

The inhibitory effect of glycomacropeptide on dental erosion

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Abstract – Throughout the world, the consumption of acidic foods, soft drinks and fruit juices has increased and has been associated with a rise in dental problems such as caries and erosion. The aim of this study was to investigate the protective effect of glycomacropeptide (GMP) against dental erosion and to compare the effect with that of caseinophosphopeptides (CPP). To assess the contribution of the calcium content of these peptides and their protection, calcium-reduced GMP (GMP-ca) and calcium-reduced CPP (CPP-ca) were studied. Hydroxyapatite was used as the tooth model. At a pH of 3, the protective effect of GMP was superior to that of CPP; however, CPP performed better at a pH of 4.5. The effect of GMP-ca and CPP-ca was evident only at a pH of 4.5, when compared with native GMP and CPP. These findings indicate that the calcium content of the peptide may have a role in protection against mineral loss from teeth. However, the pH of the erosive solution was the most influential factor contributing to erosion.

glycomacropeptide / caseinophosphopeptide / calcium / dental erosion

摘要 – 酪蛋白糖巨肽对龋齿的预防作用。随着酸性食品、软饮料及果汁消费量在全世界的增加，龋齿等一系列的牙科问题也凸显出来。本文旨在研究糖巨肽 (GMP) 在预防龋齿方面的作用，并将其与酪蛋白磷酸肽 (CCP) 进行了对比。此外，为了评价这些多肽中钙含量对牙齿的保护作用，分别用低钙的 GMP-ca 和 CCP-ca 进行了对比研究。羟基磷灰石是牙科模型的原材料。pH 3 时，GMP 的预防作用高于 CCP，但是 pH 4.5 时 CCP 的效果更佳。与天然的 GMP 及 CCP 相比，GMP-ca 及 CCP-ca 只有在 pH 4.5 的条件下效果才是显著的。这些结果证明了钙含量可以预防牙齿的矿物质流失，但是腐蚀液的 pH 值仍是影响腐蚀程度最重要的因素。

糖巨肽 / 酪蛋白糖巨肽 / 钙 / 龋齿

Résumé – Effet inhibiteur du glycomacropeptide sur l'érosion dentaire. La consommation mondiale d'aliments acides, de boissons non alcoolisées et de jus de fruit a augmenté et a été associée à l'élévation des problèmes dentaires tels que caries et érosion. Le but de cette étude a été d'examiner l'effet protecteur du glycomacropeptide (GMP) envers l'érosion dentaire et de le comparer à celui des caséinophosphopeptides (CPP). De plus, pour évaluer la contribution de la teneur en calcium de ces peptides à leur effet protecteur, des GMP et CPP à teneur réduite en calcium ont été également étudiés. L'hydroxyapatite a été utilisée comme dent modèle. À pH 3, l'effet protecteur du GMP était supérieur à celui du CPP. Le CPP donnait cependant de meilleurs résultats à pH 4,5. L'effet de la réduction du calcium était évident seulement à pH 4,5 par comparaison aux GMP et CPP natifs. Ces résultats indiquent que la teneur en calcium du peptide peut avoir un rôle

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dans la protection contre la perte minérale des dents. Cependant, le pH de la solution érosive est le facteur d'influence majeur contribuant à l'érosion.

glycomacropeptide / caséinophosphopeptide / calcium / érosion dentaire

1. INTRODUCTION

In the recent decades, the consumption of acidic beverages has increased in many countries, which is thought to be responsible for the demineralisation of the hard tissue of enamel and dentin [8]. According to one study, 20–60% of children and adolescents have dental erosion and this has been attributed to the increasing consumption of acidic drinks [3]. Many attempts have been made to modify drinks to reduce the erosive potential, though some of these modifications may change the flavour, texture and pH [5]. For example, fluoride might even cause adverse effects if added to different drinks, leading the total uptake to reach high or overdose levels [3].

