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Chicken as a Bioreactor for Production of Human Tissue Plasminogen Activator

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Abstract

The fibrinolytic system of blood vessel endothelial cells includes human tissue plasminogen activator (t-PA), a serine protease that selectively cleaves plasminogen. t-PA, which converts plasminogen to plasmin, is used in clinical practice to treat thrombosis. Thrombotic disorders are among the most common diseases affecting people. Cardiovascular disease, particularly thromboembolism, poses a considerable risk to human health due to its steady annual increase. As a result, it is necessary to create thrombolytic medications that are more effective, targeted, affordable, and secure. Despite strong demand for thrombolytics, a number of issues, including strain instability, cost-effectiveness, and a lack of infrastructure, result in insufficient supply

from manufacturing units. Producing human tissue plasminogen activator from mammalian cell cultures is challenging and expensive. As a result, this paper focuses on an efficient and cost-effective approach for generating human tissue plasminogen activator in a chicken bioreactor. Because of the chicken's fast generation time and simpler protein composition, human tissue plasminogen activator can be produced and isolated in transgenic chicken.

Keywords: human tissue plasminogen activator, plasmin, cardiovascular disease, plasminogen.

Introduction:

Tissue plasminogen activator (tPA) is a serine protease. It is thus one of the most critical components of blood clot breakdown. Its main role is to catalyze the conversion of plasminogen into plasmin, the enzyme that dissolves blood clots. Recombinant biotechnology has permitted the laboratory manufacture of t-PA, also known as recombinant tissue plasminogen activators (t-PA). These medicines have been changed in a variety of ways to optimize their pharmacokinetics and pharmacodynamics, most notably by lengthening their short half-life in circulation and improving their fibrin specificity to prevent an undesirable fibrinolytic condition. These medications include tenecteplase, reteplase, and alteplase.

Fibrinolysis is a highly controlled procedure in which plasmin, a protease, degrades and remodels a fibrin-rich thrombus. This procedure is regulated by plasminogen activators and inhibitors, with the conversion of plasminogen to plasmin resulting in fibrinolysis. The interaction of plasminogen activators (t-PA) and urokinase (uPA) with their major inhibitor, plasminogen activator inhibitor-1 (PAI-1).

The tissue plasminogen activator protein (t-PA) contains 527 amino acid residues, including 35 cysteine residues that contribute to the formation of 17 disulfide bonds. ht-PA has five distinct structural domains: an N-terminal finger domain (F domain, residues 4-50), an epidermal growth factor-like domain (E domain, residues 50-87), two kringle domains (K1 domain, residues 87-176; and K2 domain, residues 176-256), and a serine protease catalytic domain (P domain, residues 276-527). The F and K2 domains control its binding to fibrin and, as a result, protease activity. ht-PA converts plasminogen to plasmin, which has been utilized to treat thrombosis (Long et al., 2015; Allahverdipoor et al., 2024; He et al., 2023).

t-PA is largely produced by vascular endothelial cells and is continuously secreted into the plasma as well as acutely released. The latter occurs when certain endothelial cell receptors are activated. Different sections of the vascular system secrete variable amounts of t-PA. The upper extremities produce four times as much t-PA as the lower extremities (Emeis et al., 2024; Nakano et al., 2021).

t-PA is removed from the bloodstream by the liver's rapid clearance mechanism. Free t-PA has a half-life of approximately 4 minutes, although this can be considerably shortened if PAI-1 levels rise, as is the case in many thrombotic patients. When an isolated rat hepatic perfusion system was studied, it was shown that the t-PA/PAI-1 complex is eliminated twice as quickly as free t-PA (Agersnap et al., 2022; Sillen and Declerck, 2021). t-PA is classified into two types: single-chain (sct-PA) and two-chain (t-PA). The single-chain molecule is the native form of t-PA released by endothelial cells, whereas the two-chain form is the result of plasmin proteolysis. Both versions are catalytically active and show equal enzymatic activity in the presence of fibrin.

Blood coagulation is an enzymatic process that starts with chemicals from injured tissues and concludes with the formation of fibrin monomers, which form clots. After a few days, the fibrinolytic enzyme system breaks down the fibrin clot. The glycoprotein plasminogen, which is found in plasma and most extravascular fluids, is the system's

primary enzyme. Plasminogen is a serine protease zymogen that is partially degraded by a plasminogen activator before being converted into the active form, plasmin. Plasmin is engaged in numerous biological processes, including cell migration, proliferation, inflammation, and tumor invasion, although its primary function is believed to be fibrin breakdown in the vasculature. The human body has two forms of plasminogen activators: tissue (t-PA) and urine (u-PA). t-PA is the primary activator of plasminogen in blood, whereas u-PA primarily functions in tissue-related proteolysis and is thought to be secondary to t-PA in the elimination of intravascular fibrin (Liu et al., 2022; Ting Mei et al., 2020).

Chicken as a Bioreactor

Today, many biopharmaceuticals are created using expression systems derived from bacterial, yeast, or mammalian cell cultures. Furthermore, the growing significance of genetically modified chickens as a bioreactor system for producing protein-based medications is discussed. The avian bioreactor enables the expression of target genes in ovarian cells, resulting in the production of gene expression products in egg whites. Many glycosylated therapeutic proteins are currently made using animal cell culture methods. Although mammalian and bacterial cell cultures have been shown to be effective in the creation of recombinant proteins, they do have some limitations. It is widely accepted that bacterial cell culture methods are less capable of post-translational modification and glycosylation than eukaryotic systems (Raju et al., 2000). Chinese hamster ovary (CHO) cell lines, which are widely used for mammalian cell lines, often allow for appropriate post-translational modifications (Houdebine, 2009), although they are costly to generate. This has spurred researchers to consider possible alternatives, such as transgenic animal bioreactor systems, which have the ability to overcome the challenges of standard cell-based production systems. Transgenic sheep, goats, and cows were created as animal bioreactors to generate high-quality proteins on a wide scale, perhaps with less capital and production costs than cell culture methods (Lillico et al., 2005). Despite enormous effort and financial investments, just a few trials proved commercially successful (Niemann and Kues, 2003). However, employing a secretory organ, such as the mammary gland, as a bioreactor incurs exorbitant expenditures for raising and maintaining a large population of mammals.Furthermore, the diverse nature of numerous milk proteins makes it challenging to purify recombinant therapeutic proteins. The use of chicken eggs can avoid these issues while also providing benefits such as significantly quicker generation times, high fecundity, and cheaper costs for breeding and maintaining large populations (Ivarie 2003). Most importantly, the therapeutic recombinant protein may be purified considerably more easily since egg white has a simpler composition than milk. Furthermore, compared to mammalian livestock systems, the glycosylation patterns of avian proteins are more akin to those of their human homologues (Raju et al., 2000). The development of laying hens as bioreactors is dependent on the hen's high protein synthesis ability. The hen egg has around 3.5 g of protein in its white. Egg white serves as a substrate for the large-scale production of a desired protein, which is then extracted for purification. The ovalbumin promoter can be changed to regulate the expression of a gene of interest in the oviductal cells that generate egg white.

Conclusion

According to current studies, enormous efforts are being made to modify avian eggs and their associated cell cultures in order to give a suitable platform for the synthesis of therapeutic antibodies, recombinant proteins, and vaccine manufacturing. The similar glycosylation pattern as humans, cost effectiveness, shorter breeding cycle, and ease of protein purification make chicken bioreactors useful for producing human tissue plasminogen activator and other theurapeutic proteins. Thus, using this transgenic technique, avian species such as chickens serve as excellent bioreactor models in the fields of biotechnology and pharmaceuticals.

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