

Biological Protein Nanostructures and Targeted Drug Delivery

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1. Introduction

Targeted drug delivery refers to the site-specific drug delivery that directs drugs mainly to certain cell types within a tissue and to certain molecular complexes or organelles within a cell while avoiding drug loading in nontargeted cells. Targeted delivery of drugs to specific cells involves the specific interactions between drugs or drug carriers and the cell-surface proteins through ligand–receptor interactions or antigen–antibody interactions. Targeted drug delivery to specific molecular complexes or organelles within a cell requires the specific interactions of drug with the targeted complexes to lead to the therapeutic effect. In the biological systems, these interactions generally occur on various types of biological nanostructures of protein origin. Understanding and utilization of these biological nanostructures could lead to significant improvement in drug targeting and drug carriers.

The biological protein nanostructures primarily include protein–lipid, protein–protein, protein–carbohydrate, and protein–nucleic acid complexes. Proteins, one group of the most important biological macromolecules in cells, are smaller nanoscale molecules with typical size range between 1 and 20 nm (1). Through sophisticated interactions with other biomolecules, these protein nanostructures are formed and widely distributed in human body. For example, low-density lipoproteins (LDL), with a diameter of 25–28 nm, are protein–lipid complexes. They are the major circulatory nanostructures in the blood. When used as a drug carrier, these protein–lipid complexes offer a certain advantage of being endogenous nanostructures that do not trigger immunological response. They can also escape the recognition and elimination by the reticuloendothelial system (RES). On the other hand, glycoproteins, i.e., protein-carbohydrated complexes, are vital structural and regulatory proteins in viruses and can serve as important therapeutic targets for anti-viral drug development. Telomerase, a protein nanostructure formed from protein and nucleic acid, is activated only in cell immortalization and cancer progression. Thus telomerase is an ideal therapeutic target for anti-cancer therapy. Because protein nanostructures are so critical to various biological and physiopathological activities, they have received wide attentions in recent years in the development of drug-targeting strategies, either as drug carries or as therapeutic tar-

gets. This chapter will focus on two aspects of biological protein nanostructures regarding their involvement in targeted drug delivery: (1) biological protein nanostructures as targeting drug carriers and (2) biological protein nanostructures as therapeutical targets for new drug development.

2. Protein–Lipid Nanocomplexes (Lipoproteins)

Lipoproteins are biological protein–lipid complexes in the nanoscale range. They have spherical shape consisting of a hydrophobic core and a polar shell that is incorporated with receptor-active proteins. The hydrophobic core contains triglycerides and cholesteryl esters, whereas the polar shell contains phospholipids, unesterified cholesterol, and one or several apolipoproteins. A schematic cross-section diagram of lipoprotein is shown in Fig. 1. Lipoproteins are commonly classified based on their densities, which can be determined through gradient ultracentrifugation. The classification thus is related to the respective amounts of lipid and protein in the complex. In an increasing order by density, lipoproteins include chylomicron, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL, and high-density lipoprotein (HDL).

Because lipoproteins are taken up in varying amounts through ligand-receptor interactions by different type of cells, they may be utilized as biocompatible nanoscale carriers for targeted drug delivery. For example, in hepatocytes, remnant receptor and asialoglycoprotein receptor can recognize chylomicrons and lactosylated HDL, respectively, in a molecular-specific manner. LDL receptor on the cell surface can specifically recognize LDL and its expression can be upregulated or downregulated depending on the type and state of these cells. By incorporating bioactive molecules into lipoproteins or modified lipoproteins, targeted drug delivery may be achieved, resulting in more bioactive molecules taken up by a select type of cells, e.g., cancer cells. Each class of lipoproteins has its unique biological property and thus can be utilized individually for targeted drug delivery.

2.1. Chylomicron for Drug Targeting

Chylomicrons are the largest lipoprotein complexes (80–500 nm) in the human body. Their main function is to transport dietary lipids from the intestine to the liver and adipose tissue. Assembled in the intestine from the absorbed dietary lipids and the apolipoproteins synthesized by the intestinal epithelium, they are transported out of the epithelial cells to the tissue fluid and further carried by lymphatic system for general circulation. When they enter the blood stream, their compositions of phospholipids and proteins are changed greatly through the hydrolysis of triglycerides and the component exchange with other lipoproteins in the plasma to form chylomicron remnants. Chylomicron remnants mainly are taken up by parenchymal cells in liver (2). When they are oxidized, chylomicrons can be taken up by liver endothelial cells and Kupffer cells (3). The uptake of chylomicron remnant by various cells is LDL receptor-mediated, which requires apoE protein as the ligand on the chylomicron-remnant particles (3,4).

