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RESEARCH ARTICLE

Enzymosomes as lipid bio carrier for targeted drug delivery system

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ABSTRACT

Enzymosomes are specially engineered lipid structures that provide a suitable microenvironment for the enzyme to be immobilised inside lipid structures or covalently coupled to the lipids' outer surfaces. Its main use is to make permeation easier as well as to deliver targeted drugs to tumorous cellular sites. These vesicular systems provide a great deal of versatility in drug development, allowing for the elimination of a variety of side effects and bioavailability issues. The use of nano systems to deliver drugs to the CNS through the BBB is a promising lead for improving treatment methods for various types of malignancies. Enzymosomes, including ethosomes, transferosomes, pharmacosomes, virosomes, and non-lipoidal ones like niosomes, aquasomes, and others, are lipid bio carriers. Various studies have shown that each carrier is successful in its operation. Enzymosomes can thus serve as a assuring carrier nucleus in the development of a new generation of flexible drug design systems in the future.

INTRODUCTION

Enzymes play roles in catalysis, site-specific pharmacological action, and the activation of prodrugs, among other things. However, their low lipid membrane penetrability is a major disadvantage; if an enzyme is encased on the liposome's surface, degradation and transmutations may be reduced, extending the enzyme's half-life and allowing for targeted action [1]. Enzymosomes are a novel drug delivery mechanism that can deliver the active form of a drug to the site of action while also rapidly degrading it to allow for easy uptake [2].

Enzymes can be bound to the liposome surface in two ways: by connecting functional hydrophobic compartments with the enzyme, such as long chain fatty acids, or by associating the enzyme with the liposome layer's phospholipids. Carrying the therapeutic agent to the destined tissue receptors, particularly seen on an organ or system, is visualised using the rational approach to improve therapeutic effectiveness and reduce side effects. For the sheathed drug molecule, such mutated vesicles enhanced solubility, stability, and therapeutic index [3].

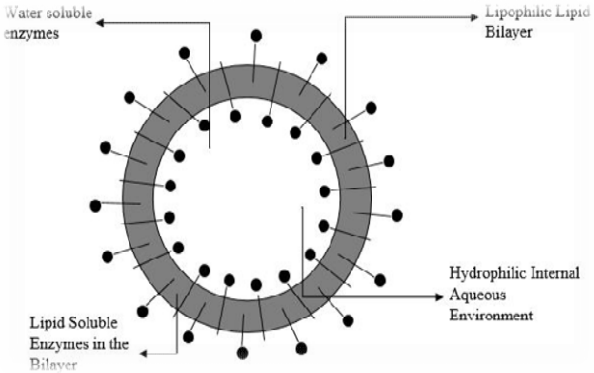

Figure 1: Enzymosomes

Novel drug delivery systems (NDDS) are being used in a variety of sectors, including pharmaceutical, food, and cosmetics. It primarily achieves and overcomes the limitation of traditional drug delivery systems, namely, delivering the entire active moiety of the drug to the site of action.

It also transports the drug through the body's proper channels at a rate determined by the body's needs. Vesicular carriers are the most recent vehicle of choice, for most of the drugs with bioavailability issues, and also include cells and genetic modification, diagnostic tests, and immunology techniques. Vesicular drug delivery aids in the maintenance of drug release at a predetermined rate, reducing the risk of drug toxicity and thus becoming useful in the treatment of ocular diseases.

Enzymosomes are a novel targeted vesicular drug delivery mechanism that is currently in development. Enzymosomes are essentially enzymes with a specific catalytic role for a substrate that are integrated into cell-like structures with a high lipid history. They produce newly engineered liposomes in which the enzymes are covalently attached to the lipid molecules' surfaces. The liposomes were designed in such a way that the enzymes inside them would be incapacitated. These vesicles have a user-friendly structure and can hold both hydrophilic and lipophilic drugs. Most carriers have orderly concentric congregates of lipid bilayers in which different sites are used to enclose hydrophilic and lipophilic drugs, eventually resulting in amphiphilic form. Bingham first announced the vesicles in 1965, referring to them as Bingham bodies. By spatial induction of drug bordering the diseased organ or tissue, especially by chemical derivatization, the novel carriers localise their action. Various treatments for glaucoma, ulcerative colitis, colon disorders, NSAIDS, and insulin-like drugs have been shown to have increased bioavailability and duration of action in humans in studies. Vesicular drug delivery lowers treatment costs and improves pharmacodynamics, especially for poorly wetting drugs. It has the potential to set significant milestones in traditional chemotherapy approaches, where drug penetration and cell permeability are severely limited [4].

