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Review Article

Nanogel Based Artificial Chaperone Technology: an Overview

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ABSTRACT

The purpose of this review article is to potentiate the use of nanogel based artificial chaperones as therapeutic approach in delivery of various molecules. Nanogel is a nanoscale drug delivery systems with enhanced surface area not only are capable of transmitting hydrophobic drugs *in vitro* and *in vivo* with improved drug bioavailability but also help to reduce side effects of drug. Nanogel chaperone technology is important in the regeneration of proteins from protein aggregations (inclusion bodies). Generally in living system an artificial host (chaperone) with a nanocage which selectively traps denatured proteins or their intermediates in order to prevent their irreversible aggregation with the aid of ATP and other co-chaperones, host chaperones releases proteins in its refolded form. Nanogel ensures a better carrier as artificial chaperones as they effectively prevent protein aggregation by forming nanogel protein complexes during protein refolding. Nanogel interacts with denatured proteins more strongly than native proteins. Nanogel system has been effective in assisting the refolding of proteins and renaturation of the inclusion body of recombinant protein. Current approaches used are amphiphilic polysaccharide nanogel as artificial chaperones in cell-free protein synthesis, heat shock protein-like activity of a nanogel artificial chaperone for citrate synthase etc. Thus the article basically focuses on the general approaches and a review of recent developments in this aspect.

Keywords: Nanogel, Chaperone, Refolding Protein.

INTRODUCTION

In recent years, various stimuli-responsive materials have been developed and applied in biological and medical fields for drug delivery systems and tissue engineering[1-7]. In particular, nano-scale polymer hydrogels (nanogels) in various responsive materials have attracted much attention[8-11]. Nanogels are nanosized networks of chemically or physically cross-linked polymers that swells in a good solvent[12]. Nanogels are cross-linked nanoscale particles made of flexible

hydrophilic polymers. They are soluble in water and allow spontaneous loading of drugs in aqueous media. The nanogel collapses to form dense nanoparticles after adding the drug molecules. Nanogels possess large surface area tunable sizes and a network to allow incorporation of molecule. They have been used to incorporate drugs, DNA/RNA and inorganic molecules such as quantum dots. Nanogels are very promising in drug delivery applications due to their high loading capacity i.e. unique for pharmaceutical nano-carriers. Unloaded nanogels in a swollen state contain considerable amount of water. Loading of biological agents is often achieved through self assembly mechanisms involving electrostatic, van der walls or hydrophobic interactions between the agent and the polymer matrix. As a result nanogel collapse forming stable nanoparticles in which biological agents is entrapped. Current approaches used for preparation of nanogels can be divided in to[9,13]:

1. Chemical synthesis by polymerization (Co-polymerization).
2. Chemical cross-linking of polymeric chains.
3. Physical self assembly of polymers.
4. Using liposomes as a templates.
5. Photo-fenton reaction.
6. Pulse radiolysis method.
7. Photopolymerization.

These systems include simplicity of formulation with the drugs, high loading capacity and stability of the resulting formulation in dispersion. These systems allow immobilization of biologically active compounds of diverse structure including charged drugs, low molecular mass hydrophobes and biopolymers.

Advantages of Nanogels

1. Highly biocompatible.
2. Biodegradable.
3. Non immunological responses.
4. Invasion by reticuloendothelial system is prevented.
5. Release of therapeutics can be regulated by cross-linking densities.
6. Good permeation capabilities due to extreme small size.
7. Applied to both hydrophilic and hydrophobic drugs and charged solutes.
8. Good transport characteristics.

Disadvantages of Nanogels

1. Expensive technique to completely remove the solvents and surfactants at the end of preparation process.
2. Surfactant or monomer traces may remain and can impart adverse effects.

Chaperones are proteins that assist the non-covalent folding or unfolding and the assembly or disassembly of other macromolecular structures, but do not occur in these structures when the structures are performing their normal biological functions having completed the processes of folding and/or assembly. Molecular chaperones recognize and selectively bind non-native proteins to form relatively stable complexes. In most cases, the complexes are dissociated by the binding and hydrolysis of ATP. In addition, there are specific molecular chaperones that typically are involved in the assembly of particular multi-protein complexes. Protein folding involves transition through folding intermediated stabilized by molecular chaperones. Protein misfolding can occur, resulting in prefibril formation. Normally, the prefibrils are degraded but under pathological conditions they may accumulate and lead to disease through production of amyloid fibrils. Production of excessive amounts of degradation fragments can also lead to disease[14].