When associated with chylomicrons, many lipophilic drugs and xenobiotics can be absorbed via the intestinal lymphatic system (5). This route can circumvent the first-pass effect in the intestine and, more importantly, can be used for drug targeting to liver cells because the liver is the destination of chylomicrons. Targeted drug delivery to the liver can help treat many critical diseases such as alcohol-induced liver disorders,

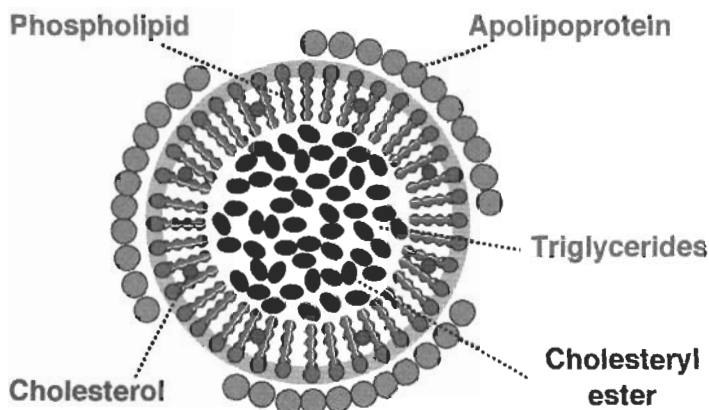


Fig. 1. Structure of lipoprotein.

chronic liver diseases and cirrhosis, virus-induced liver diseases (hepatitis), liver tumor, familial hypercholesterolemia, and type III hyperlipoproteinemia. To effectively treat viral infection, antiviral drugs or prodrugs can be incorporated into chylomicron and targeted to the liver. For example, by incorporating a nucleoside analog iododeoxyuridine into recombinant chylomicrons, the anti-viral drug was delivered selectively to liver parenchymal cells (6). Liver is also an excellent target for gene therapy for the diseases caused by metabolic defect. Gene therapy involving viral vector is in general limited by the high immunogenicity and poor safety profile. Utilizing the hydrophobic core of the chylomicrons, Hara et al. incorporated a hydrophobic DNA complex into reconstituted chylomicron remnants and the DNA was successfully delivered to liver cells (7,8).

2.2. VLDL for Drug Targeting

VLDL particles have a size range of 30–80 nm. They are assembled in the endoplasmic reticulum (ER) and matured in Golgi apparatus of hepatocytes before secretion (9). After entering into the plasma, VLDL particles are catabolized by a series of biochemical actions including apolipoprotein exchange with apoC-I, apoC-II, apoC-III, and apoE; lipolysis by triglyceride lipase; and cell-surface receptor-mediated uptake by cells. As lipolysis proceeds, VLDL particles become smaller and smaller and eventually are converted to IDL. Some of the IDL particles are rapidly taken up by hepatocytes via a receptor-mediated mechanism and others undergo further hydrolysis before being converted to LDL.

The catabolism cascade of VLDL suggests the possibility of using VLDL as a drug carrier for targeted delivery. Because some cancer cells overexpress the receptors for apoE, a protein ligand present on the surface of VLDL, VLDL can potentially serve as an antineoplastic drug carrier. In vitro experiments demonstrated that VLDL could effectively incorporate cytotoxic drugs, 5-fluorouracil (5-FU), 5-iododeoxyuridine (IudR), doxorubicin (Dox), and vindesine, and the resultant complex showed effective cytotoxicity to human carcinoma cells (10). By mimicking the composition and structure of VLDL, Shower et al. developed a VLDL-resembling phospholipid nanoemulsion system that could carry a new antitumor boron compound for targeted delivery to cancer cells (11).

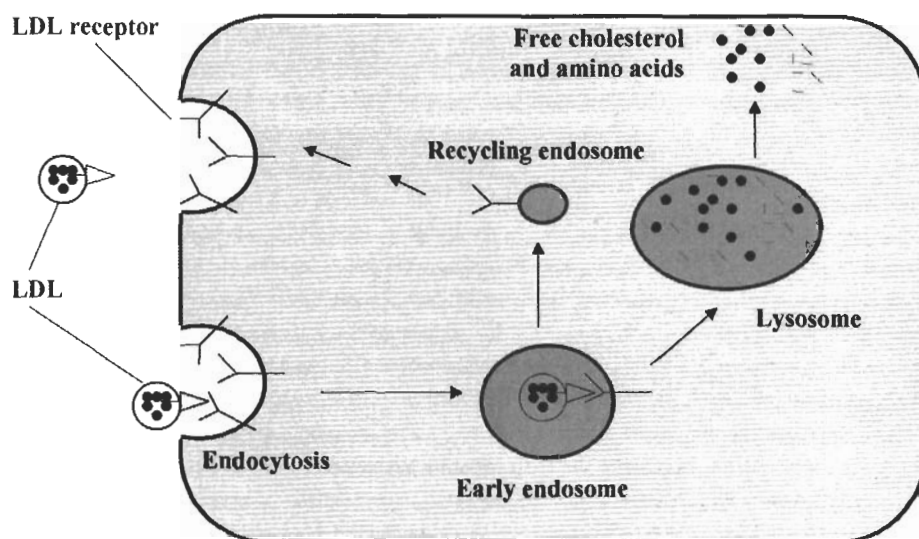


Fig. 2. LDL receptor-mediated endocytosis.

