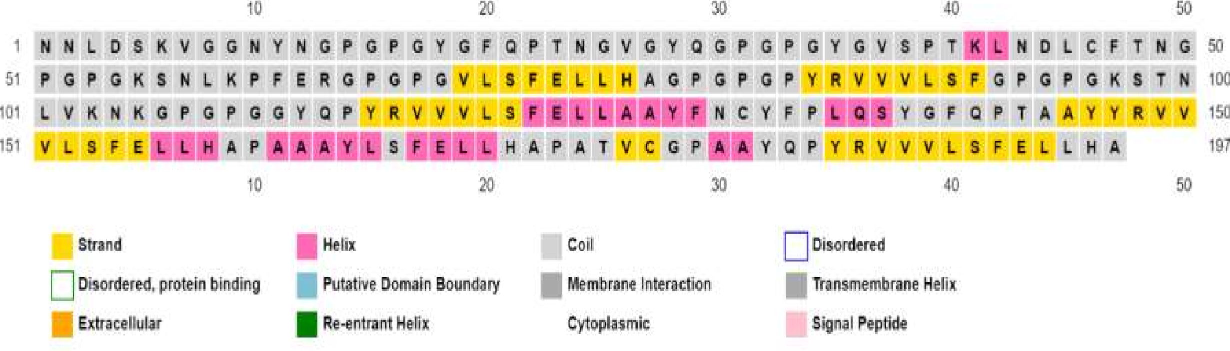

Fig. 2. Prediction of Secondary structure

Fig. 3. 3D structure of designed multi epitope vaccine

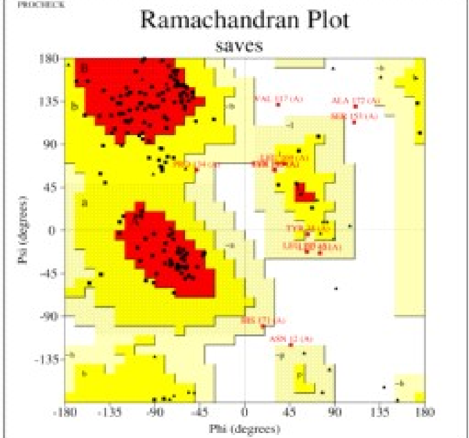

Fig 4. Validation of predicted 3D structure of multi epitope vaccine peptide

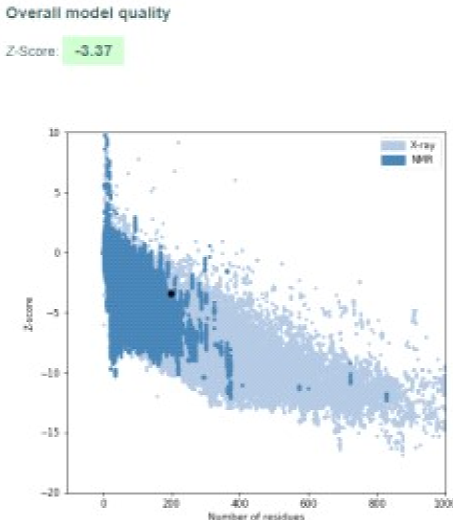

Fig. 5. Predicted z-score for designed multi epitope vaccine - by ProSA web server (-3.37)

evaluated by using this improved DNA sequence. Critical step in restriction cloning is the identification of recognition sites which are unique to the insert fragment and a vector. In this analysis, it was observed that the enzyme PdmI and PstI restriction enzymes has site specific at N and C terminal of the DNA insert sequence (Fig – 6) and present in pBluescript (+SK) vector consists of 2960bp (Fig – 7). The procedure

