Purification and Functional Characterization of Alpha-1-Antitrypsin for Autoimmune Disease Intervention

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Abstract. Type 1 diabetes (T1D) is an autoimmune disease mediated by autoreactive T cells. Current treatments primarily target symptom control and struggle to fundamentally regulate the abnormal immune response. Therefore, developing novel biotherapeutic strategies that combine immunomodulation and tissue protection is crucial. Recent studies have shown that alpha-1 antitrypsin (AAT) can delay or even reverse disease progression in non-obese diabetic mice and improve transplanted islet survival, demonstrating its potential for treating autoimmune diseases. This article systematically reviews the gene structure, biological function, and the impact of N-glycosylation on the activity of AAT. It focuses on analyzing the production advantages of CHO cell-derived recombinant AAT (rAAT) and key technologies such as ion exchange chromatography, affinity chromatography, and continuous purification. The study demonstrates that CHO cells can achieve glycosylation modifications close to those of natural AAT, and a multi-step chromatographic strategy can yield high-purity, high-activity rAAT products. In the future, the purification process should be further optimized, the functional evaluation system should be improved, and its therapeutic value in T1D and other autoimmune diseases should be verified through clinical research, so as to provide a theoretical basis for the development of novel immunomodulatory biological agents.

Keywords: Alpha-1-Antitrypsin; autoimmune disease; type 1 diabetes.

1. Introduction

Before introducing the AAT and the protein purification technique, this essay first introduces the type 1 diabetes, the Autoimmune disease, to highlight the importance of the new therapy: The type 1 diabetes is a typical example of an autoimmune disease. The autoimmune disease is a bunch of disorders that are caused by the immune system mistakenly attacking self-tissues, leading to chronic inflammation and organ damage. Type 1 diabetes (T1D) is a typical example of the typical example of autoimmune disease because autoreactive CD4+ and CD8+ T cells destroy insulin-producing pancreatic β-cells, resulting in lifelong insulin dependence and reduced life expectancy by 10–12 years [1]. and the type 1 diabetes has become a prominent example that disrupts millions worldwide. However, the current treatment of type 1 diabetes has obvious limitations, it could be either technical or clinical: The Current treatments primarily manage symptom-such as insulin injections or immunosuppressants, the question is the current treatment do not have the ability to address and resolve the underlying autoimmune process that disrupt the insulin producing cells Plus, the current treatments often brought significant side effects, including severe hypoglycemia or broad immunosuppression [2] and the high economic cost of the insulin injection and monitoring device.

Therefore, in order to basically maximize the reduction on the symptoms, the new therapy, the Alpha-1-antitrypsin (AAT) edited protein therapy was introduced. AAT is a glycoprotein that has a 52-kDa acute-phase, the AAT protein, primarily synthesized in the liver. It was categorized as the serine protease inhibitor superfamily; therefore, it performed as the inhibitor to suppress and control the physiological operations, with neutrophil elastase as its primary physiological target. Beyond its classical role as an inhibitor, AAT also has the capability to exert the potent immunomodulatory effects: For instance, the AAT could suppress and control the pro-inflammatory cytokines, inhibit neutrophil degranulation, and expand regulatory T cells [3]. During the Preclinical studies related to the immunomodulatory effect of AAT, the research results have demonstrated that AAT therapy can prevent or reverse autoimmune diabetes in non-obese diabetic (NOD) mice and prolong islet allograft survival [4].

Currently, the AAT or the edited AAT already have limited usage: Plasma-derived AAT (pdAAT) is FDA-approved for congenital AAT deficiency (AATD)-related emphysema [5], but its application in autoimmune diseases is limited by supply constraints, batch-to-batch variability, and potential viral contamination risks. Recombinant AAT (rAAT) produced in Chinese hamster ovary (CHO) cells offers a scalable, well-defined alternative. CHO cells enable proper N-linked glycosylation, which is essential for AAT's protease inhibitory activity and serum half-life [6]. Recent advances in chromatographic purification have achieved high purity (>99%) and yield of rAAT from CHO cultures [6].

However, comprehensive purification workflows and functional characterization of CHO-derived rAAT specifically for autoimmune disease intervention remain underexplored. Most existing studies have focused on AATD rather than immunomodulatory applications. Therefore, this study aims to establish an integrated purification strategy and functionally characterize CHO-derived rAAT as a candidate for autoimmune disease treatment, with particular emphasis on elastase inhibitory activity, glycosylation profile, and thermal stability. The findings will support the development of a scalable, well-characterized rAAT biotherapeutic for T1D and other autoimmune conditions.

