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"Enzyme -responsive drug delivery for system for inflammatory diseases"

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ABSTRACT :

The immune system's complex biological reaction to pathogens (such bacteria and viruses), cellular damage, poisons, and natural or artificial irritants is known as inflammation. This defence system is crucial for removing damaged cells, getting rid of the injury's source, and starting the healing process. Although inflammation is an essential part of the body's defence and healing process, its dysregulation can have harmful effects and contribute to a number of acute and chronic illnesses, including cancer, autoimmune disorders, metabolic syndromes, cardiovascular diseases, neurodegenerative conditions, and other systemic complications. Pharmacological medicines used to treat inflammatory illnesses include corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), antihistamines, biologics, and colchicine. Nevertheless, these traditional therapies frequently have drawbacks, such as negative side effects, long-term toxicity, and medication resistance. On the other hand, because of their high specificity, catalytic efficiency, and capacity to modify inflammatory pathways with fewer adverse effects, enzyme-based therapies have become a viable substitute. Their therapeutic potential is further enhanced by their function in tissue regeneration and repair. The majority of naturally occurring anti-inflammatory enzymes are hydrolases like trypsin, chymotrypsin, natto kinase, bromelain, papain, serrati peptidase, collagenase, hyaluronidase, and lysozyme, as well as oxidoreductases like catalase and superoxide dismutase. Botulinum neurotoxin type A (Bo NT/A) and Tobacco Etch Virus (TEV) protease are two examples of engineered enzymes that have shown great promise in targeted anti-inflammatory treatments. Their use in the treatment of inflammatory illnesses has been further increased by recent developments in enzyme engineering, nanotechnology-based enzyme delivery, and biopharmaceutical formulations. This article offers a thorough summary of the formulations of both natural and artificial enzymes used as anti-inflammatory treatments. It discusses their mechanisms of action, clinical uses, and possible future breakthroughs in enzyme-based biomedical therapies, highlighting improvements in stability, effectiveness, and specificity as well as reduced immunogenicity.

Keywords: inflammation; therapeutic enzymes; pro-inflammatory mediators; oxidoreductases; hydrolases; protease; engineered enzymes

1] INTRODUCTION :

Biological systems use enzymes as chemical catalysts. They enable organisms to efficiently and selectively catalyse vital metabolic activities as well as self-replicate. With the exception of ribozymes, a tiny class of RNA molecules with catalytic activity, all enzymes are proteins. These proteins can distinguish between substrates with similar structures due to their great specificity. They also have a remarkable catalytic power that speeds up the desired chemical reactions. Biochemical reactions are catalysed in aqueous solutions with extremely mild pH and temperature conditions. They are essential for the diagnosis and treatment of many different illnesses and conditions. Hydrolases, oxidoreductases, isomerases, lyases, transferases, ligases, and translocases are the seven functional divisions into which the International Union of Biochemistry and Molecular Biology (IUBMB) divides enzymes.

One of the most promising therapeutic approaches in pharmacology and medicine is the use of enzymes, which provide site-specific, effective, and targeted treatment methods that are commercially viable. Because of their great substrate specificity, metabolic and physiological processes can be precisely modulated to restore cellular equilibrium. Favourable kinetic characteristics, such as a low Michaelis constant (Km) and a high maximum velocity (Vmax), are crucial for effective enzyme action at low substrate concentrations. Therapeutic enzyme applications, however, are frequently limited by issues such high production costs, low stability, immunological reactions, and a short in vivo half-life, notwithstanding their benefits. Recombinant DNA technology has been used to create high-purity, contaminant-free enzymes in order to get around these restrictions. The medicinal efficacy and economic scalability of these recombinant enzymes are improved by their expression in genetically modified bacterial, yeast, plant, mammalian, and insect cell systems. Nowadays, therapeutic enzymes are widely used in a variety of medical applications, such as anticoagulant, antibacterial, anti-inflammatory, and anti-cancer therapies; they are also used to treat fibrinolysis, metabolic storage disorders, and mucolytic diseases. The therapeutic enzyme market is expected to grow at a compound annual growth rate (CAGR) of 12.6% from its 2023 valuation of USD 7322.4 million to USD 16,750 million by 2030. These enzymes are used to treat a variety of illnesses using a variety of administration methods, such as oral, injectable, and topical formulations, either alone or in conjunction with other treatments.