Molecular chaperones comprise several highly conserved families of unrelated proteins; many chaperones are

also heat shock/stress proteins. The ubiquitous role of molecular chaperones continues to unfold with more discoveries each year. Chaperones behave as "buffers of evolutionary change"[15]. These are promising pharmaceutical carrier which effectively prevent protein aggregation by forming nanogel protein complexes during protein refolding. Nanogel interact with denatured protein more strongly than native proteins. Chaperones trap denatured proteins selectively and so the simple amphiphilic nanogels are stimulating the same function as complex proteins are released in refolded state upon dissociation. This is similar to two step mechanism of molecular chaperones that is to capture the denatured protein and release of refolded protein. Chaperones may correct the conformational changes caused by various mutation, and make the genetical changes phenotypically silent in various organisms studied. Thus chaperones were probably not only contributors to the emerging cellular organization of primordial cells, but in parallel, they also increase genetical stability by buffering the phenotypical consequences of mutational events.

DESIGN AND APPLICATIONS OF NANOGEL-BASED CHAPERONE INSPIRED SYSTEMS

1. Amphiphilic Polysaccharide Nanogels as Artificial Chaperones in Cell-Free Protein Synthesis

The application of the cell-free protein synthesis (a promising technique for the rapid production of proteins) requires the development of an artificial chaperone that prevents aggregation of the protein and supports its correct folding. Here, nanogel-based artificial chaperones are introduced that improve the folding efficiency of rhodanese produced in cell-free systems. Although rhodanese suffers from rapid aggregation, rhodanese was successfully expressed in the presence of the nanogel and folded to the enzymatically active form after addition of cyclodextrin[16].

2. Protein refolding assisted by self-assembled nanogels as novel artificial molecular chaperone

Molecular chaperone-like activity for protein refolding was investigated using nanogels of self-assembly of cholesterol-bearing pullulan. Nanogel of cholesteryl group-bearing pullulan (CHP) selectively interact with proteins as a host and are useful as artificial molecular chaperones and drug carriers such as cancer immune therapy[17].

- Nanogels effectively prevented protein aggregation (i.e. carbonic anhydrase and citrate synthase) during protein refolding from GdmCl denaturation. Enzyme activity recovered in high yields upon dissociation of the gel structure in which the proteins were trapped, by the addition of cyclodextrins. The nanogels assisted protein refolding in a manner similar to the mechanism of molecular chaperones, namely by catching and releasing proteins. The nanogels acted as a host for the trapping of refolded intermediate proteins. Cyclodextrin is an effector molecule that controls the binding ability of these host nanogels to proteins. The present nanogel system was also effective at the renaturation of inclusion body of a recombinant protein of the serine protease family.
- CHPA nanogels were cross-linked with PEGSH to prepare a biodegradable hydrogel (CHP-PEG gel). Gelation occurred within 10 minutes when the final concentration of CHPA nanogel was 30mg/ml in hydrogel. The nanogel structure was maintained after gelation and nanogels distributed homogeneously in the hydrogel. The CHP-PEG hydrogel was an efficient delivery system for bone anabolic agent, PEG2 and also cytokines.

3. Polysaccharide nanogel-cyclodextrin system as an artificial chaperone for in vitro protein synthesis of green fluorescent protein

Polysaccharide nanogels have been demonstrated to aid the refolding processes of chemically or thermally denatured proteins, a function that is similar to that of natural molecular chaperones. In this study, the possibilities of using the nanogel chaperone system to mediate protein folding in a cell-free (in vitro) protein synthesis system containing transcription/translation factors are examined. High-performance liquid chromatography showed that a polysaccharide nanogel comprising cholesteryl group-bearing pullulan (CHP) trapped unfolded or partially folded green fluorescent protein (GFP) expressed in the cell-free system. The protein release and refolding processes, which are induced by ATP in natural molecular chaperone systems, were also simulated by methyl-β-cyclodextrin (M-β-CD). The CHP nanogels dissociate on complexation with M-β-CD to yield dissociated CHP. Thus, the dissociation of the CHP nanogel–protein complex subsequently allows for the release and folding of GFP. The folding kinetics in the presence of the CHP nanogel and M-β-CD was comparable to that of spontaneous folding in the absence of CHP/M-β-CD, indicating that the CHP nanogels did not affect protein synthesis in the cell-free system, providing correctly folded active proteins[18].