2. Structural and Functional Properties of AAT

2.1. Gene Sequence and Basic Structure of AAT

The main character of the modernized method that has the potential to reduce the symptoms of autoimmune disease. The original version of AAT could became actively produced by the human body, the genes used to produce the AAT are encoded by the SERPINA1 gene, located on chromosome 14q32.1 in humans. The gene sequence length spans approximately 12.2 kilobases and contains seven exons, of which exons II–V encode the mature secreted protein [7]. The gene is predominantly expressed in hepatocytes and some small parts of the extrahepatic expression, the hepatocytes perform as the primary source of circulating AAT, although small proportion of the extrahepatic expression has been documented in monocytes, macrophages, intestinal epithelial cells, and bronchial epithelial cells [8].

After the human body processed the mature AAT protein, The mature AAT protein consists of 394 amino acids with a molecular weight of approximately 52 kDa, which increases to 55–65 kDa upon glycosylation [7]. Structurally, AAT belongs to the serine protease inhibitor (serpin) superfamily, characterized by a conserved tertiary fold comprising three beta-sheets (A, B, and C), nine alpha-helices (hA–hI), and a mobile reactive centre loop (RCL) that serves as a pseudosubstrate for target proteases [9]. The RCL contains the P1–P1' scissile bond, where Met358–Ser359 forms the critical recognition site for neutrophil elastase (NE). Upon protease engagement, the RCL is cleaved and inserts into beta-sheet A, trapping the protease in a covalent acyl-enzyme intermediate and irreversibly inhibiting its activity [9].

This conformational change—often referred to as the stressed-to-relaxed (S-to-R) transition—is a defining feature of the serpin mechanism and confers both high specificity and potent inhibitory activity. The structural integrity of AAT is particularly sensitive to point mutations in the SERPINA1 gene; the Z allele mutation (Glu342Lys), for example, destabilises the protein and promotes intracellular polymerization within hepatocytes, leading to AAT deficiency (AATD) and associated pulmonary and hepatic pathologies [10].

2.2. Functional Properties of AAT: An Immunomodulatory Protein

In the past, the original AAT protein was characterized primarily as an anti-protease, in other words, these proteins play a role as protect pulmonary tissue from proteolytic degradation by neutrophil elastase released during inflammatory responses. However, accumulating evidence over the past two decades until now has substantially expanded the functional landscape of AAT and the structural landscape as the gene sequence has been found, establishing it as a important pleiotropic

immunomodulatory agent with broad anti-inflammatory properties that extend well beyond protease inhibition [11].

The cellular analyzation of the AAT has explained why the AAT could become the immunomodulatory agent; AAT exerts immunomodulatory effects through multiple distinct mechanisms in order to edit the immune effect. At the beginning, AAT reduced and suppress the production of pro-inflammatory cytokines, including interleukin-1 beta (IL-1β), tumour necrosis factor-alpha (TNF-α), and iIL-6, by interfering with NF-κB signalling pathways and downstream transcriptional activation [12]. Concurrently, AAT are able to do something more: promotes the processing and production of anti-inflammatory mediators, most notably instance is the production of IL-10, thereby shifting the immune effect in cells to an equilibrium towards a tolerogenic immune environment [12].

Except for the immunomudulatory function of AAT, AAT has been shown the function to inhibit neutrophil degranulation and reduce the release of reactive oxygen species (ROS), finally enable the cell to limit oxidative tissue damage at inflammatory sites [13]. Third, and of particular relevance to autoimmune disease, AAT promotes the generation and function of regulatory T cells (Tregs). In some research done In vitro and in vivo studies have demonstrated that AAT treatment enhances the frequency and suppressive capacity of CD4+CD25+FoxP3+ Tregs, which plays a central role in maintaining immune self-tolerance and preventing autoimmune activation [14].

Additionally, the research in recent years also approved AAT have the ability to protect pancreatic beta-cells directly from apoptosis induced by pro-inflammatory cytokines and nitric oxide. This cytoprotective effect is partly mediated through inhibition of caspase-3 activity and modulation of the intrinsic apoptotic pathway [15] finally protect the pancreatic beta cells. In conclusion as the AAT has both the protective and the immunomodulatory function, these properties render AAT an attractive therapeutic candidate for conditions characterised by dysregulated immune responses, including type 1 diabetes (T1D), graft-versus-host disease (GvHD), and inflammatory bowel disease (IBD).

2.3. Role of N-Linked Glycosylation in AAT Activity

Molecular biology has one central dogma the structure determines function, so the AAT's special function means the glycoprotein has its own special structure: AAT is a glycoprotein carrying three N-linked glycosylation sites, located at Asn46, Asn83, and Asn247 of the mature protein [16]. Each site is modified by complex-type bi-, tri-, and tetra-antennary oligosaccharide chains, which together constitute approximately 12–15% of the total molecular mass of native AAT [16]. In addition, the glycan groups play a functional role in the biological mechanism, pharmacological properties, and effectiveness of action of these proteins.