2] THERAPEUTIC ENZYMES :

Therapeutic enzymes are biocatalyst medicines that catalyse the conversion of target substrates into their appropriate products by binding to them with high affinity and specificity. Therapeutic enzymes have been developed over the last 50 years to treat a variety of illnesses, such as cancer, digestive disorders, acute poisoning, inherited enzyme deficiency syndromes, and cardiovascular ailments. In order to improve protein stability, lower immunogenicity, lessen renal ultrafiltration, and occasionally allow the enzyme to be targeted to the proper cellular compartment, chemical modifications of the native enzyme (such as conjugation with polyethylene glycol) are frequently used in the manufacturing process. Nevertheless, parenterally or intramuscularly delivered therapeutic enzymes still have limited metabolic and clinical efficacy. Short circulatory half-lives, hypersensitivity reactions, and immunogenicity are the main causes of this. The physicochemical properties of the protein, the delivery method, the level of exposure, and the use of immunosuppressive medications during administration are some of the variables that affect immune responses. Additionally, host factors may be involved. For instance, people with congenital enzyme deficits may not be able to identify a therapeutic protein as "self" and may be more susceptible to developing an immune response while receiving a therapeutic replacement enzyme. An immunological response to a therapeutic enzyme can result in anything from severe, perhaps fatal diseases to a brief development of antibodies with no clinical after effect.

On the other hand, enzyme inhibitors have been created to alter the activity of enzymes for medical reasons. In HIV treatment, protease inhibitors like ritonavir and saquinavir target viral protease to stop viral maturation and replication, hence delaying the course of the disease. The therapeutic potential of

enzymes has been further increased by the development of enzyme-based gene-editing methods like CRISPR-Cas9. CRISPR-based methods have potential for treating inherited diseases and creating tailored health plans since they allow for precise genomic alterations. Enzymes are also essential biomarkers for monitoring and diagnosing diseases. Elevated blood serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are suggestive of liver injury and inflammation, offering important information on hepatic function and the course of the disease. Enzymes have demonstrated enormous promise in controlling inflammatory reactions beyond their conventional uses.

3] INFLAMMATION AND DISEASE :

Patients with bacterial, viral, fungal, or parasitic infections, anaphylaxis, environmental diseases (smoke inhalation, asbestos exposure, etc.), rheumatoid arthritis, gout, autoimmune diseases, intestinal diseases, endocrinological or autoimmune diseases, and chronic conditions like diabetes all exhibit inflammation (see, for instance, any internal medicine textbook). However, it has also been clear over the last three decades that a far wider range of illnesses exhibit distinctive cellular and molecular signs of inflammation. These include myocardial ischemia, acute cerebral stroke, persistent Alzheimer's disease, chronic arterial and venous illness, and, more recently, cancer and arterial hypertension. Clinical observations and, more recently, molecular evidence of inflammation in osteoarthritis have been present for several decades. Shock and multiorgan failure, which have one of the highest mortality rates, are associated with a severe form of inflammation. Patients with depression may exhibit indicators of inflammation even if they do not have overt inflammatory symptoms or are just exposed to environmental dangers. There is a long and expanding list of illnesses linked to molecular indicators of inflammation. The c-reactive protein (cRP), which is synthesized during inflammation in the liver along with other proteins like fibrinogen, is one of the effective and affordable measurements that have been introduced to detect signs of inflammation in the plasma of larger groups of patients. Since then, a wave of large-scale clinical studies has provided supporting evidence for the earlier smaller experimental studies (30–33). Inflammation is statistically associated with obesity (34, 35) and infrequent (moderate but not extreme) exercise (36, 37). Additionally, inflammation is a major concern in biomaterials that deal with the interactions between living tissue and non-living implants or living grafts (38, 39) as well as in the creation of blood substitutes (40). Inflammation is therefore a major problem in tissue engineering. The holy grail of human disease research today is inflammation. There may be further options for intervention if anti-inflammatory therapies that have been demonstrated to be successful in one condition also prove to be successful in another.

