Acta Scientific Dental Sciences

Volume 2 Issue 6 June 2018

Review Article

Statherin-Role in Biomimetic Early Caries Management

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Received: March 30, 2018; **Published**: May 21, 2018

Abstract

Dental enamel is a highly mineralized acellular tissue comprising of individual crystallites which are larger and more oriented than other mineralized tissues of the body. Morphologically dental enamel is formed by matrix-mediated biomineralization. Precipitation of enamel crystallites from a supersaturated solution within a well-delineated biological compartment lead to enamel lattice crystallization. The enamel crystal comprises of carbonated apatite which dissolves by organic acids (lactic and acetic) produced by the cellular action of plaque bacteria on dietary carbohydrates. Demineralization occurs when pH levels fall to 5.7. Salivary proteins slow down enamel demineralization by inhibiting calcium hydroxyapatite (HA) demineralization. Remineralization allows loss of calcium, phosphate, and fluoride ions to be replaced by fluorapatite crystals which are more resistant to acid dissolution and are substantially larger than the original crystals, thereby providing a more favorable (smaller) surface to volume ratio. Hence larger apatite crystals in remineralized enamel are more resistant to enamel breakdown by the resident organic acids. Statherin (StN43), a 43-residue phosphorylated salivary protein with primary sequence similarities to osteopontin and caseins, binds calcium and HA. The identification of the minimum length of the functional domain of the statherin molecule augments the understanding of cariostatic functions by measuring the efficacy of peptides of progressively shorter length (i.e. containing only the N-terminal 21 (StN21), 15 (StN15), 10 (StN10), or 5 (StN5) residues) to reduce HA demineralization rates (RDHA). The mechanism by which statherin-like peptides reduce Hydroxyapatite demineralization rates may be associated with their binding to HA surfaces. Overview of previously published binding energies of statherin to HA also suggest that statherin-like peptides containing 21 and 15 N-terminal residues or more are required for binding suggesting a link between binding and demineralization reduction. The short chain statherin N-Terminal residues do not exert a cariostatic effect owing to the inability to undergo helical changes on adsorption to hydroxyapatite crystals. Different remineralization strategies using fluoride, electrolytic deposition, hydroxyapatite nanoparticles, amorphous calcium phosphates, and hydrogen peroxide have been developed recently and synthetic peptides are being employed in biomimetic hard tissue remineralization processes. Further *in vivo* studies are needed to develop salivary biomimetic peptides for anti-caries applications.

Keywords: Statherin; Biomimetic; Hydroxyapatite

Introduction

Dynamic Processes of Enamel

Dental enamel is the hardest acellular tissue unlike other mineralized tissues of the human body (dentine, cementum and bone) and comprises approximately 96% carbonated hydroxyapatite (HA) mineral with exceptional flexural strength and hardness [1]. In a healthy environment of the oral cavity, enamel stays relatively stable, with a dynamic equilibrium between processes of demineralization and remineralization at the interfaces between tooth-salivary pellicle and plaque-saliva [2]. The imbalance between demineralization and remineralization renders the enamel susceptible to permanent damage by a dynamic disease process called dental caries [3]. Hence remineralization of early small caries lesions before they progress into cavities is given emphasis to for inhibiting irreversible destruction of enamel.

Different remineralization strategies using fluoride, electrolytic deposition, hydroxyapatite nanoparticles, amorphous calcium phosphates, and hydrogen peroxide have been developed recently [4-8] and synthetic peptides are being employed in biomimetic hard tissue remineralization processes [9-11]. Some methods are however not applicable clinically subject to stringent conditions, adverse reactions or controversial therapeutic efficacy [12].

Dental caries are treated conventionally by the mechanical removal of the affected part and filling it with a resin or metal alloy. In early small lesions this method is not beneficial since healthy tooth must be removed disproportionately for accommodating the alloy or resin. Various non-surgical methods for managing noncavitated carious lesions include therapies such as fluorides (F) in the form of (toothpaste, gel, varnish, mouthrinse, and combination), seal-

ants (S), xylitol (X) in the form of (lozenges, gum, or in combination with F and/or xylitol) Chlorhexidine (CHX) alone or in combination with F, casein phosphopeptide amorphous calcium phosphate (CPP-ACP) or in combination with calcium fluoride phosphate [12]. Hence in situ HA remineralization in physiological oral conditions is a preferred alternative pathway for restoring caries by repairing early carious lesions via nanocrystalline growth and minimizing natural enamel tissue preparation.

Enamel is constantly exposed to the oral salivary fluid which exhibits a crucial role in remineralization process by providing a multitude of proteins. These proteins maintain the integrity of teeth and regulate caries risk [13]. Statherin including cystatins, histatins and the acidic Proline rich proteins are major salivary proteins which maintain salivary homeostasis of the supersaturated state of calcium phosphate salts and impart a reparative and protective environment crucial for the integrity of teeth [14].

Statherin

Statherin is a 43-residue, dephosphorylated, asymmetric, tyrosine-rich, acidic salivary peptide with a molecular weight of 5380 Daltons and a concentration of 10 - 40 μM in the human saliva [15]. It is secreted by the acinar cells of the submandibular and parotid salivary glands and the primary structure comprises of tyrosine-rich residues and vicinal phosphoserines [16]. The primary sequence is similar to casein and osteopontin [17].

