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# Anticariogenic Effect of Amorphous Calcium Phosphate Stabilized by Casein Phosphopeptid: A Review Article

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**Abstract:** The anticariogenic potential of casein phosphopeptid-amorphous calcium phosphate nanocomplexes (CPP-ACP) has been demonstrated using laboratory, animal and human *in situ* caries model. The CPP-ACP has also been shown to remineralize enamel subsurface lesions *in vitro* and *in situ* when delivered in sugar-free gum. The proposed anticariogenic mechanism for CPP-ACP is the localization of ACP at the tooth surface which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel reducing demineralization and enhancing remineralization. The CPP-ACP therefore has exciting potential for use in oral care products and foods to help prevent dental caries and repair early stages of diseases, a development which will add significantly to dentists armoury of tools in preventive dentistry. This review article presents a brief on mechanism of action and role of CPP-ACP in caries prevention, remineralization of enamel lesions by CPP-ACP and use of CPP-ACP in different dental products like glass ionomer cement, tooth paste, mouth wash, tooth mousse and etc.

**Key words:** Milk, CPP, ACP, anticariogenic, enamel remineralization

## INTRODUCTION

Dental caries is initiated via the demineralization of tooth hard tissue by organic acids from the fermentation of dietary sugar by dental plaque odontopathogenic bacteria. Even though, in most developing countries the prevalence of dental caries has deceased through, the use of fluorides, the disease remains a major public health problem. Except for some reports associating nursing bottle caries with milk consumption, dairy products have been recognized for over 50 years as exhibiting an anticaries effect. Using laboratory, animal and human *in situ* caries models it has been shown that Casein Phosphopeptid Amorphous Calcium Phosphate complexes (CPP-ACP) exhibit an anticariogenic activities.

The CPP have remarkable ability to stabilize calcium phosphate in solution and substantially increase the level of calcium in dental plaque. Through their multiple phosphoryl residues the CPP bind to forming Clusters of Amorphous Phosphate (ACP) in metastable solution preventing their growth to the critical size required for nucleation and precipitation. The proposed mechanism of anticariogenicity for the CPP-ACP is that they localize ACP in dental plaque which buffers the free calcium and phosphate ion activities thereby helping to maintain a state of supersaturation with respect to tooth enamel depressing demineralization and enhancing remineralization. The CPP-ACP, unlike fluoride can be added to sugar containing foods and therefore have commercial potential as an additive to foods as well as to tooth pastes and mouthwashes for the control of dental caries (Reynolds, 1995, 1998).

## ANTICARIOGENICITY OF DAIRY PRODUCTS

The components of dairy products like milk, milk concentrates, powders and chesses, which are responsible for their anticariogenic activity, are mainly casein, calcium and phosphate. The bovine milk phosphoprotein, casein, which is known to interact with calcium and phosphate and is a natural food component is an obvious candidate for an anticariogenic food and tooth paste additive (Reynolds, 1995, 1998, 2001).

In the early studies on the anticariogenecity of casein, the insoluble acid form (Casein HCl) was used which required very high levels for activity.

More recently, lower levels of soluble caseinate have been shown to be anticariogenic in a rat caries model when added to the drinking water or as an ingredient or supplement in confectionery. The level of

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caseinate (17% w $w^{-1}$) which significantly reduced the cariogenecity of the chocolate confection was considered too high by the manufacturers due to the unpalatability of the caseinate, at a much lower, palatable level (2% w $w^{-1}$) the caseinate did not significantly change the confection's cariogenecity. It was concluded that casein's adverse organoleptic properties and large amount required for efficacy precluded its use as a food or toothpaste additive to lower the risk of caries (Reynolds and Black, 1987).

## ANTICARIOGENIC CASEIN PHOSPHOPEPTIDS

Using a human intro-oral caries model, it has been shown that digestion of caseinate with trypsin did not destroy the protein's ability to prevent enamel sub-surface demineralization. tryptic peptides of casein were found incorporated into the intro-oral appliance plaque and were associated with a substantial increase in the plaque's content of calcium and phosphate. It was concluded that the tryptic peptides responsible for the anticariogenic activity were the calcium phosphate sequestering phosphopeptids. As the CPP are not associated with unpalatability or antigenecity of the caseins and have the potential for a specific anticariogenecity at least ten times greater on a weight basis, then their potential as a food and tooth paste additive is considerably better than that of the intact proteins.

A simple and efficient purification procedure of the CPP has recently been developed involving ultrafilteration of amorphous calcium phosphate nanoclusters stabilized by the multiple phosphoseryl-containing peptids from a tryptic digest of casein (Reynolds, 1987, 1998, 1999).