Enzymosomes-based Drug Delivery:

One use of these lipid nanocarrier systems is in the treatment of central nervous system (CNS) disorders such as epilepsy and seizures, as they act as natural attractants of the blood brain barrier (BBB) due to their lipophilicity. The enzymes, which function as therapeutic proteins, are delivered through polymeric carriers such as liposomes and lipoplexes, with the attachment of enzymes to exposed areas of liposomes showing the best results. The vesicular delivery system loaded with drugs produces accurate results at the site of infection or inflammation, with minimal drug toxicity and side effects, making it effective for centrally acting drugs that must pass through the BBB, which is critical to the brain's homeostatic function [5]. It also helps to improve drug bioavailability, or the amount of drug concentration available for systemic circulation, by lowering the cost of purchase. When studied in vitro and in vivo, the covalently bound enzyme and liposome result in minimal changes in the enzyme's function, and the enzyme-loaded in a vesicle retains its structural integrity and enzymatic activity. Advances in the use of enzymosomes can be applied to a variety of areas, including the manufacture of new recombinant proteins, biotechnological products, and so on [6].

Preparation of Enzymosomes of Surface-Exposed Superoxide Dismutase (SOD):

While directly binding enzymes to the lipids of liposomes is a difficult task, it is currently used as a convincing method in the treatment of immune-mediated diseases or antibodies. SOD is an example of a direct conjugated therapeutic enzyme (Super Oxide Dismutase). Cu, Zn-superoxide dismutase (SOD) has been established as an innate defence mechanism that reduces the teratogenicity of toxic free radicals. It causes the toxic superoxide radical anion O_2^{2}_ to dismutate into O_2 and H_2O_2, disrupting a variety of biochemical inflammatory processes triggered by the free radical. By using non-steroidal anti-inflammatory drugs and avoiding their side effects, SOD tends to be a potential replacement for traditional anti-inflammatory therapies. Owing to its finite properties, short half-life in the bloodstream, and low penetration into cells, the enzyme was not clinically suitable [7].

Many studies were conducted to improve the substrate on which the enzyme was loaded with an improved half-life and liposomal incorporation performance, as well as clinical trials in particular risk groups such as obesity, Type 2 diabetes, and so on. Targeting protein to cell penetrating peptides (CPPs) or protein transduction domains is the most popular application for intracellular SOD delivery to date (PTDs). Regardless of the functional benefits of enzyme transduction technology, the main focus of this strategy is on the inefficient escape from the endosome to the cytosol, resulting in CPP-

tagged cargoes being trapped in intracellular vesicles. Proteins are essential for biological functions, and modern encapsulation techniques have made it possible to regulate the delivery of these peptides, allowing for their delightful use in the treatment of a variety of diseases [8].

Construction of Nanocarriers:

Polymer colloids that encompass nanocarriers are prepared using techniques such as carbon nanotubes and crosslinked nanogel matrices. The biological conditions as well as the intermolecular force of attraction determine how active these systems are. A nanocarrier must be able to transport drugs to the active site without deactivation, the drug must be released according to kinetic rules, the drug must be stable after administration, and the drug must be actively delivered with site specificity. Chemical ligation, either covalent or non-covalent, was used to modify the molecules. Another choice was for proteins and nanocarriers to self-assemble. Nanoparticles can be gathered from a variety of materials and arranged into desirable geometries and configurations, gaining useful functionalities and properties in the process. Conventional chemotherapy therapies have a number of side effects, as well as the failure to reach the disease's heart. As a result, nano-sized polymeric carriers are able to deliver drugs to cells selectively and precisely. Since the release of the enzyme from the liposomes at the site of inflammation may not be needed to achieve therapeutic action, launching SOD over the liposomal surface is thought to be more effective than encapsulation of the enzyme in liposomes.