2.3. LDL for Drug Targeting

LDL (25–30 nm) is not directly synthesized in human body. Instead, most of them are formed through the VLDL pathway. LDL is the major circulatory lipoprotein for the transport of cholesterol and cholesteryl esters. Cholesterol required for cell-membrane construction is mainly obtained from LDL. LDL can be internalized by cells via LDL receptor-mediated endocytosis, a process that is determined by the availability of LDL receptors (12). Apolipoprotein apoB-100 is the major ligand in LDL for recognition and binding by LDL receptor (13). Once it is internalized, LDL is distributed to lysosomes in which cholesteryl esters are hydrolyzed (Fig. 2). Because cholesterol is required for cell growth and LDL is the main carrier for cholesterol in blood circulation, most cells can take up LDL through a receptor-mediated mechanism. It is estimated that 60–80% of LDL can be cleared from plasma by LDL receptor-mediated pathways (14,15). As compared with chylomicron, VLDL, and IDL, LDL has a longer serum half-life of 2–4 d (16). Thus, among various lipoproteins, LDL has a distinctive advantage to be used as drug carrier for targeted delivery and has been widely studied.

2.3.1. LDL for Anticancer Drug Targeting

It has been demonstrated that many tumor cells overexpress LDL receptors for the uptake of LDL particles to meet their increased requirement for cholesterol in cell-membrane construction (17–22). Subsequently, a significant amount of work has been carried out to examine LDL as a candidate for antitumor drug carriers. Many lipophilic anticancer drugs have been incorporated into LDL particles for the purpose of drug targeting to various tumors (10,23). When the antineoplastic drugs, methotrexate and floxuridine (FdUrd), were oleyl-derivatized and incorporated into LDL particles, they were effectively delivered into the hepatocellular carcinoma cell line Hep G2 (24). The serum half-life of these drugs carried by LDL particles was considerably prolonged as compared to the free drug. Photodynamic therapy (PDT) of tumors is a recently developed therapeutic approach. It is based on the generation of highly cytotoxic oxygen

species through the irradiation of photosensitizer such as porphyrins, chlorins, and phthalocyanines at selected wavelength. The efficacy of this therapy is dependent on the specific uptake of these photosensitizers by tumor cells. Using LDL as a carrier, photosensitizers were successfully targeted to tumor cells (25–27).

2.3.2. LDL for Brain Drug Targeting

The blood–brain barrier (BBB) is a semi-permeable barrier that allows certain types of molecules to pass through but not others, depending on the lipophilicity, molecular size, and electric charge. It is a significant barrier for many drugs such as antibiotics, neuropeptides, and antineoplastic agents. In order to overcome this barrier, a number of methods have been employed including the use of prodrugs, antibody and drug-carrier systems such as liposomes (28–30). Because the brain involves a variety of receptor-mediated transport systems to control the entry and exit of hydrophilic molecules and macromolecules, such systems can be utilized for brain drug targeting and transport. It is known that LDL receptors exist on endothelial cells of brain capillaries for LDL endocytosis (31–33). Thus LDL potentially can be used as carriers for those drugs that are unable to pass through BBB freely.

2.3.3. Acetylated LDL for Drug Targeting

When chemically altered lipoproteins appear in the circulation of human body, the RES system is activated to remove these altered lipoproteins if they are recognized as foreign substances. The process involves the scavenger receptors on the cell surface of human macrophage (34). Unlike T4 lymphocytes, which lead to collapse of the immune system when they are infected by human immunodeficiency virus (HIV), HIV-infected macrophages allow HIV to replicate for a long period of time. Macrophages play a very important role in HIV dissemination to various organs and to other parts of the immune system (35,36). Experiments have shown that when antiviral drugs, e.g., AZT, are incorporated into chemically altered LDL, such as acetylated LDL, the HIV-infected macrophages can be targeted (37).

2.3.4. Lactosylated LDL for Drug Targeting

In liver, Kupffer cells play a critical role during inflammation through enhanced expression of adhesion molecules, often resulting in the harmful infiltration of neutrophils into the liver. In addition, the production of inflammation mediators, such as interleukins (IL) and tumor necrosis factors (TNF) by Kupffer cells, causes a cascade of events that are related to serious physiological problems (38). Because only Kupffer cells express galactose particle receptors in the liver, lactosylated LDL became a good candidate for drug targeting to Kupffer cells (39). A cholesterol-conjugated oligonucleotide, which is a potent inhibitor to the gene expression of intercellular adhesion molecule-1, was associated with lactosylated LDL and the antisense oligonucleotide was efficiently delivered into Kupffer cells (40), indicating the specific uptake of the encapsulated content by Kupffer cells.