Concerning their inhibition of proteases, there has been research indicating that N-linked glycosylation helps maintain the correct conformation of the AAT reactive center loop by preventing its aggregation or degradation.[17]. Deglycosylation studies using PNGase F, which cleaves all N-linked glycans, consistently demonstrate a reduction in the inhibitory activity of AAT against neutrophil elastase, confirming that intact glycosylation is required for full protease inhibitory function [17].

Glycosylation also exerts a profound influence on AAT's serum half-life and circulatory retention. The most significant is the sialytion changed the composition and receptors, this terminal sialylation of N-linked glycan chains prevents recognition by hepatic asialoglycoprotein receptors, therefore the hepatic receptors would not be over recognized and overwhelm, the recombinant and the glycosylation would protect circulating AAT from rapid hepatic clearance. Accordingly, recombinant AAT preparations with deficient or heterogeneous sialylation exhibit markedly shortened serum half-lives compared to plasma-derived AAT [18]. That is why the recombinant version of the AAT is getting considered. The main consideration is the development of rAAT produced in non-human expression systems, where glycosylation patterns may diverge from the human glycotype.

It is worth noting that CHO cells have been established as the optimal mammalian system for rAAT production due to their ability to undertake complex N-linked glycosylation like human cells, including addition of sialic acids. [19]. However, AAT obtained from CHO cells may display differences in glycosylation and sialylation compared to the plasma AAT protein, which can influence the pharmacokinetics of the drug and its immunomodulatory strength. The full characterization of the glycosylation profile is usually done through mass spectrometry after PNGase F treatment—is therefore an essential component of the functional validation of recombinant AAT preparations intended for therapeutic use [19].

3. The Strategies of Purificationtrategies of Purification

3.1. Chromatographic Approaches

In order to purify the recombinant version of rAAT from CHO cell culture supernatants requires a multi-step chromatographic workflow designed to achieve high purity that is approximate to 99 percent, this process begin with the retention of biological activity,for instance removal of process contaminants such as HCPs, DNA, endotoxins, and adventitious agents. The following segment in the chapter of the purification technique would discuss the major chromatographic methods used for purification of rAAT, with emphasis on separation principles, operational parameters, and specific advantages for this target protein.

3.1.1. Ion exchange chromatography (IEX)

The Ion exchange is prevalent in the biological process and cellular process, basically, the cell would use the ion exchange to separate the nutrition, therefore the Ion exchange chromatography (IEX) still applicable in protein separation based on differences in their net surface charge at a given pH, exploiting electrostatic interactions between charged amino acid residues on the protein surface and oppositely charged functional groups on the chromatographic resin. IEX is widely employed as a capture or intermediate purification step in biotherapeutic manufacturing due to its high binding capacity, scalability, and ability to resolve target proteins from a broad spectrum of process impurities [20].

AAT has an isoelectric point (pI) of approximately 4.8–5.0, rendering it negatively charged under physiological pH conditions (pH 7.0–8.0) [20]. This charge characteristic makes anion exchange chromatography (AEX) particularly well-suited for AAT capture from CHO conditioned medium. In AEX, the resin carries positively charged quaternary ammonium (Q) functional groups that bind AAT and other anionic proteins, while cationic impurities and unbound species are removed during the wash phase. AAT is subsequently eluted by increasing the ionic strength of the buffer (typically using a NaCl gradient), exploiting differential desorption kinetics to achieve selective elution.

Alternatively, cation exchange chromatography (CEX) can be applied at lower pH values (below the pI of AAT) where the protein carries a net positive charge. CEX is particularly effective for the removal of negatively charged impurities and serves as a useful complementary polishing step when used in conjunction with AEX [20]. Combined AEX-CEX workflows have been reported to achieve substantial reduction in host cell proteins and nucleic acid contamination, yielding rAAT preparations of high chromatographic purity.

3.1.2. Affinity chromatography (His-Tag or immunoaffinity)

Different from the ion exchange chromatography technique that separate the recombinant protein and containment molecules. through the electronegative difference. Affinity chromatography exploits highly specific biomolecular recognition interactions to selectively capture a target protein from a complex mixture, typically achieving substantial enrichment in a single step. Two primary affinity strategies are applicable to rAAT purification: immobilized metal affinity chromatography (IMAC) using polyhistidine (His-tag) fusion constructs, and immunoaffinity chromatography employing AAT-specific antibodies.

IMAC relies on the coordination of hexahistidine (6×His) tags, incorporated at the N- or C-terminus of the recombinant protein, to transition metal ions (typically Ni2+ or Co2+) immobilized on the resin matrix. His-tagged rAAT binds with high affinity under near-physiological conditions and is eluted by competitive displacement using imidazole gradients or by acidification [20]. IMAC offers considerable advantages in terms of rapid capture efficiency and simplicity; however, it introduces the requirement for subsequent tag removal if the His-tag is deemed immunogenic or otherwise detrimental to therapeutic application. Tag cleavage using site-specific proteases (e.g., thrombin or factor Xa) to make the protein edited and specific to be easier to purify, but this chromatography introduces additional processing steps to separate and the risk of incomplete cleavage or off-target proteolysis.