## REMINERALIZATION OF ENAMEL LESIONS BY CPP-ACP

The effect of CPP-ACP solutions on remineralization of artificial lesions in human teeth has been investigated. A series of solutions containing various amounts of CPP (0.1-1%), calcium (6-60 mM) and phosphate (3.6-36 mM) at different pH values (7.0-9.0) have been studied. The associations between the activities of the various calcium phosphate species in solution and the rates of enamel lesion demineralization for this series of solutions were then determined. The activity of the neutral ion species $CaHPO_4^{\circ}$ in the various remineralizing solutions was found to be highly correlated with the rate of lesion remineralization. ACP-CPP acts as a reservoir of calcium phosphate ions including the neutral ion pair $CaHPO_4^0$ which are formed in the presence of acid. The acid can be generated by dental plaque bacteria, under those conditions, the CPP bound ACP would buffer plaque pH and so doing would dissociate to calcium phosphate ions including $CaHPO_4^0$. The increase in plaque calcium and phosphate ions and ion pairs would offset any fall in pH thereby preventing enamel demineralization (Reynolds, 1987, 1995, 1999, 2001).

This therefore, could explain why the CPP-ACP solutions are such efficient remineralizing solutions as they would consume the acid generated during enamel lesion remineralization by generating more $CaHPO_4^0$ thus, maintaining its concentration gradient into the lesion, these results are therefore consistent with the proposed anticariogenic mechanism of the CPP being the inhibition of enamel demineralization and enhancement of remineralization through the localization of ACP at the tooth surface (Reynolds, 1999; Zapanta Le Geros, 1999).

Schupbach and Neeser (1996) and Yamanaka *et al.* (2003) demonstrated that incorporation of Caseinoglycomacropeptid commerce peptide and caseinophosphopeptide into the salivary pellicle inhibits adherence of mutant streptococci.

They have shown the protective effects of milk and milk products against dental caries, this showed that this effect was mediated by micellar casein or casein peptide derivatives. A reduction in the streptococcus sobrinus population in the oral microbiota of animals fed diets supplemented with these milk components was consistently observed. A possible explanation for these findings is that milk components are incorporated into the salivary pellicle, thereby reducing the adherence of *S. sobrinus* and *S. mutans*. It was suggested in this study that calcium and phosphate-rich micellar casein or caseinopeptides are incorporated into the pellicle. The resulting ecological shifts, together with the increased remineralization potential of this biofilm, may explain its modified cariogenic potential.

Rose (2000) studied the effects of an anticariognic casein phosphopeptide on calcium diffusion in streptococcal model dental plaques, using Dibdin's effusion system, calcium diffusion was measured in streptococcal model plaque. They demonstrated that by providing a large number of possible binding sites for calcium, 0.1% CPP-ACP reduced the calcium diffusion coefficient by about 65% at pH 7 and 35% at pH 5.

Hence, CPP-ACP binds well to plaque, providing a large calcium reservoir within the plaque and slowing diffusion of free calcium. This is likely to restricts mineral loss during a cariogenic episode and provide a potential source of calcium for subsequent remineralization. Overall, they concluded that once in place, CPP-ACP will restrict the caries process.

Renolds and Shen (2001) and Shen and Cai (2001) Studied the ability of CPP-ACP sugar-free chewing gum to remineralize enamel subsurface lesions in human *in situ* model. They demonstrated that addition of CPP-ACP to either Sorbitol-or Xylitol-based gum resulted in a dose-related increase in enamel remineralization.

Poitevin *et al.* (2004), evaluated the clinical effectiveness of tooth mousse containing CPP-ACP for treatment of tooth hypersensitivity and showed that the tooth mousse to be effective in reducing tooth hypersensitivity.

## INTERACTION OF CPP-ACP WITH FLUORIDE

The additive anticariogenic effect of the 1% CPP-ACP and 500 ppm F in the rat caries experiments led to the investigation of the potential interaction between CPP-ACP and F.

Analysis of the solution containing 1.0% CPP, 60 mM $Cacl_2$ 36 mM Sodium phosphate and 500 ppm F (26.0 mM NaF) at pH 7.0 after ultrafilteration revealed that nearly half of the fluoride ions had incorporated into the ACP phase stabilized by the CPP to produce a novel amorphous calcium fluoride phosphate phase of composition. The identification of this novel Amorphous Calcium Fluoride Phosphate (ACFP) phase led to the proposition that the formation of this phase is responsible for the observed additive anticariognic effect of CPP-ACP and F.

The proposed anticariogenic mechanism of the CPP-ACP is the localization of ACP at the tooth surface such that in the presence of acid, the ACP dissociates to release calcium and phosphate ions increasing the degree of saturation respect to hydroxyapatite preventing enamel demineralization and promoting remineralization (Kariya and Sato, 2004; Reynolds , 1998; Shen and Cai, 2004).