2.3.5. Oxidized LDL for Drug Targeting

Atherosclerosis is responsible for more deaths than any other disease in Western countries. One important hallmark of this disease is the appearance of lipid-loaded macrophages in the vessel wall. Currently available therapies such as percutaneous angioplasty and bypass surgery are limited by recurrence or worsening of the atherosclerotic process. Photodynamic therapy involving various photosensitizers was con-

sidered to be a promising new therapy in recent years (41). One obstacle to this therapy is how to efficiently deliver photosensitizers into macrophage cells. It is known that a high level of scavenger receptors are expressed on the cell surface of macrophages with the atherosclerosis plaque (34). These scavenger receptors can be good candidates for targeted delivery. It has been shown that photosensitizer aluminum phthalocyanine chloride associated with oxidatively modified LDL (OxLDL) was delivered selectively to macrophages (42).

2.3.6. Surface-Modified LDL for Gene Delivery

The success of gene-therapy is dependent on a safe and efficient gene-delivery system. Most of the current gene-therapy protocols are based on viral gene-delivery vectors, which may cause long term safety problems (43). Although many nonviral gene-delivery vectors have been widely investigated, most of them were limited by low transfection efficiency. Lipoprotein has been used to construct a new gene-delivery system to increase safety and efficiency. Kim et al. developed a Terplex system, which had a diameter about 100 nm. The Terplex system was formed through the balanced hydrophobic and electrostatic interactions among LDL, lipidized poly(L-lysine), and plasmid DNA (44,45). This system has demonstrated its efficiency by delivering both plasmid DNA and antisense oligonucleotide to smooth muscle-cells and lung fibroblasts. As an endogenous nanoparticle, LDL played a key role in the internalization of the Terplex system into the target cells via LDL receptor-mediated endocytosis.

2.4. HDL for Drug Targeting

HDL is the smallest lipoprotein with a diameter of 7–11 nm. It shares common structural characteristics with other lipoproteins. However, its polar shell contributes more than 80% of the total mass. Newly synthesized HDL hardly contain any cholesteryl ester molecules. Cholesteryl esters are gradually added to the particles via enzymatic reaction by lecithin:cholesterol acyltransferase (LCAT), a 59-kD glycoprotein associated with HDLs. The cholesteryl esters in HDL can also be transferred to VLDL and LDL via another associated protein, cholesteryl ester transfer protein. The uptake of HDL into cells appears to occur in a similar way to that of LDL. However, cholesterol uptake from HDL also involves more selective means than wholesale uptake because its cholesteryl esters can be transferred into the cells (46). Although the function of HDL in the human body is not well-defined, in general it transports excess cholesterol and cholesteryl esters from various tissue cells to the liver. The major advantage of utilizing HDL for drug delivery and targeting is its small size and fast internalization by tumor cells. Among various lipoproteins, HDL has the smallest size. This makes it easier to pass through the vascular pores to reach the target tissue and quicker to be internalized by the cells. HDL has mainly been used for the incorporation of water-insoluble anticancer drugs for targeting (47,48). When the anticancer drug Taxol was incorporated into HDL, stable complexes were formed for cancer-cell targeting (48).

2.5. Artificial Lipoprotein for Drug Delivery and Targeting

Endogenous lipoprotein for drug delivery has usually been purified from plasma by gradient ultracentrifugation. These lipoproteins are limited in availability and loading additional transport or gene-transfection enhancers has been problematic. In order to overcome such limitations, the concept of artificial lipoprotein can be utilized. Previously, several research groups have attempted to develop artificial lipoproteins (49–

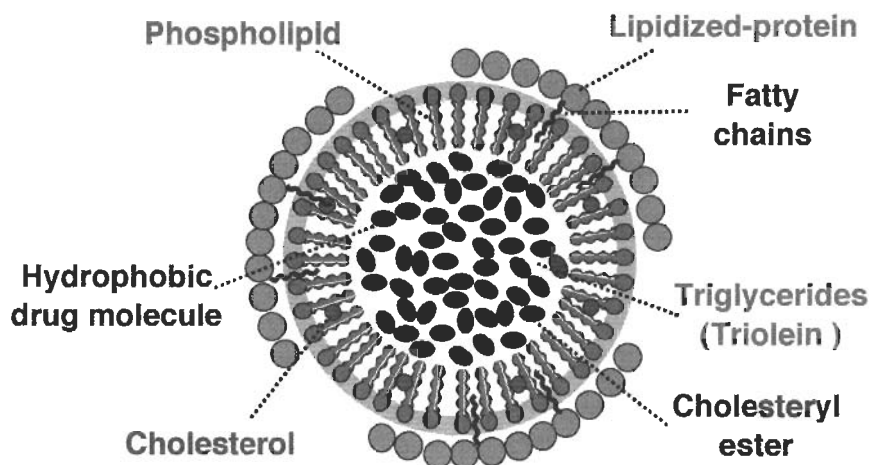


Fig. 3. Structure of artificial lipoprotein.