Immunoaffinity chromatography employs anti-AAT antibodies covalently coupled to a solid support, enabling highly selective capture of AAT regardless of its charge or size relative to co-purifying species. This approach is particularly advantageous when processing complex feedstocks with low AAT titre or high impurity burden, as single-step purification factors of several hundred-fold can be achieved [20]. The primary limitations of immunoaffinity chromatography are the high cost of antibody production and immobilization, ligand leaching concerns under acidic elution conditions, and the requirement for rigorous validation of antibody absence in the final product. For therapeutic-grade rAAT, immunoaffinity resins must meet stringent regulatory criteria, including demonstration of ligand stability across multiple cycles and absence of immunologically active leachatetes.

3.2. Integrated and Streamlined Approaches in Purification

In the industrial perspective to do the highly efficient practice and to be less complicate, the purification of CHO-derived rAAT to therapeutic-grade purity necessitates the integration of multiple chromatographic steps into a coherent, sequential workflow designed to maximise yield, purity, and process efficiency while minimising the number of unit operations and associated costs. The general paradigm for rAAT purification follows the capture–intermediate purification–polishing (CIP) strategy widely adopted in biotherapeutic manufacturing [20].

A representative integrated workflow for rAAT purification begins with an initial capture step using anion exchange chromatography (AEX), which binds AAT from the clarified CHO cell culture supernatant and achieves substantial volume reduction and removal of major host cell protein contaminants. This is followed by an intermediate purification step, often employing either IMAC (for His-tagged constructs) or a second IEX step in orthogonal mode (e.g., CEX following AEX), to further reduce process-related impurities including residual HCPs and nucleic acids. The workflow is completed by a final polishing step using SEC, which removes protein aggregates and ensures the homogeneity of the monomeric rAAT product [20].

Recent advances in continuous and integrated bioprocessing have begun to transform conventional batch chromatography workflows. Periodic counter-current chromatography (PCC) and simulated moving bed (SMB) chromatography enable continuous processing with improved resin utilisation and higher volumetric productivity compared to traditional batch-mode IEX [20]. In addition, the inclusion of PAT techniques within the process, such as in-line UV and conductivity measurements, allows for more precise fractionation control and minimizes batch-to-batch variation, which is an important quality attribute in biopharmaceuticals.

Membrane chromatography, the use of functionalized anion-exchange or mixed-mode membranes has also gained importance as an effective alternative to bead-based resins in flow-through modes for removing trace HCPs and endotoxins during polishing.[20]. The high convective stream of the biomolecules like nutrients and the recombinant proteins would be achievable with membrane adsorbers, combined with their disposable format, offer practical advantages in terms of process speed, elimination of cleaning validation, and reduced risk of cross-contamination in multi-product manufacturing facilities.

Together, the incorporation of orthogonal chromatographic techniques, continuous processing systems, and sophisticated analysis constitutes an effective method for producing rAAT on a commercial scale with efficiency, reliability, and economic viability. The optimization of each step with respect to buffer conditions, loading density, elution gradients, and step yield is essential to achieving a final product that meets the purity, potency, and safety requirements stipulated by regulatory agencies for clinical application.

4. Conclusion

This article systematically reviews the application value of AAT in the treatment of autoimmune diseases and its recombinant protein purification technology. Studies have shown that AAT not only exerts its antiprotease effect by inhibiting neutrophil elastase but also downregulates the expression of pro-inflammatory factors such as IL-1β, TNF-α, and IL-6, promotes regulatory T cell proliferation, reduces oxidative stress damage, and protects pancreatic β-cells from inflammation-induced apoptosis, thus demonstrating significant immunomodulatory potential in autoimmune diseases such as type 1 diabetes. Meanwhile, the CHO cell expression system can achieve N-glycosylation modification closely similar to natural AAT, providing a reliable platform for obtaining recombinant AAT with good biological activity and in vivo stability. Through the combined application of various purification techniques such as ion exchange chromatography, affinity chromatography, and gel filtration chromatography, high-purity, high-activity rAAT can be prepared, meeting the requirements of biopharmaceutical production. Overall, CHO-derived rAAT has advantages such as scalable production and well-defined quality control, making it an important candidate biological agent for the treatment of autoimmune diseases. Further optimization of continuous purification processes, in-depth analysis of the relationship between glycosylation structure and immune regulation function, and more clinical studies are needed to verify its safety and efficacy, so as to promote the clinical translation and industrial application of rAAT.

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