In this respect, milk peptides have also been investigated for their protective potential against dental erosion. Caseinophosphopeptide (CPP) is a peptide derived from casein by the action of trypsin. Results indicate that it is able to suppress demineralisation and/or improve remineralisation, and therefore, it would be useful if added to acidic drinks [9, 14]. Glycomacropeptide (GMP), a casein-derived peptide, has also received much attention because of its biological activities, including the reduction of dental caries. During cheese making, rennet (chymosin) cleaves κ -casein in bovine milk at Phe105-Met106 amino acids, producing two separate parts, para κ -casein and GMP. Para κ -casein is the N-terminal and consists of amino acids 1–105. The GMP, also called caseinomacropeptide, is the C-terminal and includes the rest of the amino acids (105–169). Para κ -casein remains with the curd and GMP is released into the whey [4].

One study concluded that GMP prevents adherence of bacteria to tooth surfaces [17]. Furthermore, GMP can be used to produce “high protein-reduced sugar” drinks, which are beneficial to general health in terms of providing the body with nutrients and helping it fight viral and bacterial infections [6].

The aim of this study was to investigate the protective effect of GMP with or without chemical modification, against dental erosion in comparison with CPP.

2. MATERIALS AND METHODS

2.1. Materials

Sodium caseinate (Product No. C8654), bovine trypsin (E.C.3.4.21.4; Product No. T8642), crystalline hydroxyapatite (HA) suspension (Type 1; 25% solids, Product No. H0252), the BCA (Bicinchoninic Acid kit) for protein determination and Dowex 50 (H^+) ion exchanger resin (Product No. 422096) were obtained from Sigma Chemical Co. (Poole, Dorset, UK). Commercial CPP (CPPc) and commercial GMP (CGMP-10) were provided by Arla Foods Ingredients a.m.b.a (Surry, UK). All the chemicals used were of analytical grade.

2.2. Methods

2.2.1. Preparation of HA

Six grams of original HA suspension was mixed with 240 mL of water and centrifuged for 10 min at $3000\times g$. The supernatant was discarded and washed again to remove soluble phosphate. Washed HA was resuspended in 108 mL

of distilled water. This suspension was placed on the magnetic stirrer and while it was being stirred continuously, 1100 μL of suspension (20 mg dry weight) was withdrawn and added to a series of 1.5 mL ependorfs, arranged in four groups of 15 and four groups of 9. After pipetting ($n = 96$), HA samples were centrifuged at $3000\times g$ for 10 min and then the supernatant was discarded and HA was ready to use [9].

2.2.2. Preparation of CPP

Sodium caseinate (10 g) was dissolved in 100 mL of $0.05 \text{ mol}\cdot\text{L}^{-1}$ Tris-HCl buffer, pH of 7.5 and 50°C . This was followed by the addition of 12.5 mg of trypsin and incubated at 37°C for 2 h. The reaction was terminated by heating to 90°C for 5 min. The solution was cooled and pH was adjusted to 4.5 using $1 \text{ mol}\cdot\text{L}^{-1}$ HCl, after stirring for 10 min it was centrifuged for 30 min at $3000\times g$. The pH of the supernatant was adjusted to 7.0 by adding $2 \text{ mol}\cdot\text{L}^{-1}$ NaOH. To the final solution, calcium chloride (0.8 g) was added followed by an equivalent volume of 95% aq. ethanol to precipitate the CPP fractions. After 1 h, the solution was centrifuged for 30 min at $3000\times g$. The supernatant was removed and the pellet was washed and resuspended in 30 mL of 50% (v/v) aq. ethanol. The washed solution was removed by centrifugation (10 min, $3000\times g$). The pellet was spread on a plastic dish and allowed to dry at room temperature and designated laboratory prepared CPP (CPPlp) [9, 10].

2.2.3. Calcium reduction of peptides

One gram of each peptide (GMP, CPPc and CPPlp) was dissolved in 10 mL of distilled water and added to 24 g of washed Dowex 50 ion-exchange resin. The suspension was stirred for 40 min and left for 10 min to precipitate the resin. The supernatant was removed and centrifuged for clarification, for 5 min at $3000\times g$. Then,

20 mL of water was added to the resin and stirred for 2 min and the supernatant was removed as above. The pH of the supernatant was adjusted to 7.0 with $1 \text{ mol}\cdot\text{L}^{-1}$ NH_4OH .

