Accelerated manufacturing of subunit vaccines for a rapid pandemic response

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CY15055-08Jul20-AN

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A robust pandemic response and biodefense strategy is necessary to protect the public health in case of a national security threat. To support the United States government mission to protect the United States and its military personnel by quickly responding to novel threats, scientists from GE's Fast Trak Services (previously Xcellerex™) and a team of partners worked with the US Department of Defense to develop a platform for rapid manufacturing of critical protein-based therapeutics that are essential for the military. This application note describes a process for rapid production of recombinant anthrax protective antigen (*r*PA) in short process time for the manufacture of an anthrax subunit vaccine. Histidine-tagged *r*PA was produced in *Pseudomonas fluorescens* grown in the single-use Xcellerex XDR-50 MO fermentor system. The produced fusion protein was captured directly from crude extract using immobilized metal ion affinity chromatography (IMAC). After an additional purification step, using hydrophobic interaction chromatography (HIC), the histidine-tag was proteolytically cleaved off and removed by subtractive IMAC. Using the described process, *r*PA could be processed to a purity of more than 99% at a recovery of about 40% in about 12 h from cell harvest to purified *r*PA. The described process was developed within the Accelerated Manufacture of Pharmaceuticals (AMP) program funded by the Defense Advanced Research Projects Agency (DARPA)/Defense Threat Reduction Agency (DTRA).

Note: this work is released for public distribution by DARPA/DTRA and is reported in "Xcellerex DARPA AMP project summary, [DISTAR case 11745,12104,12166] "A" (approved for Public Release, Distribution Unlimited)"

Introduction

The objective of the DARPA/DTRA-funded AMP program is to accelerate availability of critical protein-based therapeutics that are essential for the military. The goal is to develop platforms capable of manufacturing at least 3 million doses of cGMP-quality monoclonal antibodies or subunit vaccines within three months of receiving the DNA sequence. The platform needs to be flexible, as it should be easy to adapt to the manufacturing of different protein-based therapeutics or vaccines. The project plan to meet the objective was divided in three phases. In phase I, the project teams needed to demonstrate proper structure and function of the produced protein, a pathway to scale-up, and a path to meet the cost goals of phase III. In phase II, production of a protein that meets set quality requirements needed to be demonstrated, starting with a 30 L fermentation (or equivalent). In phase III, the teams needed to present a platform for production of 3 million doses that meet the cost goal of ≤ 1 USD/dose as well as a plan for process transfer to a manufacturer capable of producing a final product suitable for FDA approval.

One of the project teams funded by this program constituted scientists from GE's Fast Trak Services team, Dowpharma (now Pfenex), deltaDOT, and Biopharm Services. Within this program, the team proposed a vaccine platform based on the manufacturing of an anthrax subunit vaccine, and a process for production and purification of *r*PA was developed. The protein expression construct was based on SUMOpro™ gene fusion technology (LifeSensors), and clone selection was based on the *Pf*enex Expression Technology™ platform (*Pf*enex). The *r*PA protein was produced as a fusion protein in *P. fluorescens* using the XDR-50 MO microbial fermentor system.

Here, we describe the developed method for production and purification of *r*PA to high purity and yield in only 12 h from cell harvest to purified protein. The process development goal was a purification platform capable of producing ≥ 10 vaccine doses/L/wk, each dose containing 40 µg *r*PA/dose, from a 30 L fermentation. Criteria for the purified *r*PA were a solubility of above 95% and more than 95% correctly folded protein with less than 1% fragments in the final product.

Materials and methods

Upstream production

$(His)_6$-SUMO-tagged *r*PA was produced in *P. fluorescens* grown in 30 L fermentation broth, using the XDR-50 MO microbial fermentor system. Cell were grown for two days, and then harvested. Produced fusion protein was released by incubating the cells in lysis buffer, containing detergents, lysozyme, Benzonase™, and protease inhibitors, for 2 h at room temperature (RT), creating a crude extract.

