REVIEW ARTICLE

# Casein Phosphopeptide- Amorphous Calcium Phosphate (CPP-ACP): An adjuvant for Caries prevention

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## ABSTRACT

Bioactive peptides are specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence health. Casein phosphopeptide is a bioactive peptide released on tryptic digestion of milk protein and shown to have anticariogenic effects. Reynolds showed calcium-phosphate-stabilizing phosphopeptide was the active agent responsible for this activity. The phosphopeptide contains phosphoseryl cluster sequence Ser(P)-Ser(P)-Ser(P)-Glu-Glu which is critical for binding of calcium phosphate. The interaction of CPP-ACP with fluoride is synergistic. Currently, it is available as chewing gum and topical cream and has shown to exert preventive and curative effect against dental caries. This systematic review sought to evaluate CPP-ACP & its ability to prevent caries.

**KEYWORDS: CPP-ACP, Bioactive peptides, anti-cariogenic**

## INTRODUCTION

Dental caries is the localized disease cause by dissolution and destruction of the tooth tissue by specific dental plaque bacteria that ferment dietary sugar to organic acids. It is a multi-factorial disease that includes the participation of cariogenic and non-cariogenic bacteria, salivary components (proteins, enzymes, calcium, phosphate, fluoride) and dietary sources of fermentable carbohydrates (sucrose, glucose). Dental caries is a big public health problem with a huge economic burden on the health care system.

Bioactive peptides are small protein fragments that have biological effects once they are released, in vivo a great number of bioactive peptides from milk and soy proteins have been identified and characterized for the health benefits such as antioxidant, antihypertensive, hypo-cholesterolemic, immunomodulatory, or opioid activities.) Casein phosphopeptide is a bioactive peptide released on tryptic digestion of milk protein casein which exerts many physiological roles e.g. enhancement of mineral solubility and absorption in digestive tract, immunomodulatory activity, in particular the enhance-ment of IgA levels, bone calcification, in addition to this recent research has indicated its potential role as a anticariogenic agent.[2,3]

## MILESTONES IN CASEIN PHOSPHOPEPTIDE DISCOVERY AS AN EFFECTIVE ANTICARIO-GENIC AGENT

Lady Mellanby proposed that milk affects Pre-eruptive tooth mineralization & Post-eruptive caries resistance.[4] Shaw JH demonstrated that dairy products have anti-caries activity.[5] Bavetta reported that acid casein was able to reduce caries when used as toothpaste. Unfortunately, the levels of casein needed to reduce caries, made the toothpaste unpalatable.[6] Reynolds quoted that soluble sodium caseinate in the drinking water of rats was able to reduce the extent of pit and fissure caries. Harper reported that milk and milk products were found to reduce the numbers of mutans streptococci.[(7)]E.C. Reynolds showed that including sodium caseinate in chocolate confectionary reduced cariogenicity, but the levels of caseinate required (16.6%) made the confectionary unpalatable.[8] Later he showed that trypsin digestion of caseinate did not destroy its ability to prevent enamel demineralization & quoted that tryptic peptides responsible for the anticariogenic activity were the calcium-phosphate-stabilizing phosphopeptide.[9,10] The CPP-ACP complex is a patent of University of Melbourne, Australia, and the Victorian Dairy Industry Authority, Abbotsford, Australia. Its manufacturing and marketing rights are with Bonlac Foods Limited. [11]

## SOURCES OF CASEIN PHOSPHO-PEPTIDE

The major source for casein-phosphopeptide is milk apart from milk it can also be obtained from Cheese, Yoghurt, Food containing Phosphoprotein (Soya bean,Cereals, Nuts, etc.). in addition to these sources it can also be prepared synthetically.

**Structure & composition of casein-phosphopeptide:**

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The major casein phosphopeptides released by trypsin that sequester calcium phosphate are:-

**$\alpha_{s1}$(59-79)**Gln[59]-Met-Glu-Ala-Glu-Ser(P)-Ile-Ser(P)-Ser(P)- Ser(P)-Glu-Glu-Ile-Val-Pro-Asn-Ser(P)-Val-Glu-Gln-Lys[79]

**β(1-25)**-Arg[1]-Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Ser(P)-Leu-Ser(P)-Ser(P)-Ser(P)- Glu-Glu-Ser-Ile-Thr-Arg.[25]

**$\alpha_{s2}$(46-70)**Asn[46]-Ala-Asn-Glu-Glu-Glu-Tyr-Ser-Ile-Gly-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser(P)-Ala-Glu-Val-Ala-Thr-Glu-Glu-Val-Lys.[70]

**$\alpha_{s2}$(1-21)**-Lys[1]-Asn-Thr-Met-Glu-His-Val-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Ile-Ser(P)-Gln-Glu-Thr-Tyr-Lys.[21]

The major tryptic CPP are **β (1-25)** and **$\alpha_{s1}$ (59-79)** with smaller amounts of **$\alpha_{s2}$ (46-70)** and **$\alpha_{s2}$ (1-21).** All contain cluster sequence Ser(P)-Ser(P)-Ser(P)-Glu-Glu which remarkably stabilize calcium phosphate (9,15,16,17,18, 19,20,21). CPP can stabilize over hundred times more calcium phosphate than is normally possible in aqueous solution at neutral or alkaline pH before precipitation.[15]

CPP-ACP has amorphous nature which has been shown by diffraction images using scanning electron microscopy (SEM) & transmission electron microscopy (TEM).[22] Nuclear magnetic resonance has shown CPP-ACP nano-complexes are particles with hydrodynamic radii ranging from 1.526 ± 0.044 nm at pH 6.0 increasing to 1.923 ± 0.082 nm at pH 9.0 in the case of casein phosphopeptide β-casein (1-25)(22, 23).

