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Design of a peptide for immunodetection of IgA antigliadin antibody for the purpose of screening of celiac disease

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Abstract:
Celiac disease (CD) is gluten induced enteropathy which requires jejunal biopsy for diagnosis. To select the patients for endoscopoic procedure some serologic tests are popular in clinical practice for screening of CD. Although gliadin is one of the key immuno activator of the disease; serological screening by immuno-detection of gliadin is not recommended. In this context we have designed a peptide using tools of computational biology keeping molecular pathogenesis of the disease into consideration such that antigliadin antibody detection based sensitive and specific cost effective tool for screening of celiac disease can be developed. The designed peptide QPFPEP interacts in a stable manner with dimeric immunoglobin A1 molecule and its parent peptide QPFPQP are sequentially present in maximum number of gliadin epitopes. This hexapeptide is predicted to interact with dimeric IgA1, which increases in the biofluids of the CD patients.

Keywords: Celiac disease; gliadin; peptides; immunoglobulins; autoimmunity

Abbreviations: Celiac disease CD, Tissue transglutamase, Immunoglobulin A IgA, antigliadin antibody AGA, Immunoglobulin G IgG

Background:
Celiac disease (CD) is a chronic illness which is characterized by mucosal lesion of the small intestine and impaired nutrient absorption by the bowel that improves on gluten free diet. Its diagnosis is based on demonstration of villous atrophy in jejunal mucosa. The jejunal tissue is generally obtained by endoscopoic biopsy, which is an invasive procedure. To select the subjects for invasive jejunal tissue biopsy there exist a handful of serologic screening test that are commonly used in routine clinical practice **[1].** In CD the role of tissue transglutaminase (TTG) and gliadin is proved beyond any doubt. The disease involves antibody formation against both of these proteins. Diet containing gluten supplies gliadin, a glutamine rich protein to the intestine. The elevated level of activated TTG in CD deamidate gliadin and Immunoglobulin A (IgA) antibody is generated against both TTG and gliadin, which are used for serologic screening of the disease **[2].** Gliadin is recognized as one of the key immune activator in CD **[3].** Although gliadin is a key pathogenic molecule of CD, serum IgA antigliadin antibody (IgA AGA) is not observed to be always an efficient tool for screening of CD. Some studies advocate its good sensitivity and specificity for such screening purpose **[4],** while other studies express an opposite view **[5].** Currently no authority is advocating the use of serum IgA

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AGA as a screening tool for CD **[6].** Similar is the case with the status of salivary IgA AGA estimation for CD. Some studies demonstrate positive result for the purpose and others exhibit the opposite **[7].** Contrary to IgA AGA estimation, in both serum and saliva IgA TTG seems to be promising for the purpose of screening for CD **[8].** The reason for such disparity of immuno-elevation of IgA-AGA and IgA-TTG in CD is currently not understood.

Some aspects are often ignored in the immunodetection of IgA-AGA in the context of CD. For immunodetection of IgA-AGA, generally gliadin antigen is immobilized in a well over which the sample is applied and that follows addition of secondary labeled antibody. It is supposed that gliadin antigen will react with sample IgA antigliadin antibody **[9]**. In this modality of immunodetection, a CD specific fundamental change of gliadin molecule is ignored. In CD tissue trans-glutaminase acts on glutamine residues of gliadin and convert it to glutamate. Tissue trans-glutaminase is significantly elevated in CD and it is known to cause deamidation of gliadin molecule and this altered gliadin molecule evokes immune response in CD **[8]**. Therefore it is quite possible that the generated IgA antigliadin antibody will better react with the altered gliadin with glutamine residues changed to glutamate. Recently it is proved beyond any doubt that IgA raised against deamidated gliadin is superior for the diagnosis/screening of celiac disease **[10]**. Supporting this view there is evidence that two stretches of nonapeptides derived from gliadins with glutamine changed to glutamate interact well with sera of CD patients,however the number of amino acids that binds with antibody can be even less than nine **[11]**. For practical reason, involvement of peptides in a diagnostic test with more number of amino acids will increase the cost of the analysis. Keeping this fundamental inappropriateness we have designed a shorter peptide using tools of computational biology that may be experimentally verified for the purpose. The designed peptide is supposed to overcome the current limitations of IgA antigliadin antibody estimation in bio -fluids of CD patients and if found satisfactory after experimental validation is expected to reduce the current cost of the deamidated peptide based diagnosis of CD.

Methodology:

Modeling of the 3-Dimesional structure of IgA

Carbon alpha co-ordinates of dimeric immunoglobin A1 is retrieved (PDB code 2QTJ) from protein data bank **[12].** The three dimensional structure is modeled using SABBAC tool **[13]** and is used for analyzing peptide-IgA interaction.

Peptide designing

It has been found from the literature that N-terminal region of gladin protein has the epitope binding site and have high content of PQ **[14]**. The sequence of gliadin has been taken from NCBI **[15]** and performed the BLAST **[16]** search against the non-redundant database. Four motifs rich in proline and glutamine which are found are given below and in parenthesis the number of hits of the consensus sequence is mentioned.

