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CONTROLLED DENATURATION OF LACTOFERRIN: AN UNEXPLORED SELF-ASSEMBLY TECHNIQUE FOR GENE DELIVERY NANOPARTICLES

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In recent years, self-assembling nanomaterials have gained much interest in the field of drug delivery. These materials possess the inherent capability to spontaneously transition from a molecular to a supramolecular state, often resulting in larger and more complex structures exhibiting emergent properties. The use of these compounds shows great potential in creating nanoparticles (NPs) customized for drug delivery purposes. Their self-assembling properties streamline NP manufacturing, providing precise control and consistency, and minimizing the need for high-energy inputs and toxic organic solvents. This not only improves the attractiveness of the final product but also enhances the efficiency of the production process. Lactoferrin (LF), a physiological multifunctional glycoprotein, is well-known for its antimicrobial, anti-inflammatory, and immunomodulatory properties, along with favorable technological features such as biodegradability, biocompatibility, targeting specificity, and blood-brain barrier permeability. Despite these well-documented traits, there exists a lesser-explored aspect of LF pertinent to drug delivery: its capacity for self-assembly through controlled heat-induced denaturation. This phenomenon offers a promising avenue for harnessing LF to obtain self-assembling nanosystems for targeted drug delivery. In this investigation, we have examined the potential of LF's self-assembling characteristic and its cationic properties to develop a nanosystem capable of complexing, stabilizing, and delivering small interfering RNA (siRNA) with precision. To our knowledge, this represents the inaugural endeavor to utilize LF's self-assembling capability for gene delivery purposes. Initially, the heat-induced denaturation process was optimized by evaluating key parameters such as LF solution pH (either 5.5, the natural pH of a LF solution, or 7), heating temperature (55, 75, or 95°C), and heating time (1, 5, 10, or 20 min), which govern protein denaturation and influence NP formation. Samples subjected to various conditions underwent characterization to assess the impact of these variables on the protein secondary structure, utilizing Circular Dichroism. Additionally, further characterizations were performed through SDS-PAGE, FTIR, and Raman Spectroscopy. Through precise optimization and control of the denaturation process, NPs were successfully formed with sizes ranging from 60 to 80 nm, uniform dispersion (Polydispersity Index, PdI, of approximately 0.2), and positive surface charge (z-potential of around +30 mV). Scanning Electron Microscopy (SEM) confirmed the particle size, narrow size distribution, and spherical morphology. Moreover, LF NPs demonstrated optimal physical stability when stored as a lyophilized powder and subsequently reconstituted as a suspension. Subsequently, the most promising LF NPs were utilized to create complexes with a model siRNA. An optimization was carried out by testing different molar ratios of LF to siRNA for each sample (LF to siRNA 5:1, 10:1, or 20:1). The optimized LF NP-siRNA complexes showed physicochemical and morphological properties comparable to those of non-loaded LF NPs, indicating that the addition of siRNA molecules did not negatively affect the optimal properties of NPs. The siRNA complexation efficiency was found to be higher than 98%, while the ability of NPs to protect the drug from the inactivating enzymes ribonucleases (RNases) is still under evaluation. Remarkably, the complexes also demonstrated high stability in terms of both physicochemical properties and siRNA complexation ability under all storage conditions tested (4°C, freeze-thawing, and lyophilization-resuspension) for up to 14 days. This research emphasizes the significance of utilizing LF's self-assembling characteristics to develop LF NP-siRNA complexes that are uniformly homogeneous, stable, and reproducible. Additional investigations are underway to assess the efficiency of siRNA stabilization by these nanoparticles. Moreover, in vitro studies will be performed to evaluate the effectiveness of LF NP-siRNA complexes in terms of gene silencing, cellular uptake, and cytotoxicity.

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