CPP consists of loops and turns. The proposed model ACP core that consists of two calcium phosphate phases.[16]

1. Meyer and Eanes suggested a calcium-poor phase, such as $Ca_3(PO_4)_2$, forming the core of the ACP particle with a Ca:P ratio of 1.5.
2. A calcium-rich phase with a Ca:P ratio of 2.0, such as $Ca_2(PO_4)(OH)$, that is in contact with the peptide.

CPP can stabilize over hundred times more calcium phosphate than is normally possible in aqueous solution at neutral or alkaline pH before precipitation. The bond between CPP & ACP is pH dependent and as the pH decreases the bond dissociates releasing free calcium & phosphate which binds to CPP.[17,18]

## MECHANISM OF ACTION OF CASEIN-PHOSPHOPEPTIDE

The proposed mechanisms of action of CPP-ACP are:

**Remineralization of enamel:** A series of solutions containing the various concentration of CPP-ACP were prepared & the associations between the various calcium phosphate species released by CPP-ACP complex and the rate of enamel lesion remineralization was evaluated. The solutions contained CPP (0.1-1.0%), calcium (6-60 mM) and phosphate (3.6-36 mM) at different pH (7.0-9.0).[14,19,20] The activity of the neutral ion species $CaHPO_4^0$ was found to be highly correlated with the rate of the lesion remineralization indicating that the CPP-bound ACP, $CPP[Ca_3(PO_4)_{1.87}(HPO_4)_{0.2}xH_2O]_8$ acts as a reservoir of calcium phosphate ions, including $CaHPO_4^0$& the remineralization process comprises of distribution of $CaHPO_4^0$ and related calcium and phosphate ions through the protein/water occupied holes of damaged surface enamel into the body of the enamel lesion leading to hydroxyapatite formation.[19] In addition to this there is a generation of acid and phosphate, including the neutral ion $H_3PO_4^0$ which diffuses out of the lesion. CPP-ACP would consume the acid by generating $CaHPO_4^0$ thus maintaining its concentration gradient in the lesion.[19,21]

**CPP-ACP on plaque biofilm**:Studies have demonstrated CPP gets incorporated in salivary pellicle and reduces the adhesion of S.Mutans & S.Sorbinus. CPP-ACP and calcium compete for the same binding sites on the surface of S.Mutans, and the affinity of CPP-ACP is twice that of Calcium, thus inhibiting the calcium supply to the bacteria and thus help in the formation of non-cariogenic plaque.[21,22,23]

**Inhibition of demineralization**[4,9]: In a study, the effect of CPP on inhibition of calcium loss by enamel subjected to demineralizing conditions was investigated. Windows approximately 1 $mm^2$ were painted onto abraded and polished bovine enamel using acid-resistant varnish. The teeth were then demineralized for 8 days at 37°C in a 0.05 mol/L lactic acid 8% methylcellulose gel at pH 4.5. A pellicle was developed on the tooth surface by immersing it in whole human unstimulated saliva for approximately 34 hrs. Following rinsing in water, the teeth were treated with either 300 µL of a 1% w/w solution of CaPCPP or a water control for one hour, followed by a sixteen-hour treatment at room temperature. Remineralizing solution contains radioactive calcium, i.e.,$^{45}$Ca. The windows were then repeatedly acid-etched and the acid extractions analyzed by scintillation counting, allowing the uptake of $^{45}$Ca into the enamel (µg/$cm^2$) to be calculated. Following pretreatment of partially demineralized enamel with CPP, the subsequent uptake of calcium under remineralizing conditions was found to be significantly reduced by about 80% compared with the control (no CPP) treatment. It can be seen that enamel which was pre-treated with CPP showed greater retention of $^{45}$Ca compared with the control treatment group.