The method of acylation by covalent linkage of palmitic acid to q-NH2 groups of SOD was used to achieve surface localization of SOD on enzymosomes (Ac-SOD). The researchers hypothesised that the procedure would produce a more hydrophobic enzyme with a higher affinity for liposomal bilayers. We also hoped to target SOD to inflammatory sites by exposing it to the external liposomal surface. Liposomes were prepared with a long circulation period for this study in order to achieve localization at inflammation sites and serve as an effective intracellular drug delivery system. Long-circulating liposomes (LCL) have been shown to preferentially localise at inflammatory sites following intravenous administration in these studies. This preferential localization may be due to an inflammatory response that causes increased capillary permeability in a specific area, enabling liposomes to pass through. As a result, LCL has promising prospects for delivering Ac-SOD to specific locations. To combine SOD and Ac-SOD, PEG-coated LCL (PEG liposomes) and non-PEG-liposomes containing stearylamine were used (SA-liposomes). Since enzymes are vulnerable to physical and chemical stress, such as thermal treatment, they are covered within lipid bilayers, where they preserve their native conformation. Incorporation performance, zeta potential, retention of enzymatic activity, and externally exposed enzyme activity were all assessed in both liposomal formulations [9]. The research done to prepare and optimise SOD enzymosomes was primarily to extend the time that human blood circulated and accumulate the enzyme at the target site, allowing the enzyme to remain flawless within the lipid structure. Therapeutic enzymes from the hydrophilic range are retained or encapsulated within the prepared vesicles' inner aqueous space. A liposome that has catalytic activity in its intact state, that is, until it is killed.

The enzymes could be bound to liposomes by two approaches:-

- By interacting with the enzyme's hydrophobic anchoring molecules, such as long chain fatty acids.
- By first connecting the enzyme to the phospholipid bilayer components of the liposome bilayer.

The enzyme is assimilated to the liposomal membrane in the first step, and the docking between the liposome bilayer and the binding reaction with the liposomal surface occurs in the next. Both the processes are having difference in;

- The stability of the enzyme-liposome complex.
- A number of enzyme molecules which are entrapped/displayed to the outer lipid bilayer.
- Since there are several molecules to which the enzyme could be docked, such as phospholipids, long chain fatty acids, or polymer-like substances attached to phospholipids, the essence of modified enzymosomes.
- The method of preparation is generally chosen based on the various cases of therapeutic needs requiring enzyme delivery through enzymosomes.

The enzyme in the interiors of the liposomes is not required for reaction when the liposomes are intact. However, when enzymes are directly bound to the lipid membrane, they are ready to catalyse long before the liposomes are destroyed. The process of acylation (adding of an acyl functional group) occurs during the unification of hydrophilic enzyme to the acyl residual chains of lipids, resulting in the formation of Ac-enzyme. As a consequence, the enzyme is diverted from a hydrophilic to a hydrophobic microenvironment. The length and number of fatty chains attached to the enzyme's surface influence the amount of hydrophobicity achieved. The conservancy of the changed enzyme's other equities must be assessed using simple conjugation strategies. When the active site of the enzyme is inhibited by the substrate during conjugation, the enzyme L-asparginase, which is used to treat acute lymphoblastic leukaemia, withholds 100% of its catalytic activity.

Different strategies are used to augment a fine dose of Ac-enzyme to the liposomal structure and allow proper enzyme release. Ac-enzyme is partially inserted into lipid bilayers or hidden within the hydrophobic lipid vesicular matrix. The electrostatic interactions between the charges associated with enzymes play a major role in the association of Ac-enzyme to the lipid bilayer membrane [10]. The ratio of catalytic values measured during the intact life cycle versus disrupted enzymosomes is used to calculate the efficiency of the Ac-enzyme incorporated into liposomes. The size of the vesicle, structure, ionic charge, and other ideal characteristics of liposomes are very useful during the construction of enzymosomes [11].