2.2.4. Assessment of protective effect of the peptides at different pH

The HA samples were prepared as described in two series of four groups. The first group was used for GMP, GMP-ca, CPPc and CPPc-ca with control and the second group for CPPlp and CPPlp-ca (-ca: calcium-reduced peptide) with control to investigate the protective effect of the peptides at different pH levels 3, 3.5, 4 and 4.5. The concentration of all peptides was adjusted to $5 \text{ mg}\cdot\text{mL}^{-1}$ using the BCA method. From the first group, the four subgroups were treated with GMP and GMP-ca, CPPc and CPPc-ca, respectively. The final subgroup was kept as control. All the samples were resuspended and mixed thoroughly with the peptide solutions for 20 min with intermittent stirring to permit maximum binding to the HA, then centrifuged at $3000\times g$, for 10 min. The supernatant was discarded and HA pellet was rinsed with 1 mL of distilled water to remove excess unbound peptide to HA particles and it was removed by centrifuging at $3000\times g$, for 10 min. To the treated HA and control, 1 mL of $0.1 \text{ mol}\cdot\text{L}^{-1}$ citric buffer at a pH of 3, was added. The mixture was resuspended and allowed to stand for 10 min, stirred once during the standing time and centrifuged ($3000\times g$, 10 min). The supernatants were rapidly decanted off into clean tubes for calcium and phosphate analysis. All the remaining groups were treated similarly but at different pH levels.

2.2.5. Assessment of calcium and phosphate in erosive solution

Calcium was measured by atomic absorption spectroscopy to measure the calcium

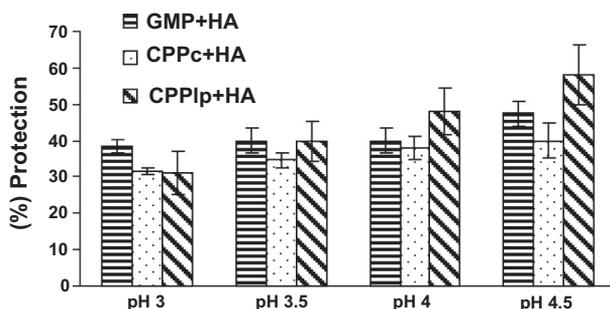


Figure 1. Protective effect of the peptides against calcium and phosphate dissolution from HA. GMP, glycomacropptide; CPPc, commercial caseinophosphopeptide and CPPIp, laboratory prepared caseinophosphopeptide.

content of the erosive sample. Different concentrations of standard calcium were prepared from calcium ion solution of $1000 \text{ mg}\cdot\text{mL}^{-1}$ (2.5, 5, 7.5, 10, 15, 20) and absorbance was measured at 422.7 nm. Samples were diluted with distilled deionised water into appropriate concentration [8].

The level of phosphate in the erosive solution was measured with colorimetric determination using the method described by Allen [2].

3. RESULTS

Hydroxyapatite suspension was used as a substitute for teeth mineral. The amount of calcium and phosphate loss from HA was used to evaluate the protective effect of the peptides against acid dissolution-erosion in comparison with the control.

3.1. Protective effect of intact peptides at different pH values

After each experiment, the calcium and the phosphate content of the citric buffer was measured and compared with the control. At a pH of 3, the HA treated with GMP showed a protective effect of 38.5% and its effect increased to 47.5% with

increasing pH. The CPPc showed around 14% less protection than GMP at different pH values, and CPPIp showed less protection than GMP at pH 3 but its effect increased with increasing pH. The protection of CPPIp was the highest at a pH of 4.5 (Fig. 1).

3.2. Protective effect of calcium-reduced peptides at different pH levels

The calcium content of each peptide sample at the same protein concentration ($5 \text{ mg}\cdot\text{mL}^{-1}$) was measured, which was $78 \text{ mg}\cdot\text{L}^{-1}$ in GMP, $19 \text{ mg}\cdot\text{L}^{-1}$ in CPPc and $1157 \text{ mg}\cdot\text{L}^{-1}$ in CPPIp. The calcium content of each peptide was measured after calcium reduction, and the calcium content of both GMP and CPPIp was reduced by 80%, and that of CPPc was reduced by 13%.