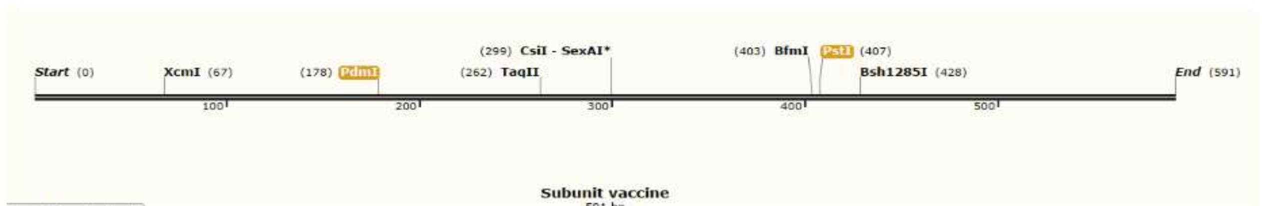

Fig. 6. Restriction enzymes site on DNA sequence

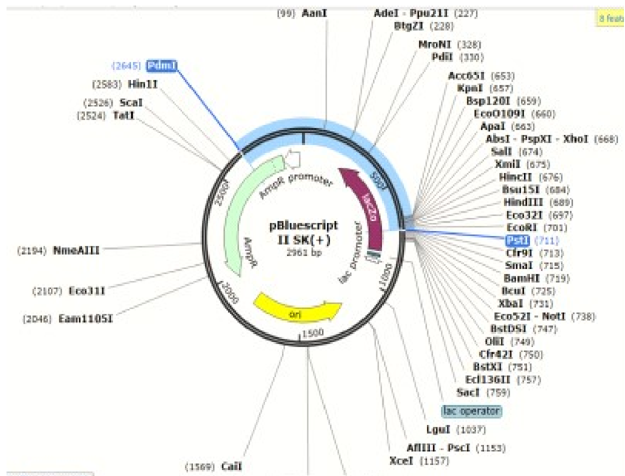

Fig. 7. Locating restriction enzymes on vector

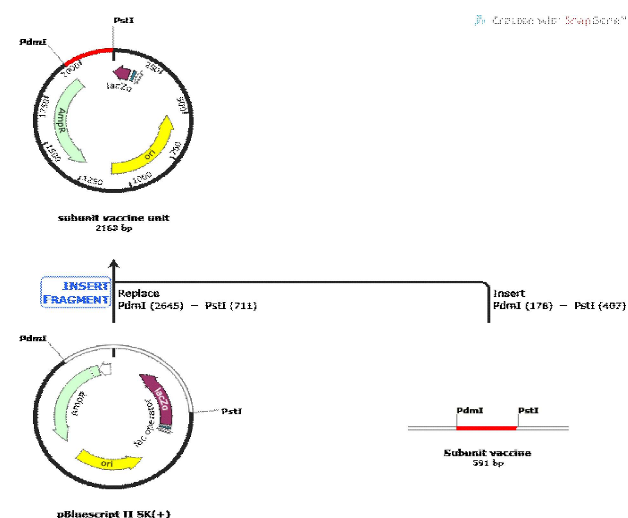

Fig. 8. Steps involved in restriction cloning

Table 4. Details of Binding energy of Peptides

S.No.	Peptide	Binding Energy	Peptide protein complex
1.	KSNLKPFER	-178.033	
2.	VLSFELLHA	-179.3	
3.	PYRVVVLSF	-202.99	
4.	KSTNLVKNK	-145.813	
5.	GYQPYRVVVLSFELL	-217.95	
6.	FNCYFPLQSYGFQPT	-238.343	

S.No.	Peptide	Binding Energy	Peptide protein complex
7	YRVVVLSFELLHAPA	**-222.833**	
8	LSFELLHAPATVCGP	**-189.843**	
9	QPYRVVVLSFELLHA	**-221.991**	
10	NNLDSKVGGNYN	**-179.502**	
11	YGFQPTNGVGYQ	**-189.938**	
12	YGVSPTKLNDLCFTN	**-184.942**	

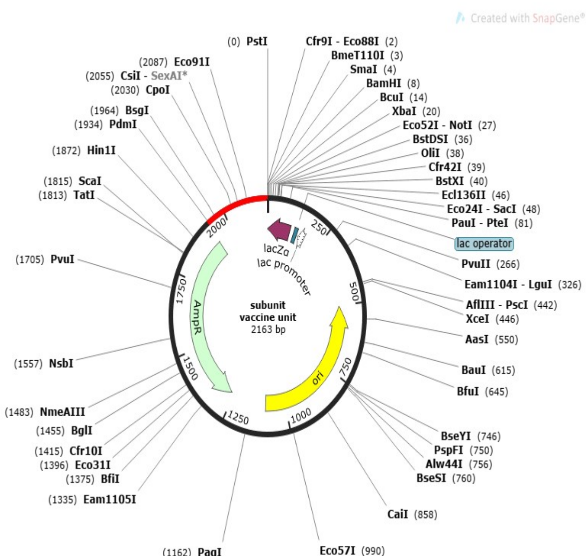

Figure – 9 - In silico restriction cloning of the designed multi epitope vaccine into the pBluescript SK (+) expression vector where the red region indicates the gene coding for the vaccine and the black colored circle represents the vector.

