Investigating Oxidative Susceptibility of Peptoid-Based Materials for Selective Biosensing Applications

Hattie C. Schunk,[1,2] Adrianne M. Rosales[1] and Laura J. Suggs[2]
Chemical[1] and Biomedical[2] Engineering, University of Texas at Austin, Austin, TX

Statement of Purpose: As the primary orchestrators of inflammation, macrophages are an attractive target for the detection of chronic inflammatory disease. In inflammatory environments, macrophages assume different activation states (*i.e.* phenotypes) in response to a variety of external stimuli. Although these phenotypes are commonly classified as M1 (pro-inflammatory) or M2 (anti-inflammatory), they exist along a spectrum associated with the release of a number of biological analytes. These analytes act via multiple degradation mechanisms, including oxidative damage by reactive oxygen species (ROS), or proteolysis by enzymes such as matrix metalloproteases (MMPs). As a result, peptide and protein biodegradability targeting ROS and MMPs is commonly exploited for inflammatory disease biosensing. However, a key challenge in designing sensors that selectively respond to *one* type of degradative mechanism is uncontrolled cross-reactivity. In a step towards selectively identifying, labeling, and monitoring macrophage phenotypes, we present a strategy using peptoids, or N-substituted glycines. Peptoids are well-known to be resistant to proteolysis as a result of the conformational changes caused by their N-substitutions. However, other modes of peptoid degradation remain to be fully explored; in particular, oxidative degradation mediated by ROS. One study has shown that polydisperse peptoid backbones were found to be more susceptible to a chemical ROS generator (H_2O_2, 0.5-50 mM, with $CuSO_4$ catalyst, 50 µM) than polyethylene glycol (PEG) or poly(2-oxazolines) [1]. This work indicates feasibility that oxidation represents a significant degradation mechanism for peptoids. Here, we aim to define the selectivity and sensitivity of peptoids to ROS species in more biologically relevant contexts.

Methods: We have conducted preliminary experiments to assess the range of biodegradability of a library of peptide and peptoid 18-mer oligomers in relevant oxidative and enzymatic environments (Fig. 1A). To probe differences in susceptibility as a function of residue type (peptide vs. peptoid), substrates were separately incubated with ROS generators and proteases using two common model conditions: H_2O_2 + $CuSO_4$ (oxidative) and trypsin (enzymatic). To identify timescales of degradation, each substrate was dissolved in PBS at a concentration of 1 mg/mL and exposed to each condition at varying concentrations (10 µM-10 mM). Samples were incubated at 37˚C and physiologically-relevant pH was maintained (pH 7.4). At set timepoints, aliquots were collected, frozen (to quench the reaction), and lyophilized. Liquid chromatography (LC) and mass spectrometry (MS) analysis was performed to determine degradation rates by decreasing chromatography peak area associated with the intact structure (Fig. 1B). To quantify degradation rates, the max absorbance of each sample was normalized against its respective control at fixed retention times (Fig.1C) for use in degradation half-life ($t_{0.5}$) calculations.

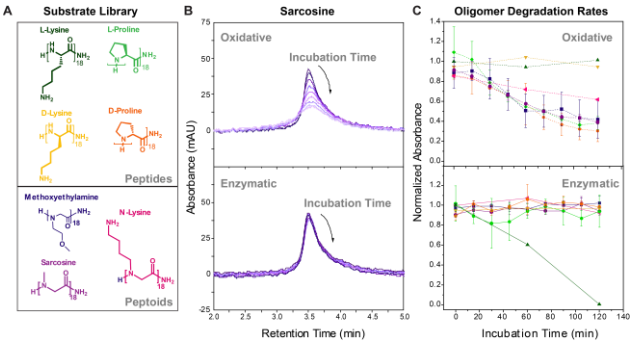

Figure 1. A) Substrate library. B) Representative LC trace of peptoid upon exposure to oxidative (10 mM H_2O_2 + 50 µM $CuSO_4$) and enzymatic (trypsin, 10 µM) stimuli. C) Comparison of oxidative and enzymatic degradation rates for peptides and peptoids. Each point is normalized to a control without ROS or trypsin (n=3).

Results: Our preliminary degradation studies established a baseline comparison of peptoids to (L)- and (D)-peptides for oxidative degradation of chemically-generated ROS. Calculated $t_{0.5}$ for sarcosine (0.55 ± 0.04 hrs) and methoxyethylamine (0.57 ± 0.02 hrs) indicate that peptoid substrates degrade at rates on par with other N-substituted molecules (L-proline: $t_{0.5}$= 0.56 ± 0.01 hrs and D-proline: $t_{0.5}$ = 0.53 ± 0.04 hrs). Interestingly, both L- and D-lysine did not degrade under these oxidative conditions (Fig. 1C), but D-lysine degraded to H_2O_2 at high concentrations (1 M H_2O_2, data not shown). Notably, all peptoids were stable to trypsin (Fig. 1C), presenting peptoids as a promising path to achieve selective oxidative degradation while maintaining susceptibilities on par with prolines.

Conclusions: Here, we have established one of the first reports on oxidative degradation of a library of peptoids. These degradative properties are particularly attractive for M1 macrophage sensing, because M1 macrophages produce significantly higher levels of ROS than non-stimulated macrophages. Furthermore, peptoid resistance to proteolysis can be leveraged to prevent cross-reactivity in cell sensing applications where a multitude of other degradative stimuli might be encountered. Ongoing work involves development of a fluorescence-based screening tool for investigating the susceptibility of sequence-defined peptoids in the presence of live cells (RAW 264.7 macrophages) stimulated to produce ROS and enzymes *in situ*. This approach will capture the synergistic interactions of multiple ROS and define peptoid backbone susceptibility to oxidative versus enzymatic degradation *in vitro*. Together, these studies may establish peptoids as molecules with targeted susceptibility that are broadly useful for biosensing applications in which selectivity for certain degradation modes is desired.

Reference:

[1] Ulbricht, J. *Biomaterials.* 2014, *35* (17), 4848-4861.