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Dynamic light scattering and zeta potential of colloidal mixtures of amelogenin and hydroxyapatite in calcium and phosphate rich ionic milieus

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ABSTRACT

The concept of zeta-potential has been used for more than a century as a basic parameter in controlling the stability of colloidal suspensions, irrespective of the nature of their particulate ingredients—organic or inorganic. There are prospects that self-assembly of peptide species and the protein–mineral interactions related to biomineralization may be controlled using this fundamental physicochemical parameter. In this study, we have analysed the particle size and zeta-potential of the full-length recombinant human amelogenin (rH174), the main protein of the developing enamel matrix, in the presence of calcium and phosphate ions and hydroxyapatite (HAP) particles. As calcium and phosphate salts are introduced to rH174 sols in increments, zeta-potential of the rH174 nanospheres is more affected by negatively charged ions, suggesting their tendency to locate within the double charge layer. Phosphate ions have a more pronounced effect on both the zeta-potential and aggregation propensity of rH174 nanospheres compared to calcium ions. The isoelectric point of amelogenin was independent on the ionic strength of the solution and the concentration of calcium and/or phosphate ions. Whereas rH174 shows a higher affinity for phosphate than for calcium, HAP attracts both of these ions to the shear plane of the double layer. The parallel size and zeta-potential analysis of HAP and rH174 colloidal mixtures indicated that at pH 7.4, despite both HAP and rH174 particles being negatively charged, rH174 adsorbs well onto HAP particles. The process is slower at pH 7.4 than at pH 4.5 when the HAP surface is negatively charged and the rH174 nanosphere carries an overall positive charge. The results presented hereby demonstrate that electrostatic interactions can affect the kinetics of the adsorption of rH174 onto HAP.

1. Introduction

Surface charge of particles in sols has been used for centuries to regulate the stability of colloidal suspensions.[1–3] The ancient Egyptians used to render many colloids, from clay to ink, stable by electrostatic means, without being aware of that.[4,5] Built on the basis of Gouy–Chapman model of the particle–solution interface, DLVO theory developed in 1940s by Derjaguin and Landau, and Verwey and Overbeek, separately, explained the stability of colloids by drawing a balance between the repulsive electric double layer forces and the attractive, short-range van der Waals forces. Ever since the propositions of this theory, it has been used as the theoretical basis for controlling the stability of colloidal dispersions in

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various technologies. An essential and easily measurable quantity used to control the intensity of the repulsive electrostatic interaction between the naturally charged colloidal particles is zeta-potential (ζ-potential).

As far as the biochemical systems are concerned, it is known that enzyme–ligand binding is favoured under conditions of electrostatic attraction.[6] Also, enzyme immobilization is known to depend not only on the chemical interaction specificity, but also on the difference in the surface potentials between the enzyme molecule and the matrix carrier.[7] Electrostatic effects have been regularly used for the electrophoretic separation of peptides, and the protein adsorption has been shown to be directly dependent on the magnitude of the difference between the ζ-potentials of the protein and the adsorbent.[8] Deviations of ζ-potential of cells from the normal range of values have been used as an indicator of membrane abnormalities.[9] Charge on the cell membrane, originating from phosphoryl and carboxyl groups of macromolecules that constitute it,[10] can be manipulated to prevent cellular aggregation, which is an effect detrimental for cellular electrophoresis techniques.[11] It was recently proposed that ζ-potential may play a role in viral–host interactions,[12] whereas ζ-potential of polioviruses was used as a control parameter during their removal from contaminated waters.[13] Zeta-potential has also been used to explain the effect of ions on coagulation in blood, including the effect of thrombosis.[14] Recently, the same concept was applied to explain the aggregation of cholesterol particles, demonstrating how a control over ζ-potential may be used to prevent the formation of pathological cholesteric deposits, including atherosclerotic plaque and gallstones.[15,16] The idea to manipulate surface charges of interacting species in order to generate complex soft matter morphologies has been, however, pursued to a lesser extent.