52). However, these studies have primarily been focused on the incorporation of natural apoB protein into lipid microemulsion for biochemical and metabolism research, and few of them on drug delivery and targeting.

By mimicking the structure of natural lipoproteins, artificial lipoproteins have been extensively investigated in our laboratory to incorporate different lipidized proteins or peptides for a diversified drug-delivery and drug-targeting strategy. The artificial lipoproteins consist of two structural portions, a hydrophobic core and a polar shell, containing surface proteins. The hydrophobic core is mainly composed of triolein and cholesterol oleate. The polar shell is composed of egg phosphatidylcholine, lysophosphatidylcholine, cholesterol, and lipidized protein or peptide. The fatty chains on the lipidized proteins and peptides serve as an anchor to interact with the phospholipid chains and to form stable protein-lipid nanocomplexes (Fig. 3). An early trial of such a system for drug delivery and targeting was through the constitution of a lipidized poly-L-lysine onto phospholipid nanoemulsion particles for gene delivery to tumor cells (53). The incorporation of sufficient amount of palmitoyl poly-L-lysine (p-PLL) molecules onto the nanoemulsion particles led to positively charged complexes that were able to interact electrostatically with negatively charged DNA molecules. As demonstrated by Fig. 4, plasmid DNA migrated toward the positive anode because they were negatively charged (Lane 1). When plasmid DNA was incubated with p-PLL (Lane 2), no DNA migration band was observed. The binding of DNA molecules by p-PLL could block the intercalation of ethidium bromide molecules into the DNA molecules and thus no fluorescence emission occurred. When different ratios of p-PLL to nanoemulsion (i.e., the p-PLL to triolein ratio) were incubated, they demonstrated different DNA carrying capability (Lane 3 to Lane 7). A high ratio of p-PLL to nanoemulsion could tightly bind all the DNA molecules and no free DNA migration band appeared (Lane 3 to Lane 6). When the ratio of p-PLL to nanoemulsion became sufficiently low (0.0625:1 as the p-PLL to triolein ratio), plasmid DNA started to escape from the complex and free DNA bands (Lane 7) appeared on the agarose gel. Because the cell surface is normally negatively charged, the uptake of exogenous particles is affected largely by the surface charge of the particles. Positively charge particles appeared to be required

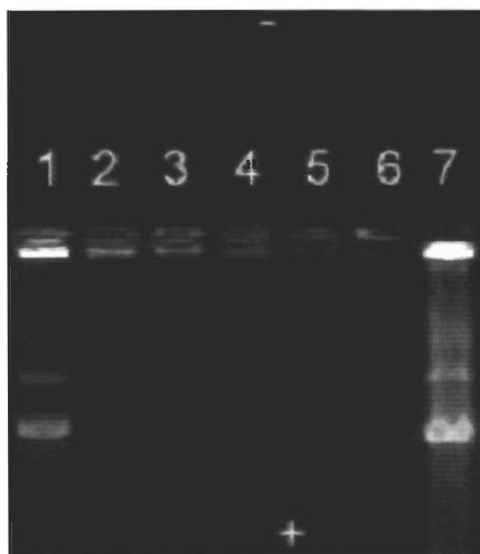


Fig. 4. 0.4% Agarose gel electrophoresis of plasmid DNA and its complexes with nanoemulsion and p-PLL stained with ethidium bromide. Lane 1: Pure DNA; Lane 2: DNA/p-PLL; Lane 3 to Lane 7 were complexes of nanoemulsion with different amount of p-PLL and DNA. The ratio of p-PLL to triolein was 1:1, 1:0.5, 1:0.25, 1:0.125, and 1:0.0625, respectively.

for the cellular uptake of the particles. However, excess positive charge on the particles could induce cellular toxicity and limit its use as DNA carrier. Thus particles with a properly balanced charge are required for the cellular uptake. The surface charge of the nanoemulsion/p-PLL/DNA complexes were measured and their zeta potentials are shown in Fig. 5. The zeta potentials of the particles increased with the increase of the amount of p-PLL when a fixed amount of DNA was used. Among the nanoemulsion/p-PLL/DNA complexes examined for the gene transfection, the complex with the zeta potential of 8.47 ± 1.85 mV resulted in the highest transfection efficiency. Such complexes demonstrated similar transfection efficiency as the Lipofectamine®, a commercial gene-transfection product. The artificial lipoprotein complex, however, had much lower toxicity (Fig. 6).