The role that chronic inflammation plays in the onset and spread of cancer is among its most worrisome effects. By encouraging genetic abnormalities, epigenetic changes, and a microenvironment that supports cancer cell survival, chronic inflammatory activities aid in the development of tumours. Reactive oxygen species (ROS) and cytokines, which can damage DNA and cause carcinogenic mutations, are released by inflammatory cells. Although genetic abnormalities are the main cause of cancer, environmental exposures (such as UV radiation and smoking), viral infections (such as hepatitis B and C viruses and the human papillomavirus (HPV)), and lifestyle variables (such as nutrition and obesity) also play important roles in the development and progression of cancer. Treatment outcomes may be impacted by the intricate interactions between inflammation and cancer, which can have both tumour-promoting and tumour-suppressive effects. By promoting tumour invasion, angiogenesis, and immune evasion, enzymes such matrix metalloproteinases (MMPs), DNA repair enzymes, and cyclooxygenases (COX-2) aid in the development of cancer. Similarly, the inflammatory signalling pathways that propel disease pathology involve COX-1 and 2, lipoxygenases (LOX), nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, nitric oxide synthases (NOS), and a variety of proteases.

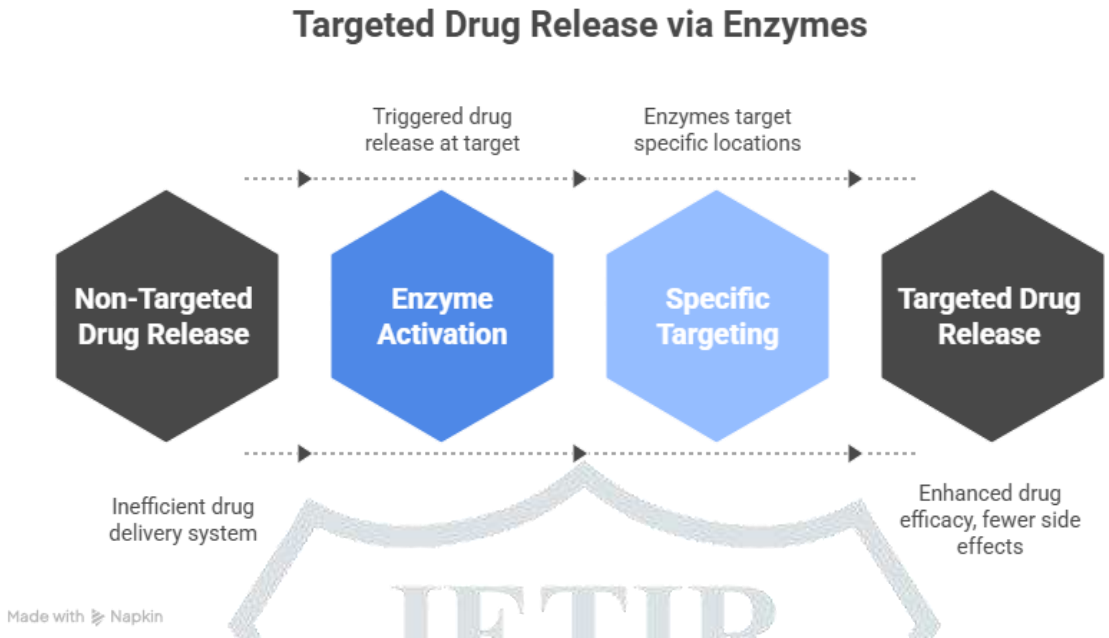
4] Biotechnological Strategies to Enhance Enzyme Therapeutics :