4. Cyclodextrin-responsive nanogel as an artificial chaperone for horseradish peroxidase

The thermal stabilization and refolding of horseradish peroxidase (HRP) upon heating were investigated using an artificial molecular chaperone consisting of cholesterol-bearing pullulan (CHP) nanogels. The CHP nanogels inhibited the aggregation of HRP under heating by complexation with the denatured HRP. The enzyme activity of HRP complexed with CHP nanogels was not detected. However, the enzyme activity recovered up to 80% of native HRP after the addition of cyclodextrin (CD) to the complex. The dissociation of CHP nanogels was induced by the formation of an inclusion complex of cholesterol groups of CHP with CD. The enzyme activity of HRP was only significantly recovered by the addition of β-CD or its derivatives. Natural molecular chaperones, such as GroEL/ES, trap, fold, and release the nonnative proteins by changing the hydrophobicity of the specific sites of the molecular chaperone that interact with the nonnative protein. The functional mechanism of the nanogel chaperon system is similar to that of natural molecular chaperones. The nanogel chaperone system is a useful tool to aid the refolding and thermal stabilization of unstable proteins for post-genome research, and in medical and biological applications[19].

5. Polysaccharide Nanoballs: A New Building Block for Nanogel Biomedical Engineering and Artificial Chaperones

Enzymatically synthesized glycogen (ESG), a highly branched (1→4) (1→6)-linked α-glucan, is a new monodisperse spherical hyperbranched nanoparticle (molecular weight, 106−107; diameter, 20−30 nm), polysaccharide nanoball. Amphiphilic ESG nanoballs were synthesized by introducing a cholesterol group to enzymatically synthesized glycogen (CHESG). CHESG assembled into a structure containing a few molecules to form cluster nanogels (approximately 35 nm in diameter) in water. The cluster nanogels were dissociated by the addition of cyclodextrin (CD) to form a supramolecular CHESG−CD nanocomplex due to complexation with the cholesterol group and CD. The CHESG nanogel showed high capacity for complexation with proteins, and the CHESG−CD nanocomplex showed high

chaperone-like activity for thermal stabilization of enzymes. CHESG has great potential to become a new building block for nanogel biomedical engineering and to act as an artificial chaperone for protein engineering[20].

6. Hybrid hyaluronan hydrogel encapsulating nanogel as a protein nanocarrier: new system for sustained delivery of protein with a chaperone-like function

Novel hybrid hyaluronan (HA) hydrogel encapsulating nanogels was designed for sustained delivery of protein. HA modified with 2-aminoethyl methacrylate was cross-linked via Michael addition in the presence of cholesteryl group-bearing pullulan (CHP) nanogels. The nanogels were physically entrapped and well dispersed in a three-dimensional network of chemically cross-linked HA (HA gel). Therapeutic peptides and proteins, such as glucagon-like peptide-1, insulin and erythropoietin, were spontaneously trapped in the CHP nanogels in the HA gel just by immersing hybrid hydrogels into the drug solutions. CHP/protein complex nanogels were released from the hybrid hydrogels in a sustained manner both in vitro and in vivo. The release was controlled by the cross-linking density and the degradability of the HA gel, modulated by the initial gelation condition. The synergy between the CHP nanogel as a drug reservoir and the HA gel as a nanogel-releasing matrix of the hybrid hydrogel system simultaneously achieved both simple drug loading and controlled release with no denaturation of the protein drugs. This is a new method of fabricating biodegradable controlled release matrix with molecular chaperone-like activity for therapeutic proteins[21].

CONCLUSION

In conclusion, nanogels are promising novel pharmaceutical carriers for small biologically active agents and biomacromolecules. The advantages of these systems include simplicity of formulation with the drugs, high loading capacity and stability of the resulting formulation in dispersion. These systems allow immobilization of biologically active compounds of diverse structure including charged drugs, low molecular mass hydrophobes and biopolymers. Nanogel cross-linking hydrogel with chaperone like activity can be used as a new hydrogel scaffold with isolated binding nano-domain of proteins or drugs for tissue engineering.

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