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IN SILICO IDENTIFICATION OF POTENTIAL AMINO ACIDS FOR ENAMEL REMINERALIZATION

Manobharathi G[1], Sandhya Raghu[2*], Ajay Guru[3], Rajalakshmanan Eswaramoorthy[4]

[1]Post graduate student, Department of Conservative Dentistry and Endodontics, Saveetha Dental college and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077

[2*]Professor, Department of Conservative Dentistry and Endodontics, Saveetha Dental college and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077

[3]Assistant Professor, Department of Conservative Dentistry and Endodontics, Saveetha Dental college and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077

[4]Professor, Department of Biochemistry, Center of Molecular Medicine and Diagnostics (COMManD), Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai - 600077

Corresponding author: [2*]Sandhya Raghu

[2*]Professor, Department of Conservative Dentistry and Endodontics, Saveetha Dental college and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077

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ABSTRACT:

Various amino acids play a role in formation of continuous organic framework, hydroxyapatite crystal formation, mineral nucleation, orientation of mineral crystallites. Self-assembling peptides have shown to diffuse into the porosities in the enamel, attract calcium and allow its saturation.

Aim: Aim of this study was to design peptides and identify potential amino acids for enamel remineralization.

Methodology: Glutamine, Aspartic acid, Phosphorylated Serine were the amino acids identified for the analysis. The influence of the positions of these amino acids at the N terminal and C terminal were assessed. 6 peptides were designed for computational analysis. Designing of peptide and receptor of amelogenin was done using Chemdraw Ultra Ver, Binding affinity was analysed in HPEPDOCK tool. Toxicity was analysed using ToxinPred and Peptide Ranker Analysis. Peptide P11-4 was the positive control.

Results: Positive control of P114 showed the highest binding affinity score of -419.327 Kcal/mol when compared with other peptides. Peptide 3 (-294.701 Kcal/mol), 4 (-294.668 Kcal/mol), and 6 (-293.411 Kcal/mol) showed the least binding affinity score. But a nearly equivalent binding affinity score compared to P114 peptide was obtained in the Peptide 1 (-327.112 Kcal/mol), 2 (-310.596 Kcal/mol) and 5 (-328.708 Kcal/mol). All the peptides showed non-toxic condition, while the peptide 2 (0.251) and peptide 5 (0.244) showed better bioavailability.

Conclusion: Newly designed peptides were stable. Binding affinity was comparable to P 11- 4. The designed peptides were non-toxic. Aspartic acid had binding affinity closer to P 11-4 and biocompatibility more than P 11-4.

Keywords: Demineralization, calcium phosphate, Amino Acids, Enamel, affinity

1. INTRODUCTION

Demineralization is a slow process that causes the hydroxyapatite crystals in enamel to dissolve gradually, causing tooth tissue to gradually disappear [1]. Dental enamel is an avascular, hard, and acellular tissue that is made up of 1% organic material, 3% water, and 96% inorganic material (hydroxyapatite nanocrystals)[2]. Ameloblasts create the enamel protein matrix, which is subsequently mineralized by crystals of calcium phosphate[3]. Recently, the use of self assembling peptides has been introduced as a strategy for enamel remineralisation[4]. Amino acids are the building blocks of peptides. The location of amino acid side chains which have a terminal –COOH or –NH2, can be designed in a way so as to control the interaction between adjacent peptides. P11-4, a self-assembling peptide molecule, has been thoroughly investigated. It is a synthetic peptide with a final design consisting of 11 amino acids that assembles into B-sheet tapes, ribbons, fibrils, and fibers using a hierarchical self-assembly process[5]. Several agents that enter an initial lesion as liquid resin (ICON caries inltrant) or encourage remineralisation by releasing calcium and phosphate have been launched in recent years (e.g, casein phosphopeptide- amorphous calcium phosphate, CPP-ACP complex). Furthermore, nanohydroxyl apatite-based products (such as BioRepair) have been proposed for "repairing" enamel . These agents, on the other hand, were unable to produce matrix-mediated mineralization in the same way as the natural process does. To combat this, the self-assembling peptide (SAP) P11-4 was developed to rebuild enamel by matrix mediated mineralization[6]. Various remineralizing agents are available for non-invasive management of these incipient lesions[7].