Therapeutic enzymes are a promising class of biologics that can be used to treat a variety of ailments as more targeted and natural substitutes for synthetic medications. However, a number of significant

obstacles, including as immunogenicity, restricted shelf-life stability, inadequate therapeutic efficacy, and issues with cost-effective scalability, restrict their wider clinical implementation. Enzymes that are immunogenic, especially those that come from non-human origins, might cause immunological reactions and the production of neutralizing antibodies, which lowers the effectiveness of treatment. PEGylation, the use of human or humanized enzyme homologs, site-directed mutagenesis-based epitope engineering, nanocarrier-based enzyme encapsulation, and co-administration with immunosuppressants are some of the methods that have been developed to overcome these obstacles. Stabilization methods like lyophilization, the addition of stabilizing excipients (sugars/polyols, amino acids, proteins, polymers, reducing agents, surfactants, cryoprotectants, antioxidants, chelators, buffers, and/or salts), bioconjugation, and protein engineering have been used to improve the enzyme's thermal and structural stability to address shelf-life stability, which is frequently compromised by environmental factors like temperature, pH, and oxidant stress.For example, PEGylation of superoxide dismutase (PEG-SOD; pegorgotein) significantly enhanced its thermal stability, circulatory half-life, and reduced antigenicity and immunogenicity .

Enzyme bioavailability and site-specific action have been increased through the use of strategies such targeted delivery systems, controlled-release formulations, and combination therapy with traditional anti-inflammatory medications. For instance, by covalently conjugating the enzyme with mannose-6-phosphate-functionalized glycol polypeptides (M6P-GP) to enable targeted lysosomal trafficking, Padhyet al. developed a novel lysosome-targeting β-glucosidase delivery system using protein engineering and bioconjugation. Likewise, Gui et al. created a delivery strategy for antioxidative nanoparticles employing SOD-loaded porous polymer nanoparticles (SOD-NPs), which showed preferential accumulation and extended retention in the synovium of murine knee joints. High-yield recombinant expression systems, such as prokaryotic hosts like Escherichia coli and eukaryotic platforms like yeast (Pichia pastoris), plants (Nicotiana tabacum, Oryza sativa, Zea mays), and mammalian cell lines (such as Chinese Hamster Ovary (CHO) cells), have been widely used to enable cost-effective scalability. These technologies make it easier to produce enzyme therapies on a large scale in a way that is profitable when paired with efficient fermentation and downstream purifying procedures. One prominent example is Taliglucerase alfa, an enzyme replacement treatment for Gaucher illness made via a plant expression system based on carrot root cell culture, which is a groundbreaking method in plant cell-expressed biotherapeutics. The therapeutic potential, stability, efficacy, and manufacturability of enzyme-based anti-inflammatory medicines are all greatly improved by these biotechnological treatments taken together.

5] TRAGETED DRUG RELEASE VIA ENZYME :



Enzyme-triggered drug release, also known as enzyme-responsive drug delivery, employs the overexpression or distinct presence of particular enzymes in sick tissues (such as tumours, inflammatory regions, or infections) as a stimulus to cause a medication to be released from a prodrug or carrier.

For example, a carrier (nanoparticle, liposome , hydrogel) may include a linker or coating that is cleaved by a target enzyme, leading to release of the therapeutic agent at the target site .This approach enhances specificity (less off-target release) and can improve the therapeutic index of the drug.

5.1] Why use enzymes as triggers

Many diseases have characteristic enzyme profiles (e.g., certain proteases in tumours , bacterial enzymes in infection sites).

Enzymatic cleavage occurs under physiological conditions (body temperature, aqueous environment), so you don't necessarily need external stimuli (light, heat).

By linking drug release to enzyme activity, you gain spatial & temporal control — ideally the drug releases only where/when the enzyme is active.

Numerous studies confirm enzyme-responsive systems can increase on-site drug concentration and reduce systemic side-effects. For example, one review states: > "Enzyme-activated prodrugs … have stronger specificity … therefore these prodrugs have greater development potential."