The additive anticariogenic effect of CPP-ACP and F therefore maybe attributable to the localization of ACFP at the tooth surface by the CPP which in effect would co-localize Ca, P and F. These results suggest that CPP may be an excellent delivery vehicle to co-localize calcium, phosphate and fluoride at the tooth surface in a slow release amorphous form with superior clinical efficacy.

Shen *et al.* (2004) and Reynolds (2003, 2004), compared the enamel remineralization ability of mouth rinse containing CPP-ACP with that of mouth rinse containing fluoride in an intraoral enamel remineralization model.

They demonstrated that the mouth rinse containing 0.4% CPP-ACP and 220 ppm F produced 19% enamel subsurface lesion remineralization compared with 8% remineralization by the 220 ppm F rinse and 14% remineralization by 0.4% CPP-ACP rinse.

The results support the role of fluoride in promoting remineralization and demonstrate an important facilitation of the effect of fluoride by CPP-ACP.

## RECALDENT

Recaldent is ingredient derived form casein, part of the protein found in cow's milk. Its technical name is casein phospho-peptids-amorphous calcium phosphate or CPP-ACP. It was discovered and patented by the school of Dental Science at the University of Melbourne in Australia. It contains xylitol and CPP-ACP, available as sticks and tablets. Recaldent works safely, strengthen teeth by delivering calcium and phosphate (the building blocks of tooth enamel) in a unique soluble form to help remineralize the enamel. Because Recaldent is milk derived, it isn't recommended for people with milk allergies, but Recaldent will not affect people with lactose intolerance. Studies have shown that Recaldent will improve the effect of fluoride, since Recaldent provides an excellent source of soluble calcium and phosphates and fluoride requires calcium and phosphate to work, so, rather being better than fluoride, or replacing fluoride, Recaldent and fluoride work together to rebuild and strengthen tooth enamel.

Iijima *et al.* (2004), investigated the acid resistance of enamel lesions remineralization *in situ* by a sugar free chewing gum containing CPP-ACP nanocomplexes (Recaldent) and demonstrated that it is superior to an equivalent gum not containing CPP-ACP in remineralization of enamel subsurface lesions *in situ* with mineral that is more resistant to subsequent acid challenge.

Yamanaka *et al.* (2003), evaluated the caries prevention potential of tooth mousse containing CPP-ACP and demonstrated its effect in preventing the enamel remineralization *in vitro* and buffering capacity to acid produced by *S. mutans*.

Cai *et al.* (2003), demonstrated the effect of CPP-ACP incorporation into a sugar free lozenge (pressed mint tablet) on enamel remineralization in a human *in situ* model. They concluded that lozenges are suitable vehicle for the delivery of CPP-ACP to promote enamel subsurface lesion remineralization.

**The products containing recaldent include:** Trident white and Trident, for kids sugarless gum containing Recaldent. Tooth Mousse is also available to dentists.

Work is currently underway to develop a range of products containing recaldent, including tooth paste and mouthwash.

## INCORPORATION OF CPP-ACP IN TO GLASS IONOMER RESTORATIVE MATERIAL

Glass ionomer cement has excellent dynamic adhesion to enamel and dentin, as demonstrated by the high long term retention rates in non-undercut carious cervical lesions.

Mqazzaoui *et al.* (2003) determined the effect of incorporation of CPP-ACP (1.56% w $w^{-1}$) into self cured glass ionomer cement Fuji IX and demonstrated significant increase in microtensile bond strength (33%) and compressive strength (23%) and significantly enhanced the release of calcium, phosphate and fluoride ions at neutral and acidic pH.

In laboratory studies conducted on extracted teeth placed in acid gel preparation, enamel and dentin subsurface lesions were developed, which were similar to those occurring in clinical caries.

When inert control restorative materials, such as resin composite without fluoride, were placed in cavities in such teeth, typical dentinal wall lesions developed, as well as sub-surface lesions. However, when teeth were restored with glass ionomer, the wall lesions were frequently reduced in size or were eliminated and the sub-surface lesions developed further away from the cavity margin and were smaller than adjacent to the control material.

The placement of Fuji IX GP containing CPP-ACP in similar cavities was associated with enhanced protection of the enamel and dentin adjacent to the restoration compared with Fuji IX GP alone.

These results suggest that Fuji IX GP containing CPP-ACP will provide enhanced protection against secondary caries, when compared with conventional glass ionomer.

## CONCLUSION

Form extensive laboratory animal and human studies, it has been shown that casein phosphopeptideamorphous calcium phosphate nanocomplexes (CPP-ACP), have good anticariogenic potential, by localizing at the tooth surface and getting incorporated into dental plaque pH, depressing enamel demineralization, enhancing remineralization, reducing the risk of caries development and act synergistically with fluoride ions to form CPP stabilized Amorphous Calcium Fluoride Phosphate (ACFP) at the tooth surface and also significantly remineralization of white spot and at the end reducing dental sensitivity by occluding patent tubules and promoting dentine remineralization.

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