The reason behind studying the interaction between amelogenin (AMG), the main protein of the developing enamel matrix, and hydroxyapatite (HAP), the main mineral component of hard tissues, lies in its relevance for the process of morphogenesis of tooth enamel, known as amelogenesis. During this process, AMG self-assembles into an intricate protein network composed of nanospheres and/or nanofibers that guide the growth of bundles of elongated HAP crystals. There are indications that the first step in the self-assembly of AMG is conditioned by a narrow window of pH values at which nanospheres of different AMGs (e.g., the full-length and the proteolytically cleaved ones) are oppositely charged.[17] A former study demonstrated that the formation of nanofibrous AMG entities was fostered under conditions at which the full-length AMG nanospheres and those composed of the largest proteolytic product of the enzymatic degradation of AMG by means of matrix metalloproteinase 20 (MMP-20), one of the two main proteases of the enamel matrix, carry opposite charges.[17] As for the protein–mineral interaction, the exact nature and conditions for protein adsorption/desorption to and from the mineral surface are not precisely defined. By understanding the mechanism of this process, an insight into the fundamental nature of protein–mineral interactions that govern biomineralization processes in general could potentially be gained, altogether with a prospect of enabling more superior clinical treatments for restoring the diseased enamel.

In our previous work, we studied the effect of pH on the particle size and ζ-potential of full-length recombinant human AMG (rH174) and the two largest recombinant products of the digestion of AMG by means of MMP-20.[18] The tendency of the protein nanoparticles to aggregate into micro-sized and easily segregating entities was observed in the mildly acidic pH range, 4–7, above and below which the protein retained the form of 20–40 nm sized spheres. The tendency of the protein particles or molecules to aggregate in the weakly acidic pH range has previously been observed for a 340 kDa blood plasma protein, fibrinogen.[19] In this study, we have focused on following a change in ζ-potential upon the addition of different ionic (Ca^{2+}, $H_xPO_4^{x-3}$) and particulate (HAP) species. The content of this work is therefore divided into two parts. In the first part, we report the effect of the addition of calcium and phosphate ions to rH174 sols. The second part is the study of the interaction between HAP and rH174 by means of simultaneous particle size and ζ-potential analyses. Since specific properties with respect to particle and/or molecular size and aggregation propensities are shared by different proteins, the purpose of this study is applicable to understanding multiple other protein–protein and protein–mineral interactions, primarily those relevant to biological mineralization.

2. Materials and methods

The protein and mineral suspensions were analysed by means of a Zetasizer Nano Series (Malvern, UK) with the measurement range of 0.6 nm to 3 μm. Unless otherwise noted, 0.2 mg rH174 was dissolved in a low pH aqueous solution containing 30 mM Tris or Bis-Tris buffer and 100 mM KCl. This sample was then vortexed and centrifuged, and the supernatant dispersion was used for the dynamic light scattering (DLS) analysis. The desired pH was reached by adding small volumes (1–10 μl) of 2 M HCl/KOH. Measurements were taken at calcium ($CaCl_2$) or phosphate (KH_2PO_4) concentrations of 0.1, 0.3, 0.5, 1, 1.5, 2, 3 and 8 mM. The pH range of 2–10 was covered at intervals of one pH unit, thus analyzing rH174 in both the monomeric and particulate form. Samples were analysed for particle size and ζ-potential in the same runs and immediately upon mixing the components, resulting in $t = 3$ min as the earliest time point in the time-dependent analyses. The volume of each suspension was 1 ml and the results of each measurement were averaged over 100 runs at acquisition times of ~10 s, with the data analysis software yielding the statistically averaged size distribution by particle number as the output. The measurement temperature was 25 °C, unless noted otherwise. Universal dip cell (Malvern, UK) with a removable palladium electrode and the spacing of 2 mm in disposable glass cuvettes was used for the measurements. The voltage was manually set to 20 V and the short-pulse monomodal measurement setting was applied. To eliminate the effect of large particulate impurities, number size distribution was used for derivation of the particle size reported hereby. The particle sizes reported present hydrodynamic diameters. The average standard deviation (SD) for size and ζ-potential of AMG and HAP particles, depending on polydispersity, impurity content and inherent instability of