Antibody-Driven Protease Chain Reaction: A Versatile Platform for Rapid Countermeasure Development

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Abstract

Introduction: The ability to rapidly develop and deploy diagnostics is critical for an effective response to novel infectious disease threats. Platform technologies that can be quickly adapted offer a significant advantage over bespoke assay development. This research introduces a modular diagnostic platform based on a Protease Chain Reaction (ProCR) that utilizes target-specific antibodies to enable the rapid creation of sensitive countermeasures.

Materials and Methods: The diagnostic platform employs a room-temperature, isothermal Protease Chain Reaction (ProCR). The core of the system is an inactive protease-inhibitor complex. Target-specific monoclonal or polyclonal antibodies are integrated into the assay to serve as modular targeting agents. In the presence of a target antigen, the antibodies aggregate the protease-inhibitor complexes, initiating a localized, self-amplifying cascade that releases the active protease and generates a fluorescent signal. To enhance performance, magnetic beads coated with capture antibodies were used to concentrate target analytes from the sample and reduce matrix interference.

Results: The platform demonstrated both speed and high sensitivity. A solution-based assay detected 100 fM of Human Serum Albumin (HSA) within 30 minutes. The integration of magnetic beads significantly improved performance, enabling the detection of 50 fM of Insulin-like Growth Factor 1 (IGF1) in under 20 minutes. The platform's rapid adaptability was demonstrated by the development of a Botulinum Neurotoxin (BoNT) assay capable of detecting 1 pM of the toxins within days of receiving polyclonal antibodies.

Conclusions: The antibody-driven ProCR system is a versatile and effective platform technology for rapid diagnostic development. Its "plug-and-play" modularity, which allows for the simple integration of new antibodies, enables the creation of novel assays in a fraction of the time required for traditional methods. This approach provides a powerful tool for developing countermeasures against emerging and unforeseen biological threats.

Background

The Department of Defense biodefense research and development efforts consistently prioritize the acquisition of sensitive diagnostic and environmental detection capabilities that can be used on existing commercial-off-the-shelf/government-off-the-shelf platforms, and have minimal size, power, and cold-chain requirements for use by forward-deployed laboratories and combat units.

Protease chain reactions (ProCRs), which are being developed by Potomac Affinity Protein, LLC, are engineered protease reactions that can produce sensitive and highly specific signal cascades during ambient isothermal reactions. The enzymatic technique involves creating an environment in which transiently uninhibited proteases act to cleave and activate other inhibitor-blocked proteases to create a visually detectable chain reaction. ProCR reaction kinetics are such that when antibody-bound proteases coalesce around soluble or immobilized emerging targets, the localized proteases become more likely to lose their inhibitors and initiate a signal cascade.

As with traditional PCR-based detection methods, ProCR assays generate sensitive and highly specific signal cascades in which signal onset inversely corresponds to analyte concentration. However, ProCR has a couple of important advantages over comparable immuno-PCR assays that make the technology better suited for forward-deployed diagnostics and detection assays.

First, protease reactions don't require thermal cycling and will produce strong end-point fluorescent signals at ambient temperatures, regardless of target concentration, making it possible to incubate the reaction vessel under ambient conditions and observe the time-dependent results with a hand-held fluorescent light.

Second, protease cascades are triggered when the antibody-bound proteases coalesce around soluble or immobilized antigenic targets. Since unbound proteases do not promote cascade initiation above background, it's unnecessary to include a wash step that removes unbound detection antibody from the reaction.

We are currently performing proof-of-concept studies in which we use existing ProCR reagents to evaluate and optimize ProCR detection and quantification assays against a subset of bacterial, viral, and toxins or surrogates. Additionally, ProCR performance is being evaluated using common platforms to demonstrate the board applicability of this technique to field-deployable units. This poster introduces the ProCR technology along with our recent findings.

Figures

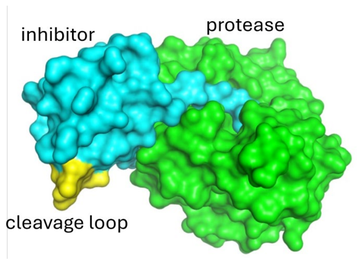

Figure 1. Basic Protease Complex. The central component of ProCR is a high-specificity protease (green) that is tightly regulated by an imidazole cofactor and a tight-binding protein inhibitor (cyan). The inhibitor can be released from the protease via a cleavage site (yellow) that corresponds to the specificity of the protease.

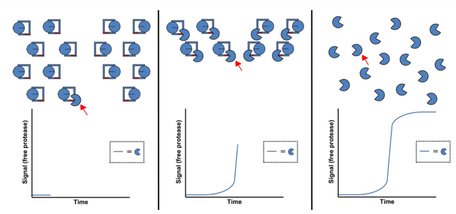

Figure 2. Protease Chain Reaction. Free proteases attack the cleavage loops of protease-bound inhibitors to free additional proteases and create an exponentially increasing signal cascade. In the first panel, a trace amount of free protease (red arrow) initiates the chain reaction and eventually causes the release of all proteases from the inhibitory complexes in panels two and three. The lag time preceding the full release of protease is determined by the initial concentration of free protease.

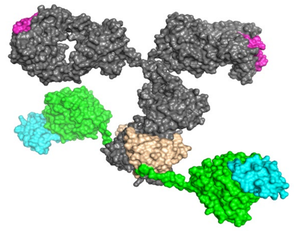

Figure 3. Antibody-Targeted ProCR Complex. To create an antigen-specific detector, the proteases (green) are fused to Protein GB Fc-binding domains (tan) and mixed with protein inhibitors (cyan) and target-specific antibodies (grey). As shown in this diagram, each antibody binds to two protease/inhibitor complexes via the duplicate Fc regions.