The glutamic acid containing N-terminus of statherin is highly charged and vital in recognizing hydroxyapatite crystals [18]. *In vitro* studies on N-terminal 15-amino-acid residue of statherin (SN15) with a structure (DpSpSEEKFLRRIGRFG, where pS specifies a phosphorylated serine) and its analog SNA15 (DDDEEKFLRRIGRFG) display high adsorption on the surface of HA [19]. N-terminal domain (residues 1–12)are in close proximity to the HA surface upon adsorption due to its α-helical structure [20]. A cysteine peptide based on the initial six-peptide sequence of N-terminus possesses a strong adsorption capacity to the tooth enamel [21]. Hence the key amino acid sequences identified in statherin are particularly important for HA adsorption and peptides based on these key residues may augment in situ enamel biomimetic remineralization. N-terminus of statherin is synthesized by fluorenylmethoxycarbonyl (Fmoc) solid-phase method [9].

The C-terminal fragment inhibit the growth of anaerobic bacteria in oral cavity [22] by binding to bacterial fimbriae via recognition receptors expressed when it is adsorbs to HAP on the mineral surface [23]. Statherin molecule is present in a solution in an unfolded state however on adsorption to HAP it undergoes a structural transition or folding to a well determined functional structure which may explain the structural basis for biological functioning [23].

Statherin function in the transport of calcium and phosphate during secretion of salivary glands and their concentration is not subject to circadian rhythms like other salivary peptides [24]; promote bacterial adhesion to enamel surfaces and provide a boundary lubricant at the enamel interface [25]. Mineral solution dynamics of enamel are maintained by inhibiting Hydroxyapatite crystallization and spontaneous calcium phosphate precipitation *in vivo* [26] and binding to hydroxyapatite *in vivo* [27] thus inhibiting nucleation and growth of hydroxyapatite crystal and their concentration. It is the only salivary protein that inhibits the spontaneous precipitation of calcium phosphate salts from the supersaturated saliva and is a very potent inhibitor of crystal growth compared to other salivary proteins [28]. It inhibits both primary as well as secondary precipitation of calcium phosphate salts [29]. The mechanism by which statherin inhibits dissolution is not known but may be associated with binding of the statherin peptide to the surface of hydroxyapatite and inhibition of coalescence of dissolution pits on the surfaces [30,31]. Structural domains of salivary statherin partly responsible for the protection and recalcification of tooth enamel have been examined with respect to charge, sequence, hydrophobicity, hydrogen bonding potential and conformation.

Solid-state nuclear magnetic resonance (NMR) studies, confirmed that the N-terminus of statherin bound strongly to HA, whereas the middle and C-terminal regions were mobile and dynamic. This may be attributed to the clustering of nearly all the negative charge at the N terminus of statherin [32]. The N terminus of statherin is suggested to be in charge for anchoring this molecule to the HA surface by assuming an alpha-helix structure upon adsorption whereas other molecular regions maybe involved in stabilizing this adsorption [33]. The N terminus of a penta peptide similar to the N terminus of statherin was found to bind strongly to HA surfaces in an extended formation [34]. One mechanism for this conformational change may be related to a change at K6 (lysine residue) from a helical structure to a random-coil structure. Additionally may be an interaction of protein side chains at the K6 position and the hydroxyapatite forming a hydrogen bond network enhancing protein–mineral recognition [35]. The alternative mechanism proposes an electrostatic interaction between the peptide and the HA surfaces [36]. which coincides with the thermodynamic studies [37]. Calculation of adsorption free-energy interactions of statherin-HA binding performed via three dimensional (3D) atomic-level modeling binding energies methods known as Monte-Carlo and energy-minimization computational methods [22] calculate solvation energies, hydrogen bonding, van der Waals interactions and electrostatic interaction. These suggest a molecular level recognition at the saliva enamel interface between the a-helix of statherin at its N terminus with the predominant 001 face of HA [38].

Correlation of a three dimensional structural model for statherin with functional activities is important because an intact molecule of statherin has been implicated in all of its functional roles in an oral cavity. Three-dimensional structural models for statherin at the enamel interface and in solution are proposed based on secondary structure predictions, circular dichroism of intact as well as synthetic fragments, and molecular modeling [39].

Treatment with a 21 N-terminal residue of a statherin-like peptide reduces the rate of demineralization in HA in caries-simulating solutions by almost 50% [29]. Another study demonstrates a similar amount of reduction in HAP demineralization in treatment with a peptide composed of only the 15 N-terminal residue of statherin. Peptides of only 10 or 5 N-terminal residues did not exert any sizable impact on reducing the rate of demineralization of HA in artificial caries like condition [17]. This suggests that cariostatic function of statherin-like peptides requires N-terminus of 15 residues or more. The computational residue–surface interaction analysis of statherin with HA proposes maximum binding en-

ergy at the N terminus, between residues 1 - 15, and in particular with the binding energy of Arg13 noted for exhibiting the strongest van der Waals interaction and hydrogen bond [38]. The inability of pentapeptide DpSp-SEE to inhibit HA demineralization is due to its inability to form an a-helix formation which may be required for cariostatic function [34]. A recent study on a cysteine-labelled peptide based on the first six-peptide sequence of N-terminus of statherin reports a strong enamel adsorption capacity [40].

Conclusion

Hence further *in vivo* studies to further develop salivary biomimetic peptides for anti-caries clinical applications. Simulating natural processes in the oral cavity shall help augment and acclimate this peptide for a biomimetic early caries management.

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Volume 2 Issue 6 June 2018