Numerous biological processes, including signal transmission, immune responses, and cellular control, depend on peptide–protein interactions. It has been discovered that small peptides mediate around 40% of protein–protein interactions. Therefore, in order to comprehend the molecular mechanism and subsequently modify the protein-protein interactions for therapeutic purposes, it is imperative to determine the structure of the protein-peptide complexes participating in these interactions[8]. However, because experimental methods are expensive and technically complex, only a small number of protein-peptide complex structures have been determined, in comparison to the enormous number of protein-peptide interactions that have been found. Thus the determination of protein–peptide complex structures has been greatly aided by computational modeling techniques such as molecular docking[9][10]. Protein-peptide docking takes a protein structure and a peptide sequence as inputs, samples potential peptide binding conformations, and ranks the potential protein-peptide complexes using an energy scoring function to predict the complex structure[5].

There are two difficulties with protein-peptide docking as opposed to protein-ligand and protein-protein docking. First, unlike protein–ligand docking in which the binding site is normally known, the information of the binding site for peptide is not available in many cases. As a result, a comprehensive search for potential binding modes throughout the entire protein is frequently necessary for protein-peptide docking[11][12]. Second, peptides lack a stable conformation prior to binding to a receptor and are far more flexible than small molecules and proteins. In protein–peptide docking, sampling all of the peptide conformations is computationally costly. In recent years, numerous cutting-edge algorithms for protein-peptide docking have been developed to address these issues. To efficiently consider the peptide flexibility in peptide docking, a hierarchical algorithm for blind and flexible peptide docking by fast modeling of peptide conformations and sequent global sampling of binding orientations, which is referred to as HPEPDOCK is used. In this docking algorithm, the peptide flexibility is considered by generating an ensemble of peptide conformations with various program. Peptideranker and ToxinPred are the servers used to predict BioActive peptides and toxicity of

peptides. The aim of this study was to design peptides and identify potential amino acids for enamel remineralization by In Silico analysis.

2. MATERIALS AND METHODS

Glutamine, Aspartic acid, Phosphorylated Serine were the amino acids identified for the analysis. The influence of the positions of these amino acids at the N terminal and C terminal were assessed. 6 peptides were designed for computational analysis. Designing of peptide and receptor of amelogenin was done using Chemdraw Ultra Ver. Binding affinity was analysed in HPEPDOCK tool. Toxicity was analysed using ToxinPred and Peptide Ranker Analysis. Peptide P11-4 was the positive control.

P11-4 Positive Control
Ac-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2
6 New Peptides
Peptide 1 - Ace-Gln-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2
Peptide 2 - Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-Gln-NH2
Peptide 3 - Ace-Gln-Gln-Aspartic acid-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2
Peptide 4 - Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Aspartic acid - Gln-Gln-NH2
Peptide 5
Ace-Gln-Gln-Phosphorylated serine-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2
Peptide 6
Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu- Phosphorylated serine-Gln-Gln-NH2

Insilico studies
HPEPDOCK
HPEPDOCK (http://huanglab.phys.hust.edu.cn/hpepdock/) is a server that uses a hierarchical algorithm to investigate protein-peptide docking. The results obtained from the protein-ligand binding affinity are expressed in kcal/mol units of free energy. In the discovery studio software, the interaction between the peptide and the receptor was visualized.

Peptide rank and toxicity prediction
PeptideRanker (http://distilldeep.ucd.ie/PeptideRanker/) is a server that uses a novel N-to-1 neural network to predict bioactive peptides. The Peptide Ranker predicts whether a peptide sequence is likely to be bioactive by giving scores ranging from 0 to 1. A peptide score of more than 0.75 indicates that a sequence has the potential to be bioactive.
ToxinPred was used to calculate the toxicity of predicted T-cell epitopes (http://crdd.osdd.net/raghava/toxinpred/). ToxinPred is a computer programme that predicts which peptides are toxic or non-toxic.

3. RESULTS

These newly developed 6 peptides were investigated for the docking studies using http://huanglab.phys.hust.edu.cn/hpepdock/ and before docking analysis the peptide structure was developed using Chemdraw Ultra Ver 12.0. The receptor of amelogenin was structured. Both the peptide structure and receptor of amelogenin in PDB format was used for the analysis in HPEPDOCK tool.