In recent years, cancer drug delivery and targeting have become a very active research area. Many cancer cells overexpress specific receptor proteins or peptides, which can recognize and bind specific ligands. For example, during the development of a tumor, angiogenic endothelial cells overexpress α_v integrin, which can specifically recognize cyclic peptides containing Arg-Gly-Asp motif (54). Many other specific ligand-receptor binding has also been found in tumor cells (55–57). In order to direct anticancer drug to the specific cellular site, an effective delivery system becomes critically important. The nanoscale size of the artificial lipoproteins and their capability of incorporating different recognition protein ligands may present a practical solution for the anticancer drug targeting and effective gene delivery.

3. Protein–Protein Complexes and Drug Targeting

Protein–protein interactions are critical events for a wide range of physiological and pathological processes. The biological formation of protein nanostructures through pro-

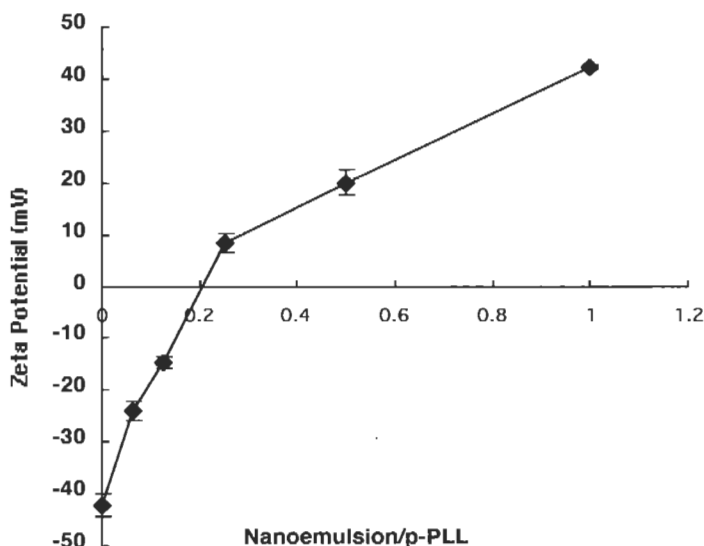


Fig. 5. Zeta potential of the nanoemulsion particles and their complexes with different amount of p-PLL and DNA (2 μ g).

tein–protein interactions must be controlled in a precise manner in order to function properly. In viruses, virtually all the cellular processes, including the formation of protein nanostructures during replication and assembly, involve protein–protein interactions (58). In the human immune system, the proper interaction between CD4 and CD8, the cell surface proteins expressed on T cells, with T-cell receptor (TCR) and major histocompatibility complex Class I (MHC-I) or II (MHC-II) is required to activate T cells (59). The importance of such protein–protein interactions, as in the examples of viral replication and assembly and immune activation in human body, makes these nanocomplexes to be ideal therapeutic targets for new drug developments.

There are many types of nanoscale protein–protein complexes, including homodimers, heterodimers, antigen–antibody complexes, enzyme–inhibitor complexes, and multicomponents such as viral-coat protein and ribosomes (60). The formation of these protein nanostructures via protein–protein interactions generally involves large and relatively flat surface areas with numerous contact sites, making it difficult to use small drug molecules to block such processes. However, these interactions require precise control in order to form pathophysiologically functional complexes. This presents an opportunity for therapeutic drug design and development. In herpesvirus (HSV), HSV ribonucleotide reductase (RR) is a tetramer ($\alpha_2\beta_2$) that consists of two large R1 subunits and two small R2 subunits (61,62). The formation of an intact tetramer through proper interactions is important for the survival of HSV. When a synthetic peptide YAGAVVNDL was introduced into cells, the activity of RR was inhibited without causing significant side effect in the host cells (63,64). The proper formation of other protein nanocomplexes in HSV, such as DNA polymerase (heterodimer) and helicase–primase complex, are also essential for the virus. Therefore, these nanocomplexes are also being considered as potential therapeutic targets.

In HIV, protease, integrase, and reverse transcriptase are all homodimer nanostructures formed by protein–protein interactions. Protease has been one of the primary therapeutic

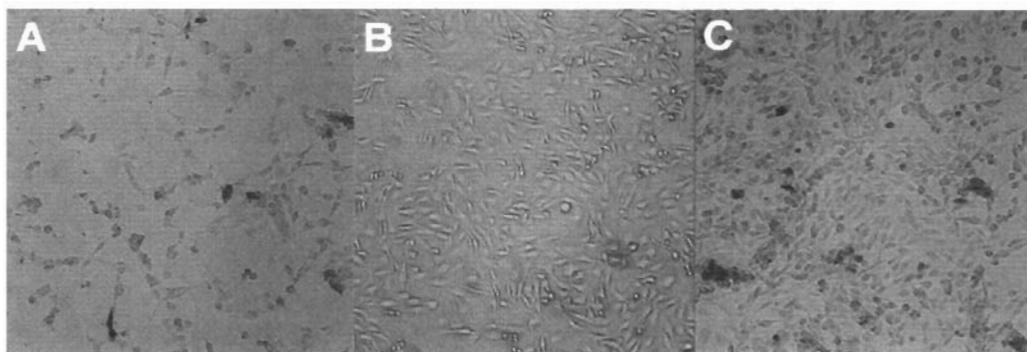


Fig. 6. X-Gal staining of glioma cells. (A) Cells transfected using Lipofectamine™ Reagent. (B) Control. (C) Cells transfected using nanoemulsion/p-PLL/DNA complex.