Acylated-sod Liposomes:

In general, SOD enzymosomes were obtained as a homogenous film by dispersing an aqueous solution containing SOD, and the non-bounded enzyme was then extruded using the ultracentrifugation separation process. The prepared enzymosomes were characterised by Gaspar MM et al's research work, which used various parameters to ensure that the enzymosomes were uniform in their characteristics. The different parameters included where;

- Liposome mean particle size (diameter) was analysed using dynamic light scattering.
- Protein coupling to liposomes was assessed after disruption of the liposomes with Triton X-100 and sodium dodecyl sulphate (SDS).
- Free amino acid groups and lipids were determined.
- Phospholipids by colourimetric assay.
- The enzymatic activity of Ac-SOD and SOD formulations was determined.
- The enzymatic ability to decrease the speed of autoxidation of epinephrine to adrenochrome was assessed.
- The total activity of enzyme within SOD or Ac-SOD enzymosome was determined by initially preparing a series of dilutions to obtain a final concentration of protein.
- The enzymatic activity of the enzyme exposed to the external surface was measured.
- Zeta potential was analysed.
- The electric field intensity and scattering angle were found.
- The thermotropic behaviour of the membrane phospholipids of enzymosomes was found.

Innovative methodologies for characterising Ac-SOD and determining whether it was superior to plain SOD and other pharmaceutical preparations were described as the results of various works in chemical alterations of SOD [12].

- The surpassing parameters for Ac-SOD evidenced higher affinity of it to the hydrophobic area of liposomal bilayer when compared to free SOD.
- The efficiency of Ac-SOD incorporation in SA-containing enzymosomes was compared to PEG-liposomes, which had a lower initial (protein/lipoprotein) ratio due to rivalry between Ac-SOD and cholesterol for phospholipid inclusion.
- The various electrostatic interactions indicated that positively charged SA-liposomes were beneficial because they decreased lipid-protein charged interactions due to PEG at the lipid surface.

As a result, Ac-SOD demonstrated significant activity for the integrated enzyme, which operates independently of the rate and extent of enzyme release, resulting in a distinct mechanism of action. Thus, the completed work confirms that the engineered enzymosome has considerable potential, as the PEG-enzymosome

converted the substrate even in the presence of surface PEG chains. As a result, it was not an obstacle, and the enzyme's release was not needed to produce dismutation action at the inflamed location. If the Ac-SOD enzymosome could be made with circular micro-reservoirs, it would be much more than adequate for expressing the enzyme's action without interruption and for a long-term release pattern. Peptides and polymers undergo similar modifications, forming an essential drug targeting pathway. As a result, the Ac-enzymosome may be a replaceable therapeutic agent for rheumatoid arthritis with a strong impact and longer-flowing active particles for reperfusion pathologies. SOD's affinity for negatively charged lipid molecules is thought to account for at least part of its ability to defend lipid membranes from oxygen-induced damage, and these molecules are useful tools for studying membrane structure and dynamics [13].

Designing of Immuno-Enzymosomes Having Enhanced Enzyme Targeting Capability and Cell Binding Properties:

Because of the inadequacy of differentiation between normal and cancerous cells, the efficacy of conventional anticancer drugs for chemotherapy treatment is restricted. After reviewing the research on immuno-enzymosomes, it was discovered that they could be used to target enzymes for site-specific activation of anticancer prodrugs [14].

The value of developing a single well-represented liposomal system that can bind to a variety of targeted ligands is high. These methods enable enzymes to be hidden within small packet-like structures and then released when they enter the action site. When combined with immunoliposomes, the enzyme -glucuronidase, which was capable of stimulating anthracyclineglucuronide prodrugs, was said to be effective against ovarian cancer cells (OVCAR-3). By cleansing the commercially available enzyme -glucuronidase (GUS), an immune-enzymosome formulation with a 2 fold increase in enzyme specific activity when incubated with ovarian cancer cells could be developed. The concept involves fusing a cell-specific antibody with a (liposome) immunoliposome enclosing the chemotherapeutic agent, resulting in the possibility of selective drug transmission and cell-specific cytotoxicity. As a result, new therapeutic protein derivatives with low immunogenicity are developed. The antibody-enzyme complex was given, followed by an injection of a non-toxic prodrug after the complex had bound to cancerous cells and had been cleared from the blood and tissues. The enzyme's activity targets the prodrug, which is converted to an active cytotoxic molecule within the confluence of tumour cells, resulting in its selective eradication [15].