P 11- 4 was the positive control (Figure 1). Peptide 1 to Peptide 6 were designed using Chemdraw Ultra Ver (Figure 2-7)

Figure 1: P11- 4 structure

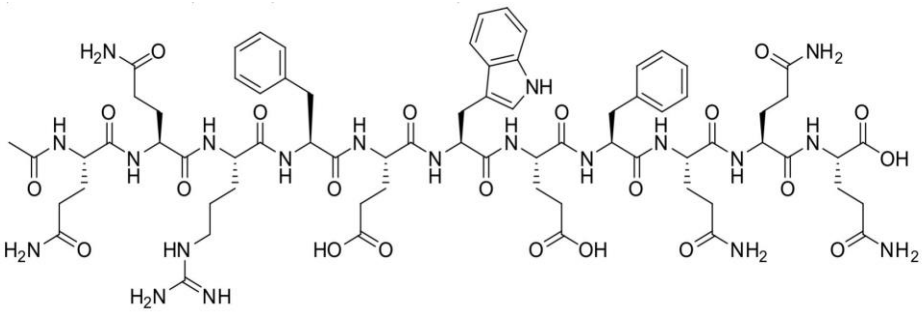


Figure 2: Peptide 1 structure

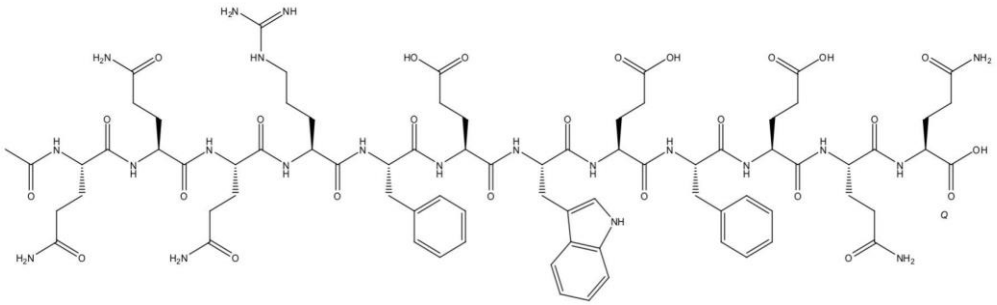


Figure 3: Peptide 2 structure

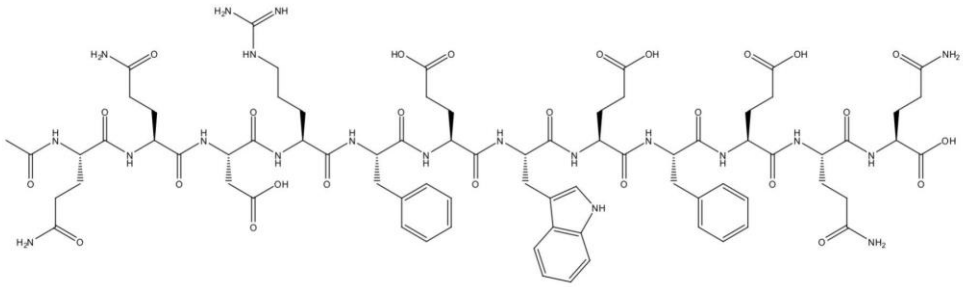


Figure 4: Peptide 3 structure

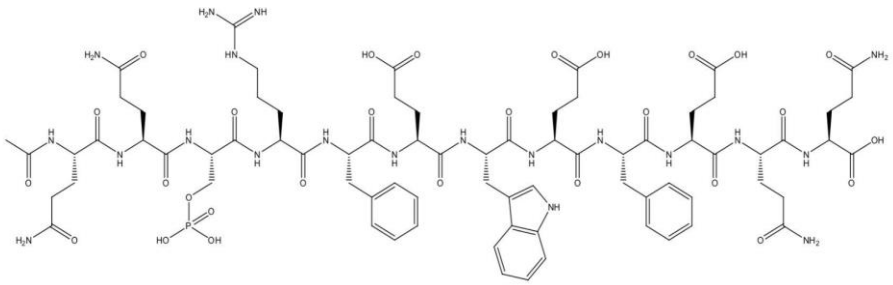


Figure 5: Peptide 4 structure

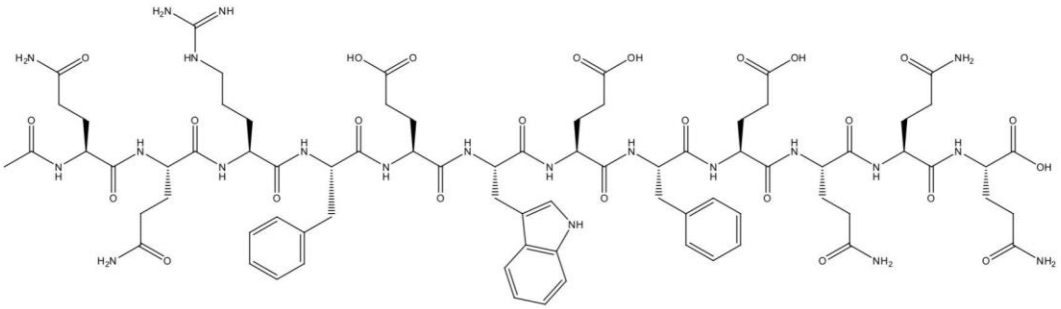


Figure 6: Peptide 5 structure

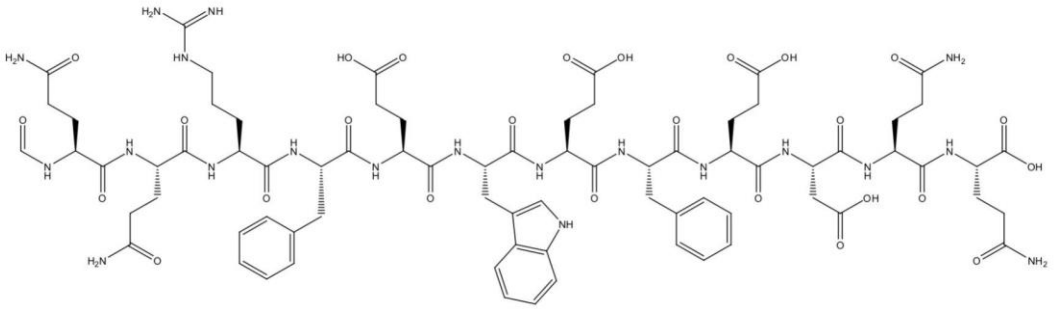
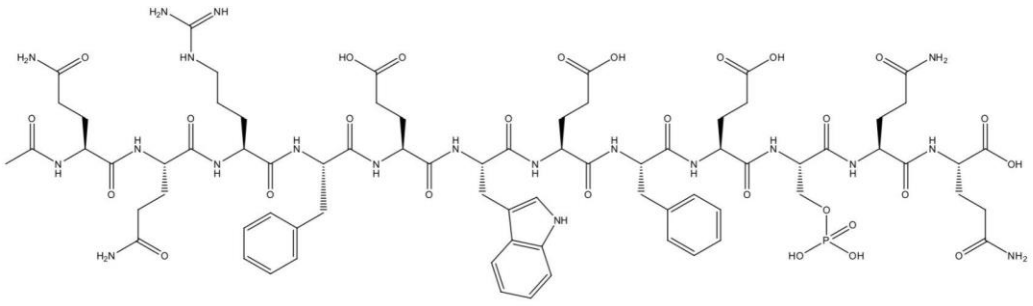


Figure 7: Peptide 6 structure



The **PeptideRanker** tool can order a set of peptides and-based on the function-structure model-assign a score for a given peptide in the range from 0 to 1. All the peptides showed non-toxic condition, while the peptide 2 (0.251) and peptide 5 (0.244) showed better bioavailability than the control peptide of P114 (0.235). These results suggest that peptide 2 and peptide 5 can show better bioavailability compared to the P11-4 peptide. P11-4 peptide[13] was used as the positive control. Based on the P11-4 peptide sequence, different peptides were self-assembled by addition of suitable amino acids into the sequence. The amino acids such as glutamine, aspartic acid and phosphorylated serine are reported to show the calcium binding effect when they are present in the specific site of the amino sequence. Based on these conditions, the amino acids were added to the sequence. These newly developed 6 peptides were investigated for the docking studies using http://huanglab.phys.hust.edu.cn/hpepdock/ and before docking analysis the peptide structure was developed using Chemdraw Ultra Ver 12.0. The receptor of amelogenin was structured. Both the peptide structure and receptor of amelogenin in PDB format was used for the analysis in HPEPDOCK tool. The results showed that positive control of P11-4 showed the highest binding affinity score of -419.327 Kcal/mol when compared with other peptides. Peptide 2 (-294.701 Kcal/mol), 5 (-294.668 Kcal/mol), and 6 (-293.411

Kcal/mol) showed the least binding affinity score of. But a nearly equivalent binding affinity score compared to P11-4 peptide was obtained in the Peptide 1 (-327.112 Kcal/mol), Peptide 3 (-310.596 Kcal/mol) and Peptide 4 (-328.708 Kcal/mol) (Figure 8). Toxicity analysis showed Peptide 3 (0.251) and Peptide 4 (0.244) had the highest biocompatibility (Figure 9).