5.2] Key design strategies & mechanisms

1. Cleavable linkers / bonds

Use of enzymatically labile bonds (peptide sequences, glycosidic linkers, esters) that are stable in circulation but cleaved by the target enzyme. For example, a review classifies various enzyme-sensitive bonds used in anti-tumour DDSs.

Example: A polymer prodrug of the anti-inflammatory drug indomethacin that is released under stimulation by cholesterol esterase.

2. Carrier destabilization by enzymes

Nanoparticles or liposomes are designed such that enzyme action destabilizes the carrier (e.g., cleaves a coating, degrades a crosslink) triggering the drug release. Example: Liposomal hydrogel for infected wound, where Phospholipase A_2 (PLA_2) from wound exudate hydrolyses lecithin in the liposome, releasing curcumin.

Another example: Liposomes loaded with antimicrobial peptide derivatives that convert from non-membrane-damaging to membrane-damaging peptides upon enzyme action, liberating payload.

3. Pro-drug activation via enzyme

The drug is masked/inactive until the enzyme reacts and "uncages" it (or converts it to active form). For instance, enzyme-activated prodrugs for cancer therapy: review summarises "enzyme reduction, enzymatic hydrolysis, enzyme-activated and nanoparticle-assisted release" mechanisms.

4. Multi-trigger or sequential triggers

Some systems combine enzyme-trigger with other stimuli (e.g., light, pH) for layered control. Example: A nano-system that uses a DT-diaphorase-responsive group plus light to release methotrexate selectively in tumour cells.

6] ANTI-INFLAMMATORY THERAPEUTIC ENZYMES :

Proteolysis of inflammatory mediators and immune complexes, modulation of cytokine networks, fibrinolysis to decrease oedema and enhance microcirculation, and reduction of oxidative stress via enzyme-mimics are some of the ways that proteolytic and other enzyme-based therapies are being studied to reduce inflammation. Plant proteases like bromelain and microbial enzymes like serra peptise are examples of enzyme therapies, as are pharmaceutical formulations that combine many enzymes (systemic enzyme therapy) and sophisticated designed enzyme-mimics (nanozymes/cluster zymes). Although there are few large, high-quality randomized controlled trials, clinical evidence indicates encouraging indications in certain musculoskeletal and post-operative scenarios. Oral bioavailability, immunogenicity, batch consistency, coagulation interactions, and regulatory classification (drug vs. supplement) are important translational concerns.

6.1] Mechanisms of anti-inflammatory action :

6.1.1] Proteolytic degradation of inflammatory mediators and debris

Proteases can digest circulating immune complexes, fibrin deposits and extracellular matrix fragments that sustain inflammation. By cleaving pro-inflammatory cytokines or activating/inactivating complement components, they may attenuate signalling cascades.

6.1.2] Modulation of cytokine networks and immune cells

Some enzymes—through direct or indirect actions—appear to reduce levels of cytokines such as TNF-α, IL-1β and IL-6, or shift macrophage/neutrophil activation states. The exact mechanisms vary by enzyme and remain incompletely characterized.

6.1.3] Fibrinolysis and improved microcirculation

Proteolytic enzymes that degrade fibrin and fibrinogen can reduce local microthrombi and interstitial protein deposition, improving capillary flow and reducing oedema.

6.1.4] Antioxidant enzyme mimics (nanozymes / cluster zymes)

Engineered nanoparticles or metal clusters can mimic superoxide dismutase, catalase, or glutathione peroxidase activities, reducing reactive oxygen species (ROS) that contribute to inflammatory signalling and tissue injury.

6.1.5] Extracellular matrix modulation and tissue remodelling

Enzymes such as collagenase or specialized proteases can modify the extracellular matrix to reduce fibrosis or scar tissue that fuels chronic inflammation.