Advantages:

- More than one enzyme moiety could be admitted in one targeted carrier system.
- Enhanced enzyme density at the cancer cell surface, thus providing efficient conversion of the prodrug.
- The enzyme GUS was preferred due to its superiority over other enzymes, which were present localized intracellular.
- They cause limited activation of hydrophilic glucoronide drugs since they have only low penetration. Thus lowering immunogenicity problem.

Because of its bulky steric hindrance, the GUS enzyme can often interfere with cell coupling. However, research has shown that simply rising the enzymatic density on the surface will result in significant enzymatic targeting. The electrosome was used in experiments by Szczupak et al. to release and act on a cascade of enzymes, overcoming the disadvantage of a small number of enzymes on the surface. GUS was purified first, and then Fab' fragments were made. The enzymosome or immuno-liposome was generated using these immuno-liposomes, and then characterised [16].

Generation of Streptavidin-Liposomal Conjugates For Targeted Ligand Specific Applications:

Streptavidin, a tetrameric biotin-binding protein isolated from Streptomyces avidinii, has a low level of nonspecific binding in immunohistochemistry. As a result, it's a must-have in a variety of detection systems. It is a member of the avidin family of antibiotic proteins, and its unique association with biotin molecules makes it useful in nonradioactive detection systems. Biotin is a vitamin that is needed by living cells for a variety of biological processes, including cell development. The biotin tag was previously used to aid the advancement of affinity purification of molecules using immobilised biotin-binding protein while biotin was attached to a molecule. Thus, the avidin-biotin interaction is used in ELISA,

immunohistochemistry (IHC), cell-surface labelling, and fluorescence-activated cell sorting (FACS), among other applications. Streptavidin attached to liposome results in a well-characterized protein-liposome conjugate under ideal conditions. The resulting targeted vesicle system has more activity in relation to its size and binds to biotinylated targeting ligands more strongly. In recent years, there has been increased interest in combining normal circulating cells with enzymes for drug delivery. Streptavidin is noncovalently coupled to biotin, a vitamin, and phosphatidylethanolamine, a phospholipid class, in the studies [17].

Because of its primary role in dissipating the negative charge formed by anionic membrane phospholipids, the phospholipid was chosen. The sample was prepared using the extrusion process, in which the lipid mixture was dissolved in a solvent and then coated over a tube with a dried to a thin film by moving a stream of suitable gas under high vacuum. The biotinylation process entails the covalent attachment of biotin to a protein or nucleic acid. Biotin in combination with streptavidin, a member of the avidin family, has a high affinity and fast action, making it useful for separating biotinylated compounds of interest in many fields of biotechnology. The liposome vesicles were incubated with streptavidin to achieve binding, and optimum coupling efficiency was obtained when a constant ratio of streptavidin to lipid molecules was preserved. Indirect targeting procedures take advantage of the efficient contact between streptavidin and biotin-containing lipid molecules.

Photoaffinity, for example, is used as an additional mechanism for molecular interactions and as a target for the binding site through light activation. The covalent binding of streptavidin to two lipid derivatives containing thiol groups to start the reaction was another approach explored in studies. The existence of long spacing reactive arms improved the cross-connection of maleimide derivatives of lipids in the research described in the reference article. The enzyme is then coupled to these liposomes and engineered to take advantage of its ability to conjugate in a particular manner with membrane-linked antigens, resulting in a variety of nanocarriers with useful physicochemical properties for drug delivery. The biotinated antibody was combined with enzyme-linked liposomes, which had a wide range of applications *in vivo* and *in vitro*. The preservation of hydrophilic drugs and fluorescent groups in water-loving compartments of targeted liposomes for cell surface activity is aided by such applications. Small-size conjugates have a longer half-life and thus maintain their potential and action longer in the plasma, varying the degree of infection and acute reactions [18].