Downstream purification

$(His)_6$-SUMO-tagged *r*PA was captured from the crude extract (without homogenization, filtration, or centrifugation) by expanded bed chromatography on an IMAC column packed with Ni-charged resin. The fusion protein was further purified on a HIC column packed with HIC-butyl resin operated on the single use ÄKTA™ ready chromatography system. Thereafter, the histidine-SUMO tag was cleaved off by incubating the fusion protein in SUMO protease solution (1:250 protease-to-target ratio) for 2 h at RT and removed by subtractive chromatography on an IMAC column packed with Ni Sepharose™ resin and operated on the ÄKTA ready system.

Analyses

Product purity was monitored by SDS-PAGE over the purification process. After the final purification step, critical quality attributes of the *r*PA was monitored by SDS-CGE using LabChip™ technology (Caliper) and *r*PA standard as reference.

Results

The cytoplasmic-expressed fusion protein was released by cell lysis, omitting the need for homogenization, filtration, or centrifugation. To ensure complete release of the fusion protein, a lysis buffer containing detergents, lysozyme, Benzonase, and protease inhibitors was used. The developed chemical release process is scalable and fits well with the single-use XDR fermentation platform. Productivity comparing two runs is shown in Figure 1. With optimization of the fermentation process from Run 1 and 2, the titer was significantly increased in Run 3. Average time to harvest was 42 h. Results from all three runs are listed in Table 1.

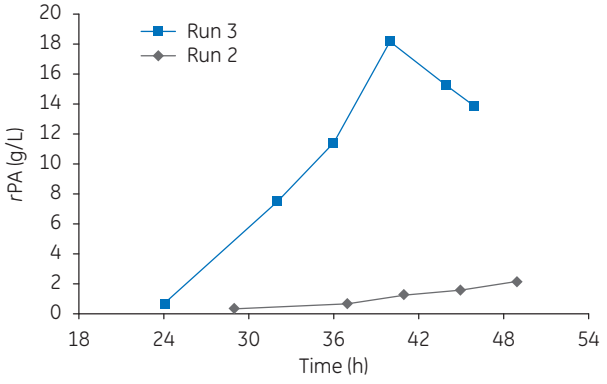

Fig 1. Productivity results from Run 2 and 3.

Table 1. Production of *r*PA in 30 L fermentations

Run no.	Peak OD_{575}	Peak titer (g/L)
1	109	1.0 (data not plotted)
2	162	2.0
3	276	18

The developed purification process is outlined in Figure 2. Expanded bed chromatography allows particulate impurities in the feed stream to pass freely through the resin. Using this technology in the capture step, *r*PA could be captured by IMAC directly from crude lysate, thereby avoiding the need for an intermediate clarification step based on centrifugation or membrane filtration. Results from SDS-PAGE analysis of fractions containing the fusion protein are shown in Figure 3. Figure 4 depicts SDS-PAGE analysis of *r*PA after cleavage of the $(His)_6$-SUMO fusion tag. As shown in Figure 5, *r*PA could be purified to high purity at high recovery using the described method. A purity of more than 99% was achieved at a recovery of approximately 40% in about 12 h from harvest to purified *r*PA.

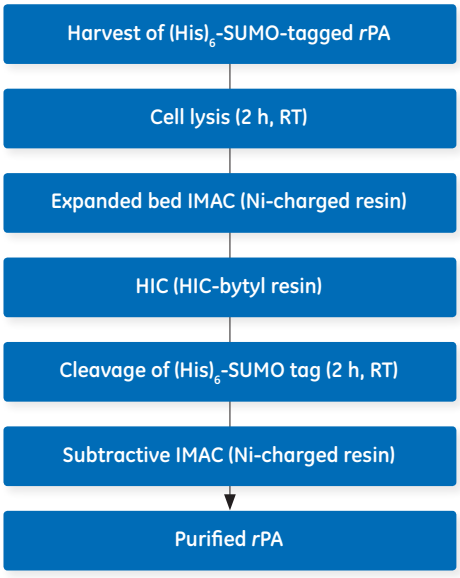

Fig 2. Process for downstream purification of *r*PA to set quality criteria in about 12 h.