6.2] Representative therapeutic enzymes and formulations

6.2.1] Bromelain (pineapple stem enzyme)

- A mixture of proteases obtained from Ananas comosus.
- Studied for anti-inflammatory, anti-oedema, and mucolytic properties. Clinical applications explored: osteoarthritis, sinusitis, post-operative swelling.
- Proposed actions: proteolysis of inflammatory mediators, modulation of immune cell function, enhancement of drug absorption.

6.2.2] Serrapeptase (serrati peptidase)

- A proteolytic enzyme originally from Serratia species. Marketed in some countries for reduction of inflammation, pain and mucus. Evidence is mixed and often derived from small trials or non-randomized studies.

6.2.3] Trypsin / Chymotrypsin and combination systemic enzyme formulations

- Enzymes of pancreatic origin or from other sources used in combination products (sometimes branded, e.g., multi-enzyme systemic therapy). These are promoted for systemic anti-inflammatory effects and recovery after surgery/trauma.

6.2.4] Collagenase and other matrix-degrading enzymes

- Collagenase is used clinically (e.g., injections for Dupuytren's contracture, Pyronine's disease) and has implications for fibrosis and chronic inflammation where extracellular matrix breakdown is therapeutic.

6.2.5] Engineered enzyme mimics (nanozymes / cluster zymes)

- Nanoparticles or atomically defined clusters that catalytically detoxify ROS or reactive nitrogen species to reduce oxidative-stress-driven inflammation. These are largely preclinical but show promise in neuroinflammation and ischemia models.

7] CURRENT CHALLENGES IN ENZYME THERAPIES :

1] Poor Bioavailability and Stability

2] Short Circulatory Half-life

3] Immunogenicity and Allergic Reactions

4] Lack of Target Specificity

5] Manufacturing and Quality Control Issues

6] Limited Clinical Evidence and Regulatory Approval

7] Interaction with Other Drugs and Coagulation Pathways

8] Cost and Scalability

9] Ethical and Pharmacovigilance Concerns

8] FORMULATIONS AND DELIVERY STRATEGIES :

1. Enzyme encapsulation & conjugation

- Encapsulating enzymes (e.g., antioxidant enzymes such as Superoxide dismutase (SOD) and Catalase (CAT)) in nano-carriers protects them from degradation, enhances stability, and prolongs circulation. For example, SOD + CAT loaded nanocarriers showed improved tissue regeneration and inflammation reduction in an IBD model.

- Conjugation of enzymes to polymers (e.g., PEGylation, block-copolymers) aids in reducing immunogenicity and improving pharmacokinetics: e.g., SOD1 conjugated to amphiphilic poly(2-oxazoline) block copolymer showed enhanced brain/neuronal uptake.

- "Enzymosomes" – liposomes or lipid vesicles with enzymes either encapsulated or covalently bound to the vesicle surface – are used for site-specific delivery of enzyme therapeutics.

2. Nanoparticles / nanozymes and enzyme-mimicking materials

- Nanozymes or enzyme-mimetic nanoparticles (e.g., metal or metal-oxide nanomaterials with SOD/CAT-like activity) serve as stable alternatives to protein enzymes, suitable for oxidative-stress mediated inflammation.

- Polymer- or lipid-coated nanoparticles designed for anti-inflammatory delivery: e.g., hydrogel nano-emulsion systems for inflammatory bowel disease combining anti-inflammatory drugs and delivery vehicle.

3. Stimuli-responsive / enzyme-responsive carriers

- Delivery systems that respond to the presence of specific enzymes (overexpressed in inflamed tissue) to trigger drug (or enzyme) release. For example, enzyme-sensitive linkers or coatings degrade when target enzyme is present, enabling site-specific release.

- Hydrogels and polymeric delivery systems tailored for anti-inflammation & tissue repair: e.g., hydrogel systems for intestinal repair in IBD that combine drug delivery + anti-inflammatory effects + tissue repair.