Antiplatelet Activity of CD 39 Enzymosomes:

The body's endothelial cells contain the enzyme CD39/NTPDase-1, which is expressed on the cell's opening side. It has the physiological capacity to quickly metabolise ATP and ADP, as well as AMP, thus reducing platelet sensitivity to the main agonists. As a result, there was inspiration to investigate therapeutic anti-platelet 'enzymosomes' formulations containing CD39 embedded within liposome lipid bilayers [19]. Initially, CD39 enzymatic activity was optimised, which appears to be contingent on either of its trans-membrane domains being released. A yeast expression system was used as a model to produce full-length human CD39, which was purified and reconstituted inside a suitable lipid vesicle. The dephosphorylation and formation of ADP and ATP were used to establish the catalytic efficiency of detergent solubilized CD39 as well as when it was reconstituted inside a lipid membrane. Platelet aggregometry was used to determine the capacity of CD39-containing lipid vesicles to inhibit platelet activation induced by ADP, collagen, and thrombin *in vitro*. The efficacy and therapeutic applicability of intravenously administered CD39 enzymosomes in limiting platelet consumption and death were investigated using a murine model of thromboplastin-induced thromboembolism [20-22].

The reconstitution of human CD39 in lipid vesicles resulted in a nearly one-order reduction in the Km value, as well as an increase in both ADPase and ATPase catalytic efficiency. Platelet activation by ADP, collagen, or thrombin was effectively inhibited by CD39 lipid vesicles, which effectively inhibited platelet aggregation and created a platelet disaggregation response when platelets were enabled. Treatment with CD39 lipid vesicles significantly reduced the drop in platelet counts induced by thromboplastin, according to reports [23-25]. When compared to its solubilized equivalent, incorporating the enzyme into a lipid bilayer significantly increased CD39 enzyme activity. As a result, studies showed that CD39 enzymo-some treatment reduced platelet

consumption and death in an animal model. CD39 enzymo-somes may thus be a valuable therapeutic alternative for complementing other anti-platelet therapies in patients with platelet thrombus formation [26-29].

Applications of Enzymosomes:

Enzymosomes are a type of lipid nanoparticulate drug delivery system made up primarily of phospholipids organised in a bilayer shape that can hold any material, regardless of solubility, electric charge, or molecular weight, and thus enhance GIT absorption and oral bioavailability [30-32]. Enzymosomes can be loaded into lipid-based nanocarriers such as liposomes and solid-lipid nanoparticles, inorganic nanocarriers such as gold nanoparticles and magnetic nanoparticles, polymeric nanocarriers such as nanogels and micelles, and protein-mediated nanocarriers such as super positively charged proteins, among other materials [33-35]. Since the cell membrane has been used as the target for therapeutic intervention, one assuring a current collection of drugs without DNA interaction exists within ether and alkyl phospholipids. These were shown to be particularly effective in clinical trials for the treatment of metastases, breast cancer, anti-inflammatory action, and other conditions [36-37].

CONCLUSION

Enzymosomes take advantage of an enzyme's basic nature, which is to bind to a specific substrate at a regulated rate and catalyse the product development process. Enzymosomes are formed when an enzyme is covalently attached to the surface of liposomes/lipid vesicles. To prepare enzymosomes with targeted action, enzymes are linked by acylation, direct conjugation, physical adsorption, and encapsulation methods. Such experimental drug delivery technologies demonstrate successful drug release while also reducing the negative side effects of traditional treatment approaches, resulting in an increase in long-term disease therapy. They are a promising alternative to gout therapy, antiplatelet exercises, and other traditional therapies. Enzymosomes are newly engineered supramolecular vesicular delivery systems that can improve drug targeting, physicochemical properties, and thus bioavailability in pharmaceutics. It shows that drugs with a limited precision have beneficial effects because targeting these drugs to their site of action enhances their overall pharmacodynamics and pharmacokinetic profile. It also reduces changes in normal enzymatic activity, improving half-life and achieving enzyme activity on specific sites like cancerous cells.

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