Quantifying the Affinity between Hydroxyapatite and a Small Peptide Probe using Surface Plasmon Resonance Imaging

Michael Weiger[1], JungJin Park[1], Marc D. Roy[1], Alamgir Karim[1,2], and Matthew L. Becker[1,2]
[1]*National Institute of Standards and Technology, Polymers Division, Gaithersburg MD 20899 USA.*
[2]*College of Polymer Science & Polymer Engineering, University of Akron, Akron, OH 44325-3909.*

Statement of Purpose: The formation of bone and teeth results from highly coordinated processes involving multiple cell types and proteins that spatially direct the nucleation and crystallization of inorganic hydroxyapatite (HA). Previous studies have shown that engineered peptide sequences can promote the nucleation process, control HA microstructure or even inhibit HA mineralization (1-5). Using phage display technology, a short peptide was identified that binds to crystalline HA and to HA-containing domains of human teeth with chemical and morphological specificity (6). However, the binding affinity and specific amino acids that significantly contribute to this interaction require further investigation. In this study, we employ a microfluidic chip based surface plasmon resonance imaging (SPRi) technique to quantitatively measure peptide affinity by fabricating a novel 4 layer HA SPR sensor.

Methods: *SPR chip*. We fabricated a HA coated SPR chip consisting of a glass substrate and thin films of Cr (≈ 0.5 nm), Ag (≈ 50 nm), TiO_2 (≈ 2 nm), and HA (≈ 15 nm) to measure the binding affinity of a previously identified peptide sequence that binds specifically to HA (6). Briefly, the films were created by sequential layer sputtering (DC and RF modes) in an argon atmosphere at 25 °C. Film thickness was determined by material deposition rates and Fresnel estimates.

SPRi System. A custom built SPR imaging system (Kretschmann configuration) composed of a 514 nm emitting LED source (LE-1G, WT&T Inc), sapphire prism (n = 1.785) and, cooled CCD camera (Retiga 2000RV, QImaging) was used to monitor real time changes in the reflectivity (R_p/R_s, the ratio of reflected intensity (R) of both p- and s-polarized light (R_p and R_s respectively)). R_p/R_s is proportional to surface density of adsorbed molecules.

Binding experiments were conducted in a PDMS based, 6 channel, microfluidic device. The channels were pre-treated with a 5 mg/mL BSA solution and rinsed to prevent depletion of the HA peptide. The device was then secured to the SPR chip which was optically coupled to the prism with immersion oil (n = 1.522). The HA specific peptide was dissolved and diluted to discrete concentrations ((200, 100, 50, 25, and 10) µmol/L) in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (25 mmol/L) at pH 7.4. Syringe pumps (Braintree Sci Inc) were used to first introduce HEPES buffer to establish a base reflectivity measure and then each peptide concentration into individual channels. Once a signal increase was detected, flow was stopped and the binding kinetics monitored under static conditions at 25 °C. Sequential images were collected every 5 s for (25 to 45) min (400 ms exposure) with imaging software (StreamPix, NorPix Inc) and reflected intensities in each channel were measured with ImageJ (Rasband, W.S., NIH, Bethesda, MD, USA, ttp://rsb.info.nih.gov/ij/).

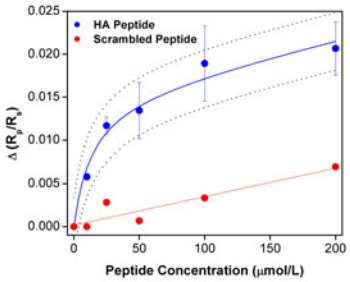

Figure 1. HA-peptide equilibrium binding curve

Results: Our novel HA coated SPR chip and integrated microfluidic format allowed us to monitor the equilibrium binding of the HA specific peptide (SVSVGMKPSPRPGGGK) at several initial concentrations (Fig. 1). Compared to a scrambled peptide sequence which bound in a non-specific manner, the HA-peptide bound specifically. In the context of a simple, one site binding model, we report a K_D ≈ 14.1 µmol/L ± 3.8 µmol/L, the combined standard uncertainty (i.e., estimated standard error of the mean).

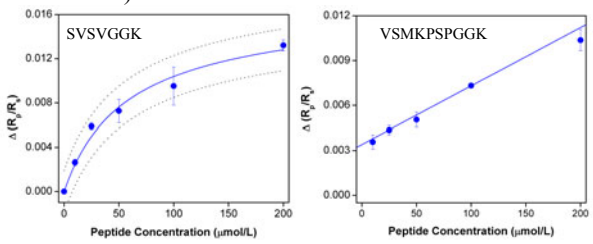

Figure 2. Equilibrium binding curves of peptide fragments

We further probed which amino acid moieties contributed to the overall affinity of the HA-peptide. We found that the peptide fragment SVSVGGK bound specifically (Fig. 2.) to HA but with lower affinity (K_D ≈ 54.4 µmol/L ± 7.6 µmol/L) than the parent. Interestingly, a second fragment VSMKPSPGGK appeared to bind non-specifically (Fig. 2.) in the current context.

Conclusions: We present a novel HA coated SPRi sensor to quantitatively measure the affinity of small peptides. Using this method, we report that our HA-peptide, a 12-mer, binds with high affinity (K_D ≈ 14.1 µmol/L ± 3.8 µmol/L) to hydroxyapatite. Further, the amino acid sequence SVSV seems to impart a significant contribution to this interaction while the MKPSP sequence may provide a conformational dependent component that enhances the peptides affinity, but by itself shows little specificity in the current context.

References:

1. Long, J.R. et al., *Proc. Natl Acad Sci*. USA 1998, 95, 12083.
2. Shaw, W.J. et al., *Am. Chem Soc*. 2000, 122, 7118.
3. Koutsopoulos, S. et al., *J. Crystal Growth*, 2000, 216, 443.
4. Koutsopoulos, S. et al., *Langmuir*, 2000, 16, 6739.
5. Wallwork, M.L. et al., *Langmuir*, 2001, 17, 2508.
6. Roy, M.D. et al., *Adv. Mater.* 2008, 20, 1830.