The protective effect of GMP-ca and CPPc-ca after reduction of the calcium contents was similar, apart from that at a pH of 4.5, where the protective effects were reduced by 31% and 26%, respectively. On the other hand, CPPIp-ca showed minimal reduction in protection at all pH levels. The data in Figure 2 compare the protection effect of reduced peptides with the original samples at different pH levels.

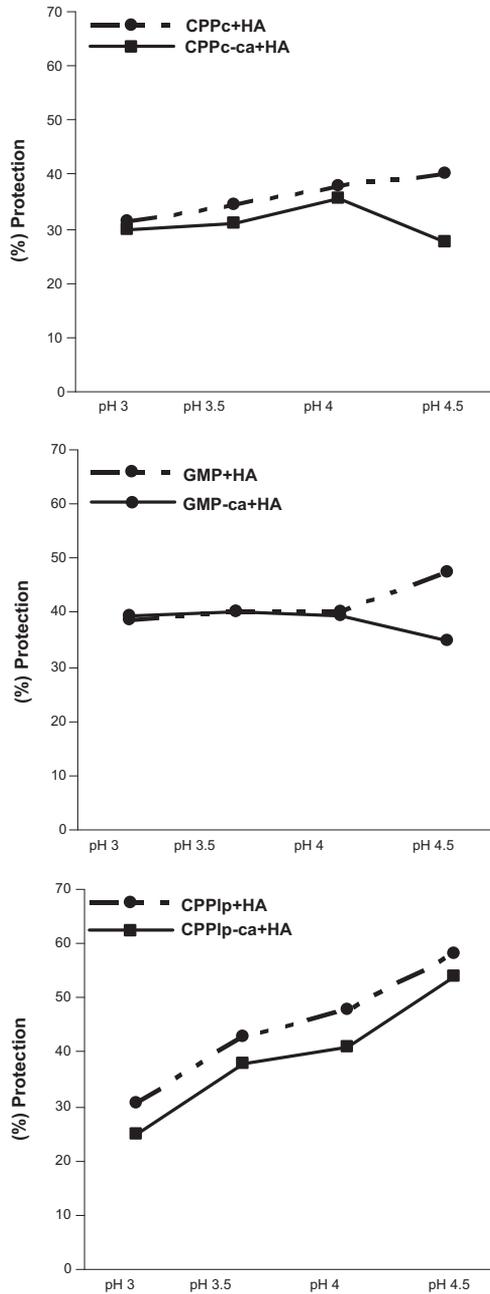


Figure 2. Protective effect of calcium-reduced peptides against calcium and phosphate dissolution from HA. GMP, glycomacropptide; CPPc, commercial caseinophosphopeptide; CPPIp, laboratory prepared caseinophosphopeptide and -ca, calcium-reduced peptide.

4. DISCUSSION

The extent of tooth erosion is determined by the number, duration of acid attacks and the pH of acidic solution [14, 19]. The role of GMP/ CPP is to coat the HA particles to suppress demineralisation and to enhance remineralisation during an acid attack. It has been suggested that CPP might replace the calcium lost from the enamel by providing fast remineralisation and also provide a “supersaturated” calcium-rich environment [14, 15]. It has been reported that CPP could localise the amorphous calcium phosphate on tooth surfaces.

The GMP inhibits the adhesion of cariogenic bacteria such as *Streptococcus* spp. *mutans*, *sanguis* and *sobrinus* to oral surfaces [4, 7, 12–14, 18], and it can modify the composition of plaque bacteria to control its acid production and in turn reduce the demineralisation of enamel and promote remineralisation [1, 11, 16]. The GMP is a source of N-acetyl-necromantic acid [17] and one study showed that dietary intake of GMP can increase the sialic acid content of saliva, with effects on its viscosity and protective function [20].

According to the results of this study, GMP demonstrated a higher mineral protective effect than CPP at lower pH levels. However, the mineral protective effect of all three peptides increased with the increasing pH of the erosive solution. Finally, since the reduction in the calcium content of the peptides can reduce their protective effect, a state of calcium saturation may be responsible for a least part of the protection effect of the peptides. Therefore, we concluded that GMP and CPP can have the ability to reduce the dental erosion.

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