## PROPERTIES OF CASEIN PHOSPHOPEPTIDE

**Interaction of CPP with fluoride -** Fluoride is a known promoter of remineralization and thermodynamically favors crystal growth.[24] Studies have shown that solution containing CPP-ACP & fluoride significantly produced remineralization superior to all formulations containing either CPP or Fluoride.[25,26] Study using 1100 ppm of fluoride has shown that not all the fluoride ions that

diffuse into subsurface lesion get incorporated into mineral phase.[19] The excess of fluoride ion presumably stays as fluoride ion in lesion fluid and perhaps gets adsorbed to crystallites. For every 2 fluoride ions, 10 calcium and 6 phosphate ions are required for the formation of flourapatite. Hence on topical application of fluorides, the availability of calcium and phosphate ions can be a limiting factor for net remineralization to occur. The presence of CPP-ACP would localize these ions thus producing higher levels of remineralization. Fluoride ion tends to promote remineralization of the surface layer, whereas CPP-ACP promotes remineralization in the body of the lesion. Thus CPP-ACP with fluoride produces more of homogeneous remineralization throughout the body of the lesion.[20]

Solutions of CPP-ACP are tasteless, stable to steam sterilization and can be stored at ambient temperature without change in appearance.[27] The commercial products delivering CPP-ACP has been classified by united states food and drug administration as generally recognized as safe, i.e., it is ***safe to swallow*** and can be used in the patient of all ages.[28,29] The appearance of the long term enamel subsurface lesion and the remineralization and inhibition of demineralization is due to the slowing of the caries progression by the CPP-ACP complexes.

**Review of studies (in situ & in vivo) regarding caries prevention:** Eight studies[30-37] were randomized clinical trials with crossover designs that determined the remineralizing effects of CPP-ACP by using in-situ caries models. In seven of these studies,[36] subjects wore custom-made removable mid-palatal acrylic appliances containing two or three demineralized enamel half-slabs covering first premolars to the last tooth in the arch. In all but one study, sound human extracted third molars were the source of the enamel. In one study, bovine enamel sections were used.[36]

Demineralized lesion was produced by polishing sound enamel and covering it with an acid-resistant coating (nail varnish or epoxy resin) and then enamel was subjected to demineralizing solutions. The subsurface lesions of the depth 80 to 110 micrometers were produced through this procedure.

At the completion of the treatment with various agents (sugar-free gum, lozenge, milk or mouthrinse containing CPP-ACP), the investigators took the remineralized enamel half-slab sand their paired demineralized control half-slabs (retained in a humidified environment) and fixed and split them. The slabs were then subjected to microradiography and computer-assisted micro-densito-metric analysis for evaluating the mean subsurface lesion depth, percentage of subsurface remineralization and/or change in mineral profile.

For every test group, the period of the study varied from 7 to21 days. In the end, all of the in-situ studies except one[37] proved the caries inhibiting properties of CPP-ACP. The remineralization of carious surface was directly proportional to the dose of CPP-ACP.

Two studies[38,39] were in vivo studies. The first study[(38)] was done on adolescents having visible white spot lesions on incisors and canines. The subjects in the test group were exposed to CPP-ACP daily for 3 months followed by daily toothbrushing with a fluoridated dentifrice for another 3 months. The subjects in the control group rinsed with 0.05 percent sodium fluoride mouthwash daily and used fluoridated dentifrice for a period of 6 months. The results after three months showed that 55 percent of the white spot lesions in test group disappeared completely as compared to 18 percent in the control group and at the end of 12 months 63 percent of the white spot lesions in the test group disappeared as compared to 25 percent in the control group and the results were statistically significant.

The other study[39] was a 2-year, double-blind, parallel-group, randomized clinical trial of 2,711 subjects aged 11.5 to 13.5 years subjects chewed a sugar-free gum (3 × 10 min/day) containing CPP-ACP as compared to the similar gum without CPP-ACP over the 24-month study period. The progression and regression of approximal caries was investigated, using standardized digital bitewing radiographs. The radiographs, scored by a single examiner, were assessed for approximal surface dental caries at both the enamel and dentine level. The difference was statistically significant in between the two groups. There was 18% less caries progression in the subjects using CPP-ACP gum.

## DELIVERY FORMS OF CPP-ACP

CPP-ACP is currently available as chewing gum & topical cream. However, it can be added to various oral health products like Lozenges, Mouth rinse, Dentifrice, Temporary cement, Glass Ionomer cement, etc. apart from this it can be added in various foodstuff such as sports drink, milk, flour, fruit juices, cereal flakes, bread cake mix, biscuits etc. which is a potential area for research & the clinical efficacy of these products needs to be proved.

## USES OF CPP-ACP

CPP-ACP has shown both curative and preventive properties. There is strong evidence that when used in the form of chewing gum it prevents progression of smooth surface caries especially in children. The topical cream has shown to be effective against decalcification around orthodontic brackets. It has also shown to be helpful in the maturation of enamel of newly erupted teeth, prevention of subsurface demineralization, erosion & treatment of hypomineralized enamel including mild cases of fluorosis, salivary gland dysfunction, these effects has been shown in-vitro or in situ studies thus accounting for weak evidence. Moreover, the anticariogenic activity in pit and fissure caries and desensitizing action on sensitive dentin is questionable.

## CONCLUSION

CPP-ACP can act as a promising adjuvant in supplementing the action of fluoride in dental caries prevention. Independent scientific evidence exists to support the use of CPP-ACP in the sugar free gum in slowing the progression of dental caries and regressing early carious lesion. Addition of CPP-ACP in products like mouth rinse, lozenges, and food fortification is a potential area for clinical research. CPP-ACP is a promising addition to armamentarium in preventive dentistry which can be used in oral health products, dental profession products, and food stuff.

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