Popular Science Article

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The Effects of Phosphorylation of Amelogenin

We use our teeth daily to eat all sorts of food with varying degrees of texture and hardness. The white part of our teeth called the enamel is the hardest tissue of the human body. Have you ever wondered what makes our teeth so hard and strong that we can chew on a chicken bone? Well, the answer is amelogenin which is the main subject of this study. Amelogenin is one of the many proteins that help build the enamel. However, amelogenin is of greatest importance to enamel formation because in its absence scientists have found that it leads to teeth defects.

Scientists have also discovered that amelogenin can be utilized in many applications such as teeth defects, wound healing, bone formation and regeneration and many others. So far, there are two amelogenin-based products on the market. These products include (i) Emdogain® for the treatment of periodontitis, an enamel defect due to lack of proper amelogenin and (ii) Xelma® used to treat leg ulcers. As a result of the numerous potential applications of amelogenin, thus, there is increasingly more research carried out on amelogenins.

So far, the most optimal production system used for the production of recombinant amelogenin is the bacteria called *Escherichia coli*. The only difference that existed between native amelogenin and recombinant amelogenin expressed using *E.coli* is that the latter lack the amino acid methionine at the N-terminus and phosphate group on Serine-16.The aim of this present study was to produce recombinant amelogenin phosphorylation mimics and to investigate the effects of phosphorylation of amelogenin on its properties. The properties of the novel phosphorylated amelogenin mimics with that of non-phosphorylated amelogenins were compared in order to examine the effects of the phosphorylation.

In this study, a total of sixteen novel recombinant amelogenin phosphorylation mimics were successfully constructed by a type of mutation called site-directed mutagenesis. This mutation was carried out by replacing one amino acid into another amino acid in amelogenin amino acid sequence. More specifically, the amino acid, serine at sixteenth position was replaced with either glutamic acid or aspartic acid in the amelogenin sequence. The site-directed mutagenesis of Serine-16 to aspartic acid or glutamic acid was performed in order to promote negative charges and to mimic the effect of the phosphorylated serine in the native amelogenin. Since the two amino acids used are negatively charged, thus, it was thought that it could be used to substitute the negatively charged phosphate group.

Various properties of amelogenin were tested to investigate the effect of phosphorylation on amelogenin. The study investigated how soluble recombinant amelogenin phosphorylation mimics are in acidic, neutral and alkaline solutions in presence of calcium and phosphate ions. Amelogenin usually change their shape and form spherical particles called nanospheres during enamel formation. Therefore, the radii of these particles and dispersity (how uniform the particles in the sample mixture

are) were measured under neutral conditions and at temperatures of 20 and 37^{o}C. The interaction between the amelogenin and a substance called calcium hydroxyapatite that constitutes ninety-percent of the enamel was also tested.

The phosphorylated amelogenins had similar solubilities to their non-phosphorylated amelogenins in acidic, neutral and alkaline conditions which indicated that phosphorylation had no effect on the solubility of amelogenin. The solubility of recombinant amelogenin phosphorylation mimics was greater than that of recombinant native amelogenin and its phosphorylation mimic counterpart. It was also found that the recombinant amelogenin phosphorylation mimics too can form nanospheres similar to the non-phosphorylated amelogenin. There were minor variations in radii and dispersity of the recombinant amelogenins at 20^{o}C and 37^{o}C which indicated that phosphorylation had not affected the amelogenins. Furthermore, temperature had no critical effect on radii and dispersity of amelogenin. The amelogenin phosphorylation mimics as well as their non-phosphorylated counterparts had a similar, strong interaction with calcium hydroxyapatite.

Although in the study, no exceptional findings were found for the effects of phosphorylation on amelogenin but at least the recombinant amelogenin phosphorylation mimics function to some extent as the native recombinant amelogenins.