tic targets for AIDS chemotherapy, being critical for viral maturation. All successful inhibitors of HIV-1 protease to date are peptide mimetics that bind to the active site of the protease. Because of specific mutation within the HIV-1 genome (65), drug-resistant proteases appeared in many HIV-1 strains. A good alternative to this therapeutic strategy would be agents that can block dimerization of protease (66,67). The assembly of other important protein nanostructures, such as integrase and reverse transcriptase, can also be inhibited by many peptides at the dimeric interface (68,69). These types of protein-protein interactions are also widely observed in other types of viruses and have been considered as the therapeutic target for drug development (70,71).

Although the formation of protein nanostructures through protein-protein interactions is critical to many physiopathological processes, it has been difficult to develop effective drug compounds to inhibit these processes. Recent design and screening strategies include rational structure-based drug design, peptide display technology, and in vivo genetic-selection systems (72–74). However, many issues relating to drug delivery, such as cell permeability, intracellular localization, and physicochemical stability, must be resolved. Subsequently, various systems including scrape loading, electroporation, and delivery systems have been investigated. Among the delivery systems, liposomes, polycationic peptides, viruses, and proteins of eukaryotic, bacterial, or viral origin have been studied (75–78). A good example of utilizing protein as delivery vehicle is the B-subunit of *Escherichia coli* heat-labile enterotoxin, which is able to deliver bioactive peptide to the cells to disrupt viral protein-protein complex (79).

4. Protein–Carbohydrate Complexes and Drug Targeting

As one of the major groups of biological molecules, carbohydrates are unique in that they can have many branches and their monomeric units can connect to each other in different linkages in contrast to proteins and nucleic acids, which are exclusively linear and have only one type of linkage (amide linkage in proteins and 3'-5' phosphodiester linkage in nucleic acids). Most carbohydrates exist as nanoscale complexes with proteins (glycoproteins) or lipids (glycolipids). The complex sugar chains of glycoproteins and glycolipids play very important roles in the control of cellular functions and cell–cell recognitions, and therefore extensive investigations into the assembly of carbohydrate complexes may yield important information for drug-targeting development.

Glycoproteins are one of the major components in the outer surface of mammalian cells. They play critical roles in many important biological processes such as cell growth, fertilization, cell adhesion, immune responses, bacterial and viral infections, degradation of blood clot, and inflammation. Majority of glycoproteins are formed by the covalent attachment of carbohydrates to nitrogen atom (provided by asparagines residue) or oxygen atom (provided by serine or threonine residue) in proteins. The proteins are glycosylated as they move through the lumen of endoplasmic reticulum (ER) and Golgi apparatus in the cells, mostly by glycosidase and glycotransferase. The type and extent of glycosylation is dependent on the type and nature of proteins, cells, and tissues (80).

The fusion of HIV envelope with host cell membranes is a critical step for HIV to enter the cells. The envelope glycoproteins of HIV are highly glycosylated. HIV-1 gp120 contains 20–25 glycosylation sites and the carbohydrates contribute about 50% of the apparent molecular weight. Blocking of the protein glycosylation can interfere significantly with the normal life-cycle of HIV (81). Many sugar analogs have been screened for the anti-HIV activity in vitro. One of these analogs, *N*-butyldeoxygalactonojirimycin (NB-DNJ) has been shown to be a potent inhibitor of infection with minimal cytotoxicity. In hepatitis B virus, although there are only two glycosylation sites on the glycoprotein, the viral replication and assembly was inhibited by the treatment of NB-DNJ. Gp41, another HIV envelope glycoprotein, also plays an important role in the fusion of HIV envelope with host-cell membranes. Corresponding peptide and nonpeptide inhibitors to gp41 have been developed (82). Knowledge about the HIV-1 envelope glycoprotein has provided insight into the possibilities for design of novel HIV vaccines (83). Protein–carbohydrate nanostructures in HIV-1 currently have become the most important therapeutic targets for the development for anti-HIV drugs.