adopted for cloning the multi epitope vaccine in to a vector is depicted in Fig – 8 and the final clone was represented in Fig – 9.

DISCUSSION

To restrain the spread of SARS-CoV2 most significant option is to design and develop appropriate vaccines. The whole process of designing and developing a vaccine is an extensive timeline consists of experimental to clinical settings. Advancements in the field of molecular immunology and epitope mapping methods facilitate the introduction of number of semi empirical approaches in vaccine design. A remarkable pace in the field of Bioinformatics is the use of tools to predict B and T cell epitopes and to develop multi subunit vaccines (Shang *et al.,* 2020). Approaches have been employed to design a multi epitope based vaccine with the major subunits of spike glycoprotein (especially the S1 domain) of SARS-CoV-2. Twelve different types of epitopes for B - cell, T - cell – HLA I & HLAII were predicted. With the default parameters the epitopes were found to be antigenic and non allergenic (Yadav *et al.*, 2020). For the purpose of immune stimulant activity, suitable adjuvant (i.e. Adjuvant sequences are experimentally proved to generate neutralizing antibodies) was added. Implications of toll – like receptor in the case of SARS-CoV-2 have been thoroughly assessed before making a decision upon the use of TLR4 agonist. Linker EAAAK was used to join the adjuvant and epitopes. GPGPG was used to connect the B cell and T cell class I type epitopes and AAY was used to join the class I and class II epitopes (Chen *et al.*, 2013). The secondary and tertiary structures were predicted and the structural stability was also be analyzed. Docking analysis showed that the screened peptides were efficiently binds with the target protein with significant energies. Results from the cloning studies also revealed that the constructed multi epitope subunit vaccine is significantly integrated with E.coli K12 strain.

CONCLUSION

In the present research work, from PDB database, SARS –CoV-2 surface spike glycoprotein sequence (PDB ID: 6M0J) was retrieved. Potential B - cell and T - cell epitopes were identified by using the spike glycoprotein sequence. Activation of B - cell plays a vital role in the humoral immune response as well as development of memory. It is generated by IgM with IgG which acts as antigen presenting cells (APCs). By using IEDB prediction server, B – cell epitopes were determined which will activates the IgG and IgM. Epitopes were selected based on their propensity score. Further, based on the IC_{50} values, class – I and class – II HLA of T – cell epitopes were also identified by using Tepitool from IEDB server. All the epitopes were subjected to allergenicity and antigenicity analysis by Vaxijen 2.0 and Allergen FP 1.0 server. Two different adjuvants were also selected for further analysis. Among them, the Matrix – M is currently reported as novel adjuvant in the design and development of various multi subunit epitope viral vaccines. Recent reports stated that the Matrix – M has more antigenic property than Alhydrogel (Ghosh, *et al.,* 2021). The adjuvant and epitoes are connected by using EAAAK linker and by using class- I HLA alleles (4), class – II HLA alleles (5) and B – cell epitopes (3) the subunit vaccine was constructed along with the GPGPG and AAY linkers (Fig – 1). Then, the 3D structure was predicted for the constructed vaccine by using Raptor – X server. Validation was carried out by using Ramachandran plot (Fig – 4) and the overall structural stability was analyzed by ProSA web server. The obtained value of Ramachanran plot score and Z - score subunit vaccine was considered to be a highly significant result for designing and resulting the immunological response. The docking studies of subunit vaccine epitopes with the TLR – 4 receptor showed that the vaccine peptide produces strong binding affinities (Table – 4). Finally the cloning studies of subunit vaccine peptide revealed their better integration (Fig – 9) for the expression in E.coli K12 strain.

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