1) QLQPFPQP (71 hits); 2) QFPQ (70 hits); 3) QLQPFPQPFPP (no hits); 4) QPFPQPFPP (no hits). Similar procedure has been applied for hordein and secalin present in barley and rye respectively, which are homologous to gliadin and also responsible for CD **[17]**. 1) QPYPQ (30 hits); 2) QPQPYPQQPFPP (6 hits); 3) QPYPQQPFPP (9 hits). After combining these outputs, we have designed 16 peptide sequences (that may bind to IgA in both monomeric and dimeric forms) as follows:

1) QLQPFPQP; 2) QLQPYPQP; 3) QPFPQ; 4) QPYPQ; 5) QLQPFP; 6) QPQPFP; 7) QQQPFP 8) QPQPFPQ; 9)QPQPYPQ; 10) QPFPQQPFPP; 11) QPYPQQPFPP; 12) QPFPQP; 13) QPYPQP; 14) QQQPYP; 15) QGSFQP; 16) QGFFQP

These peptides have been designed in extended conformation using pymol **[18]** and then energy is minimized using swiss pdb viewer **[19]** generating two different sets of minimized and non-minimized conformations.

Docking

The peptides are docked using AutoDock4.0 **[20].** Each peptide is docked against monomeric IgA at epitope binding region of Fab for first monomer, taking the grid center as the center of Fab region consisting of L and A chain and extends point upto 62 in each direction of X, Y and Z axis. Ten best conformations of peptide-IgA complexes are taken into consideration. The number of cases have been manually checked and counted where the peptide is present at variable and junction of variable-constant region of IgA and given in **Table 1**. Out of them, those are found at variable and junction of variable-constant region in higher number of cases are taken for further docking and thus seven peptides have been selected which are given in bold letters in **Table 1**. Then, the grid centre is taken on residue Tyr33 of chain A (because this residue is the approximate centre of the Fab variable region having L and A chains) and selected peptides have been docked against IgA monomer consisting of L and A chains.

The best fit peptide is docked at the second epitope binding region of the first monomer having M and B chains with grid centre Arg45 of B chain (because this residue is the mirror image of the Tyr33 and hence the approximate center of Fab variable region having M and B chains). Afterwards, to see its interaction in the dimeric form it is also docked at the other monomer at their respective epitope binding sites.

Result and Discussion

Docking and Analysis of gluten peptides with IgA

First of all, 16 peptides as mentioned in methods have been designed to check the binding with IgA. Two sets of these peptides have been made: one having the non-minimized conformation and the second one having the minimized conformation using swiss pdb viewer energy minimization tool. However two peptides, QGSFQP and QGFFQP have been rejected because no poly-proline are present in the sequences which is the main characteristic and requirement of gluten peptide to bind with IgA **[14].** So, 14 peptides have been selected for docking with Autodock with grid centre at the centre of the Fab region. The number of cases where these peptides (both minimized and non-minimized conformations) are observed at variable and junction regions of IgA are summarized in **Table 1 (see supplementary material)**. Peptide QPFPQP is interacting with better orientation than QPYPQP with IgA as QPYPQP is shifted more towards the junction region while QPFPQP is interacting at the variable region. Thus 7 peptides which are found in higher numbers at these regions have been selected for further docking with grid centre

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at Tyr33 position (because Tyr33 is the approximate center of the Fab variable region) and are given in bold letter in Table 1. Though the peptide QPFPQQPFPP is tightly fitted in the IgA molecule, only 1 or 2 residues are actually interacting with IgA and rest are away from it because of the orientation of the peptide is outwards. Peptide QPFPQ is weakly interacting with IgA because it is in the vicinity of the IgA molecule and the distance is also more. However, by addition of a Proline residue at the C terminus of peptide QPFPQ, there is a drastic improvement in the binding of the peptide with IgA. The peptide QPFPQP is properly fitted at the variable region of IgA, all residues are interacting with Fab region, side chains of the IgA molecule are also visible to be interacting with the peptide and the orientation is also accurate. Remaining 4 peptides in bold letter of Table 1 are also interacting with IgA molecule at variable region. But the peptide QPFPQP is interacting better than all of the other peptides.

So, the peptide QPFPQP is selected as the best peptide and is docked with the two monomeric and dimeric forms of the IgA molecule. The peptide QPFPQP is interacting at both the lobes of Fab region in the first monomer having L, M, A and B chains. Similarly the peptide is also docked with the other monomer having N O, C and D chains and is found to interact with variable region of IgA. Finally, the peptide is docked with the dimeric IgA molecule and is properly interacting at all the four lobes of Fab variable region.