The molecular targets for new anticancer agents include inducers of cell differentiation, cell-cycle arrest, apoptosis, and signaling pathways for growth factors and cytokines. Because the protein glycosylation pathways are ubiquitous in cancer cells, they provide excellent opportunities for anticancer drug targeting. For example, alkaloid swainsonine, a Golgi α -mannosidase II inhibitor, is the first inhibitor to be selected for clinical test (84). Because p-glycoproteins (P-gp) are multidrug transporters that result in multidrug resistance (MDR) in cancer chemotherapy, inhibitors targeting this protein have been developed (85). Protein-glycosylation pathways are not only the ideal targets for drug development to treat cancers, but are also excellent targets in other diseases. For example, platelet plays an important role in the pathophysiology of certain diseases such as acute myocardial infarction and diabetes mellitus. The platelet activation and aggregation is caused by the activation of the glycoprotein IIb/IIIa receptor. Thus, glycoprotein IIb/IIIa has been considered to be a therapeutic target in these diseases (86,87).

5. Protein–Nucleic Acid Complexes and Drug Targeting

Nucleic acids (DNA and RNA) are linear polymers of nucleotides with linkages of 3' to 5' by phosphodiester bridges. The genetic information for making all functional macromolecules are stored by the cellular DNA and accessed through the transcription of information into RNA. A typical DNA double helix has a diameter of 2 nm with varying length, depending on the organism. RNA occurs in various forms with different important biological functions such as messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), and small nuclear RNA (snRNA).

The protein–nucleic acid nanostructures resulting from the interactions between protein and nucleic acids are critical to almost all aspects of genetic activity within an organism including DNA replication, transcription, packaging, rearrangement, and repair (88). Based on the structural motif for DNA binding, there are four types of major protein–DNA complexes, i.e., helix-turn-helix (HTH), zinc finger (ZF), basic leucine zipper (B-Zip), and basic helix-loop-helix (B-HLH). Although the proteins in protein–DNA complexes are very diversified, the basic goal is to achieve a precise complementarity of the molecular shapes. This requires specific chemical recognition between proteins and their particular DNA targets. Thus, it has been proven possible to design proteins with novel recognition specificities for the purpose of breaking the normal protein–DNA binding (89). On the other hand, a specific DNA or DNA complex can also be designed to bind the protein. For example, peptide nucleic acid (PNA), a DNA mimic in which the nucleobases are attached to a pseudopeptide backbone, was used to arrest transcription within a gene sequence and to provide an artificial open complex to promote transcription (90).

Certain potent drug molecules interfere with DNA transcription by binding to the transcription factors and thus obstructing the specific DNA binding (91). For example, doxorubicin can bind to *NIL2A*, a basic leucine zipper transcription factor, to inhibit the formation of *NIL2A*/DNA complex. Echinomycin can bind to *EGR1*, a Cys2His2 ZF transcription factor, and inhibit the formation of *EGR1*/DNA complex. Intercalators such as nogalamycin and hedamycin, G/C-rich minor groove-binding drugs such as chromomycin A3, and A/T-specific drugs such as pluramycin can effectively inhibit the transcription factor–DNA complex formation (91,92).

Interaction between protein and RNA plays a very important role in posttranscriptional RNA processing and protein biosynthesis. For example, spliceosomes, complexes of small nuclear RNAs and different proteins, are responsible for the precise formation of mRNAs. Ribosomes, the complexes of proteins with RNAs, are the agents for protein synthesis. For these complexes of proteins and RNAs, either component can be the target for chemotherapy. For example, PNA can be targeted to mRNA to block protein synthesis in an antisense strategy. PNA can also be targeted to the RNA components of ribonucleoproteins (RNPs) to inhibit their enzymatic activities (90).

One of the most interesting protein–RNA complexes for drug targeting is telomerase, a protein–RNA complex that elongates telomeric DNA and appears to play an important role in cellular immortalization (93). Telomeres are nucleoprotein structures at the end of human chromosomes. They play a fundamental role in the regulation of cellular lifespan (94). The tandemly repeated DNA sequence of telomeres is specified and controlled by telomerase, which is repressed tightly in the vast majority of normal cells but becomes activated during cell immortalization and in cancers (94,95). Telomerase has received much attention as a novel and potentially highly specific target for the development of new anticancer therapeutics (96,97).

6. Concluding Remarks

Nanoscale protein complexes in the biological system, including protein–lipid, protein–protein, protein–carbohydrate, and protein–nucleic acid complexes, are ubiquitous in living systems. They play essential roles in various biochemical and genetic activities in cells and viruses. As a result, they become very important objects in pharmaceutical and biomedical research and development, especially in the development

of targeted drug-delivery systems. The understanding of the formation, structure, and function of these protein nanostructures are essential for the development of targeted therapeutic delivery systems, either using these nanostructures as drug carriers or treating these nanostructures as the therapeutic targets. With the rapid advancement in life sciences, pharmaceutical and biomedical sciences, and nanoscience and nanotechnology, more and more efficient targeted drug-delivery strategies based on protein nanostructures can be developed.

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