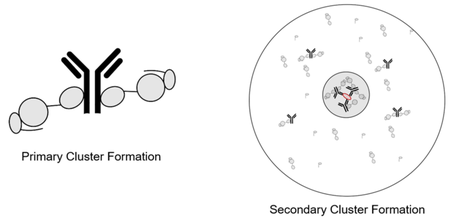

Figure 4. Activation by Clustering. The clustering of protease-complex around IgG creates a localized protease-inhibitor imbalance that initiates the chain reaction. Secondary and tertiary clustering effects are achieved when the antibody-bound proteases are further aggregated around the target and/or capture particles. This creates an accelerated cascade that is distinguishable from that generated by antibody alone. The delay in the onset of the cascade is inversely related to the concentration of the antigen in solution.

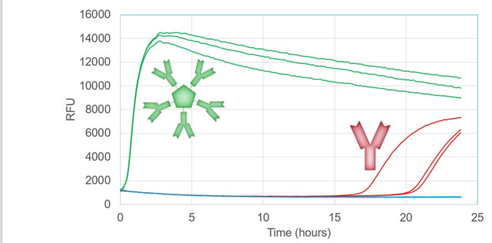

Figure 5. Primary cluster formation around an antibody. ProCR is sensitive to the presence of the antibody alone. In this experiment IgM is much quicker at initiating protease cascades than IgG because the pentameric IgM is capable of recruiting more proteases to the activation complex.

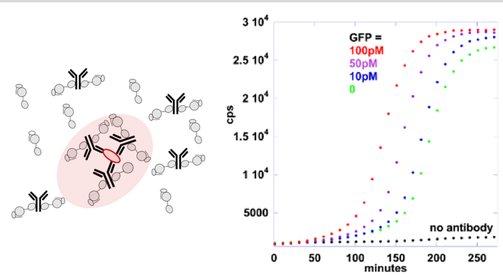

Figure 6. Secondary cluster formation around an antigen. Using GFP as a toxin surrogate, we mixed ProCR reagents with 2.5 nM anti-GFP IgG to detect as little as 10 pM protein in a 40 µL reaction. Lower levels of GFP were not distinguishable from IgG alone.

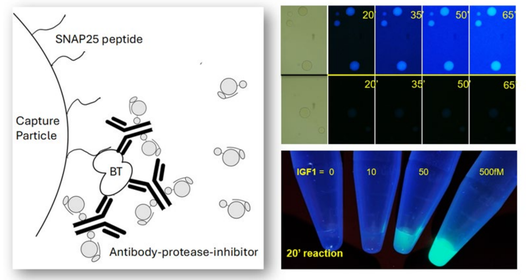

Figure 7. Tertiary cluster formation around capture particles. With minor modification, ProCR components can be combined with capture beads to greatly increase sensitivity. A capture bead concentrates the target molecule in a small volume and becomes the reaction vessel for sub nanoliter ProCR. The diagram on the left shows tertiary cluster formation could be used to develop a highly sensitive BoNT assay. In a proof-of-concept experiment, 40 µL reactions containing 40 nL polymyxin beads, 5 nM anti-Lipid A antibody, and ProCR components were mixed with or without 0.1 EU/mL endotoxin (top and bottom rows). Panels at left are viewed with visible light. Other panels in the rows show a time course of the ProCR under UV light. The diameter of the large particles ~90 microns. The magnification is 100X. In another capture particle-mediated assay (lower right), as few as 50 fM IGF1 generates a fluorescent cascade that is readable by the naked eye in under 20 minutes.

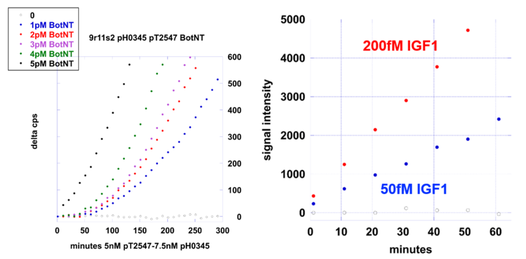

Figure 8. Obtaining accelerated results with kinetic assays. Although it can take hours for target-triggered protease cascades to achieve maximum cleavage rates, it is possible to detect target-induced protease activity during the first 15-30 minutes of the assay. This figure depicts the rapid detection of femtomolar quantities of BoNT (left) and IGF1 (right) during the early kinetic phases of solution and bead-based assays, respectively.

Learning Objectives

1. Describe the core mechanism of an antibody-driven Protease Chain Reaction (ProCR) and how it generates a rapid, amplified signal in response to a specific pathogenic target.
2. Explain how the "plug-and-play" nature of the ProCR platform allows for the rapid development of new diagnostic assays by simply integrating different target-specific antibodies.
3. Analyze the advantages of using a modular platform technology, such as ProCR, for developing rapid infectious disease countermeasures as opposed to creating bespoke assays for each new threat.

Conclusions

- ProCR has potential to enable sensitive diagnostic and detection assays against a range of targets in a variety of settings and platforms.
- ProCR could support ruggedized, low-power, isothermal assays for multiplexed pathogen detection by forward-deployed troops.
- The flexible ProCR technology is expandable to a wide range of targets and platforms.

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