Interaction between best fit peptide and IgA molecule

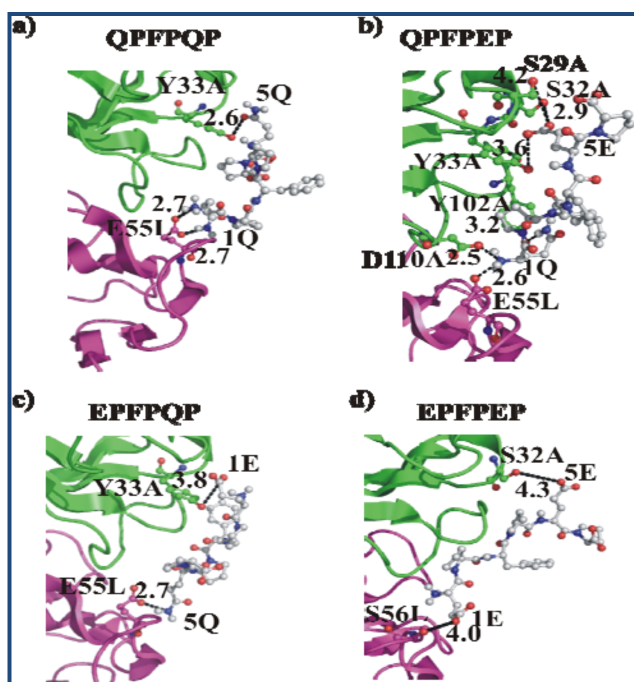

Figure 1: Interactions between the residues of peptide QPFPQP (in 1a), QPFPEP (in 1b), EPFPQP (in 1c) and EPFPEP (in 1D) with IgA molecule are shown in ball and stick mode. Green and magenta color shows the residues of IgA molecule which are interacting while white color shows the peptides with oxygen and nitrogen atoms in red and blue color, respectively. The black dashed lines indicate atoms which are in contact with distance (in Å). The interacting residues are labeled with one letter amino acid code followed by residue number and chain id.

The interactions between peptides and IgA molecule are represented in **Figure 1**. Two Gln residues of the peptide may form hydrogen bonds with polar residue Y33A and acidic residue E55A of IgA molecule as the interacting bond distances between hydroxyl and amino groups are 2.6 and 2.7v respectively **(Figure 1a).** If Q5 is substituted by E the chance of more number of hydrogen bond formation increases; thereby increasing the stability **(Figure 1b).** However substitution of either Q1 or both Q1 and Q5 decreases the chance of hydrogen bond formation **(Figure 1c, d).** It is expected that QPFPEP will intreact with the homo-dimeric IgA molecule in a similar fashion as it interacts with monomeric IgA molecule and more strongly than QPFPQP. At the variable region of IgG molecule (PDB code 7FAB) QPFPQP can bind weakly but QPFPEP cannot bind (data not shown).

The results presented clearly demonstrate that the designed peptide QPFPEP interacts in a stable manner with dimeric immunoglobin A1 molecule and its parent peptide QPFPQP is sequentially present in maximum number of gliadin epitopes. The interaction is specific for IgA AGA detection as the peptide does not even interact with IgG. In the designed hexapeptide chance of nonspecific hydrophobic interaction is less as it contains only one hydrophobic residue (F). The peptide is derived from deamidation of QPFPQP, a gliadin derived peptide and therefore in derivation of the designed peptide, the CD specific change of gliadin is kept into consideration. Earlier phage display study have emphasized role of PEQ for epitope binding **[11].** Here, if the last amino acid of the designed peptide is changed from P to Q our designed peptide will have PEQ tripeptide sequence. But in this mode of peptide engineering it will loose poly-proline, presence of which is proved to be biologically important for gliadin in the context of pathogenesis of CD **[14].** Therefore we advocate QPFPEP sequence to be tried for the purpose.

Conclusion:

The hexapeptide (QPFPEP) predicted to bind specifically with IgA antigliadin antibody. If it is proved to be a fact by experimental analysis the hexapeptide has the potential to provide a cost effective method for screening of celiac disease.

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Supplementry material:

Table 1: Table showing the number of cases where minimized and nonminimized conformations of the peptides are located at the variable and junction regions of IgA out of ten peptide-IgA complexes for each conformation of peptide obtained by using the center of the Fab region as the grid center for docking. The peptides in bold-letter are found in higher number of cases at variable and junction regions compared to rest of the peptides.

S.No.	Peptides	Number of cases			
		Minimized conformation of peptide (Out of 10 peptide-IgA complex)		Nonminimized conformation of peptide (Out of 10 peptide-IgA complex)	
		Variable Region of IgA	Junction Region of IgA	Variable Region of IgA	Junction Region of IgA
1	QLQPFPQP	0	0	1	1
2	QLQPYPQP	0	0	0	0
3	**QPFPQ**	2	2	1	2
4	**QPYPQ**	3	0	2	3
5	**QLQPFP**	0	0	3	1
6	QPQPFP	0	1	1	1
7	**QQQPFP**	1	0	2	1
8	QPQPFPQ	0	0	0	1
9	QPQPYPQ	1	0	0	0
10	QPFPQQPFPP	0	0	0	0
11	**QPYQQPFPP**	3	0	0	0
12	**QPFPQP**	3	0	3	1
13	**QPYPQP**	1	1	1	1
14	QQQPYP	0	0	0	0

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