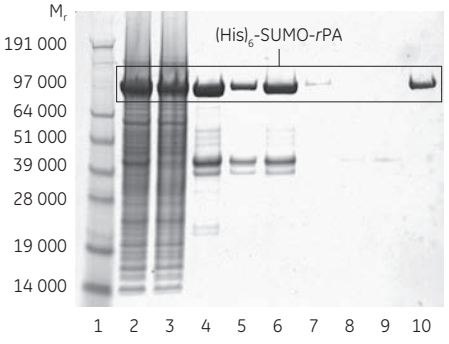

Fig 3. SDS-PAGE analysis of fractions containing $(His)_6$-SUMO-tagged *r*PA.

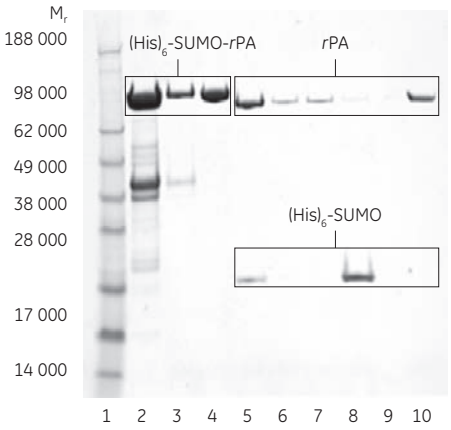

Fig 4. SDS-PAGE analysis of *r*PA after cleavage of the $(His)_6$-SUMO fusion tag.

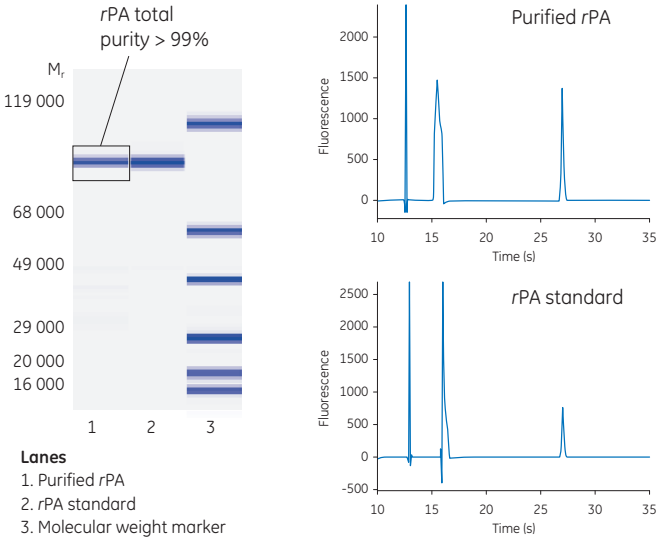

Fig 5. SDS-CGE analysis of the purified *r*PA target protein.

Results from three production batches using the described process versus set criteria are summarized in Table 2. With an average time to harvest of 42 h, 3.6 consecutive 30 L fermentations can be conducted each week. With a productivity of 18 g/L and a recovery of about 40%, the developed method meets the requirements of 10 doses/L/wk (40 µg/dose). The cost goal of < 1 USD/dose was also met: at 30 L scale, the estimated cost was 0.40 USD/dose using the described process.