4. Oral/targeted administration and local delivery

- For diseases like gastrointestinal inflammation (e.g., colitis), oral enzyme capsules or nano-formulations that allow enzyme accumulation in the inflamed tissue (colon) have been developed. Example: enzyme capsule (SOD/CAT) for oral administration in DSS-induced colitis mice, with ROS scavenging and cytokine inhibition.

- Local administration (injection, topical) of enzyme formulations for joint/muscle inflammation, or hydrogels applied locally for wound inflammation. Though specific reference in the source is less detailed, the strategy is implied in enzyme therapeutic reviews.

5. Protein engineering and formulation synergy

- Engineering enzymes for enhanced stability, modified substrate specificity, lower immunogenicity, and then formulating them in delivery systems is key. For instance, engineered enzymes are described in the anti-inflammatory therapeutic review.

- Combining enzyme therapy with delivery systems: e.g., enzyme therapy plus nanocarrier to deliver to specific cell types, or enzyme + drug co-delivery systems.

9] CONCULSIONS AND FUTURE PRESPECTIVES :

The development of enzymatic therapeutics has progressed alongside advancements in next-generation biomedicine. While the microbial production of anti-inflammatory enzymes has provided valuable therapeutic options, challenges such as immunogenicity have limited their widespread clinical use. As a result, human-derived enzymes have gained preference, particularly in enzyme replacement therapies, such as oxidase alfa for sphingomyelinase deficiency and Aval glucosidase alfa for glycogen storage disorders. Re combining enzymes produced via genetically modified organisms have also been explored; however, with the advent biologics, including monoclonal antibodies and small-molecule drugs, enzyme-based therapeutics have seen a relative decline in clinical applications. Recent advancements in anti-inflammatory enzyme production technologies, particularly using mammalian cell cultures such as Chinese hamster ovary (CHO) and human embryonic kidney (HEK) cells, offer promising avenues for generating human-like recombinant enzymes with enhanced stability, activity, and reduced immunogenicity. Combining enzyme engineering strategies with these production platforms can facilitate the development of highly efficient anti-inflammatory enzymes with optimized pharmacokinetic and pharmacodynamic properties. The combination of rational protein design, directed evolution, and post-translational modifications is crucial for overcoming challenges associated with enzymatic degradation, stability, and targeted therapeutic delivery. Moreover, innovative strategies for deimmunization and immunoreaction are required to enhance the therapeutic potential of non-human anti-inflammatory enzymes. For instance, epitope engineering has shown promise in reducing immune responses, as demonstrated by an engineered lysostaphin variant that elicited diminished antibody responses in human leukocyte antigen (HLA) transgenic mice . However, further clinical evaluations are necessary to validate these findings. PEGylation remains a widely employed technique for reducing immunogenicity, extending half-life, and minimizing the rapid clearance of therapeutic enzymes. However, the emergence of anti-PEG antibodies poses a significant challenge, necessitating alternative approaches such as glycoengineering and site-specific conjugation strategies [239]. To address limitations related to immunogenicity, target specificity, stability, and therapeutic efficacy, the development of antibody-enzyme conjugates (AECs) represents a promising approach. These conjugates involve linking enzymes to antibody fragments (Fab), Fc regions, or full monoclonal antibodies (abs) to enhance targeted delivery and bioavailability. Notably, Pallion Pharmaceuticals has pioneered the development of E-602, a first-in-class engineered bi-sialidase-Fc fusion enzyme designed to degrade sialic

acid-containing glycans on T-cell surfaces, thereby promoting immune activation. This innovative therapy is currently undergoing a phase II clinical trial for lupus nephritis . In conclusion, overcoming key challenges such as immunogenicity, shelf-life stability, therapeutic efficiency, and cost-effective scalability through advanced protein engineering, rational design, and directed evolution strategies can unlock the vast therapeutic potential of both human and non-human anti-inflammatory enzymes. By addressing these critical aspects, enzymatic therapeutics for inflammatory diseases hold immense promise for Pharmaceutics 2025, 17, 606 29 of 38 revolutionizing modern biomedicine, offering safer and more effective treatment modalities for a wide range of inflammatory disorders

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