C Terminal

Peptides	Binding affinity
Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2 (P114)	-419.327
Ace-Gln-Gln-**Gln**-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2	-327.112
Ace-Gln-Gln-**Aspartic acid**-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2	-310.596
Ace-Gln-Gln-**Phosphorylated serine**-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2	-294.701

N Terminal

Peptides	Binding affinity
Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2 (P114)	-419.327
Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-**Gln**-Gln-Gln-NH2	-294.668
Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-**Aspartic acid** - Gln-Gln-NH2	-328.708
Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu- **Phosphorylated serine**-Gln-Gln-NH2	-293.411

Binding affinity in Kcal/mol

Fig 8 : Binding affinity of newly designed peptides assessed using HPEPDOCK

C Terminal

Peptides	Toxicity	Bioavailability
Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2 (P114)	Non-toxic	0.235
Ace-Gln-Gln-**Gln**-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2	Non-toxic	0.224
Ace-Gln-Gln-**Aspartic acid**-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2	Non-toxic	0.251
Ace-Gln-Gln-**Phosphorylated serine**-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2	Non-toxic	0.232

N Terminal

Peptides	Toxicity	Bioavailability
Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2 (P114)	Non-toxic	0.235
Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-**Gln**-Gln-Gln-NH2	Non-toxic	0.19
Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-**Aspartic acid** - Gln-Gln-NH2	Non-toxic	0.244
Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-**Phosphorylated serine**-Gln-Gln-NH2	Non-toxic	0.206

Fig 9 : ToxinPred and Peptide Ranker Analysis

4. DISCUSSION

The technology of self assembling peptide P11-4 adopts a biomimetic approach where it forms a matrix scaffold for de novo synthesis of hydroxyapatite crystals[1][14]. Chemicals such as fluoride, CPP and Triethylamine used for remineralization has either the risk of fluorosis or mandatory dependance on residual hydroxyapatite crystals. Full sequence amelogenin for enamel remineralization has limitation of poor affinity of remineralized layer to enamel, increased time of contact, high cost of synthesis. Hence key functional amino acid that hence hydroxyapatite nucleation and crystal formation needs to be identified. Self assembling peptides are identified to have remineralization potential in vitro and in vivo. P11-4 peptide was used as the positive control. Based on the P11-4 peptide sequence different peptides are self assembled by addition of suitable amino acid into the sequence. The amino

acid such as glutamine, aspartic acid and phosphorylated serine are reported to show the calcium binding effect when they are present in the specific site of the amino sequence. Based on these condition, the amino acids were added to the sequence. Glutamine is an alpha amino acid, Presence of Gln in the PX33 sequence is essential to ensure correct HA crystal growth. Alpha amino acid helps in biosynthesis of proteins and it promotes crystallization kinetics of metastable ACP to nano apatite through synergistic effect. Aspartic acid[15] can promote the crystallization kinetics of metastable ACP to nano apatite through synergistic effect. The remineralized dentin obtained by this strategy can achieve mechanical and biological properties similar to that of natural teeth due to the internal and external mineralization of collagen fibers[16][17]. Serine is an Alpha amino acid, helps in biosynthesis of proteins. It is a calcium binding motif Phosphorylation of Serine increases the ability to effectively bind calcium ions. The results showed that positive control of P11-4 showed the highest binding affinity score of -419.327 Kcal/mol when compared other peptides. Peptide 2 (-294.701 Kcal/mol), 5 (-294.668 Kcal/mol), and 6 (-293.411 Kcal/mol) showed the least binding affinity score. But nearly equivalent binding affinity score compared to P11-4 peptide was obtained in the Peptide 1 (-327.112 Kcal/mol), 4 (-310.596 Kcal/mol) and 4 (-328.708 Kcal/mol). In the designed sequence, addition of aspartic acid has shown binding affinity better than the other newly designed peptides and also better biocompatibility.

This study is a computational analysis to identify the peptides that could support calcium binding. Development of peptide is expensive and time consuming. Such insilico analysis would support the identification of the peptide. The designed peptide should be synthesized and further subjected to analysis in laboratory to check its stability, binding properties, toxicity.

5. CONCLUSION

Newly designed peptides were stable and binding energy of them were comparable to P 11-4. The designed peptides were non toxic.. Equivalent binding affinity score compared to P11-4 peptide was obtained in the Peptide 1. Aspartic acid has binding affinity closer to P11-4 and biocompatibility more than P 11-4. Further Analysis needed to study the structure, mechanical properties of protein complexes and confirm the formation of hydroxyapatite bundles

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