Table 2. Results vs set criteria

Batch no.	Productivity (> 10 doses/L/wk, 40 µg/dose)	Fragments (< 1%)	Solubility (> 95%)	Correct folding (> 95%)
1	43 000 doses/L/wk	0%	100%	> 95%
2	71 000 doses/L/wk	0%	102%	> 95%
3	8700 doses/L/wk	0%	100%	> 95%

Discussion

The described process for production of *r*PA for the manufacture of anthrax subunit vaccine is fully compatible with single-use technology. By omitting the need for cleaning, steam in place, and their respective validation operations, more batches can be produced over a certain time period and changeover time between campaigns can be significantly reduced compared with using fixed stainless steel equipment. Single-use equipment also eliminates potential cross-contamination between different medical counter measure (MCM) production campaigns.

The modular and electronically controlled single-use FlexFactory™ biomanufacturing platform comprises process equipment from upstream production to downstream purification. The systems are reconfigurable in days to develop and manufacture virtually any biologic-based product from virtually any host expression system. New capacity

Fast Trak Services

GE's Fast Trak Services are specifically designed to help biomanufacturers increase their process productivity, reduce cost, and enable them to bring their product to market faster through support in process development, cGMP manufacturing, and training. The Fast Trak Services centers are equipped with the latest technologies to accelerate bioprocess development in an environment and at a scale that closely replicates the real-life industrial setting. For over 30 years, thousands of customers world-wide have been trained by GE's experienced Fast Trak leadership teams, giving customers access to industry expertise that encompasses process and analytical development, process scale-up, as well as manufacture of drug substances for use in toxicology studies or phase I and II clinical testing.

The Fast Trak Services centers are located in South Korea, USA, Sweden, India, and China, with satellite centers in Turkey, Japan, and Singapore.

can be in place within one year and existing capacity can be expanded in months. The manufacturing lines can be rapidly redeployed to new locations in weeks. The process development and manufacturing response time for a novel threat can be reduced to 12 weeks or less. With this single-use biomanufacturing solution, capital costs are reduced by roughly 50%, operating costs by 20% or more. Xcellerex single-use XDR cell culture bioreactors mirror traditional stainless steel stirred tank bioreactors and can be scaled to a working volume of 2000 L. The single-use XDR fermentors, comprising the XDR-50 MO system used in this study as well as the larger XDR-500 MO system, cover working volumes from 25 L to 500 L.

For downstream purification, ReadyToProcess™ chromatography columns are available in sizes from 80 mm i.d. to 450 mm i.d. ReadyToProcess columns are delivered prepacked and ready for use. The columns are operated on the ÄKTA ready chromatography system, with a broad flow rate range from 50 mL/min to 8 L/min and a completely disposable flow path.

FlexFactory Automation monitors and controls the FlexFactory process equipment, ensuring consistency between productions. FlexFactory Automation is based on a combination of Wonderware™ (Schneider Electric) and UNICORN™ software, recognized for engineering simplicity, operational agility, and information empowerment. The software helps protect the operational integrity of the plant, enhance the operational insight of resources, and facilitate process improvements and changes without costly revalidation work. The software can be used in a manner that complies with current good manufacturing practices (cGMP).

FlexFactory biomanufacturing solution, comprising the XDR fermentors and ReadyToProcess columns, offers a platform for production and purification of protein-based therapeutics and vaccines based on single-use technology that adds flexibility and speed to the process.

Conclusion

A robust pandemic response and biodefense system must include a capacity to manufacture medical countermeasures to be timely provided in case of a large-scale public health emergency such as an anthrax attack. Rapid, flexible, and cost-effective process development and manufacturing are key elements of an effective system. Here, we describe a process developed for production of *r*PA in about 12 h from cell harvest to purified protein for manufacturing of anthrax subunit vaccine. The results show that set goals of a purity of more than 99% at a recovery of about 40% were well met using the described method. The described process is easy to adapt to the manufacturing of other protein-based therapeutics or vaccines, is easily scaled to meet market demands, and fully compatible with single-use process equipment for efficient use of the production facility. Single-use manufacturing equipment allows addressing the requirements of pandemic response and biodefense strategies in short time, with great flexibility, and with low possible investment and operating expenditure.

Related literature

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KA2393220218AN JB55409US

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