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DNA Encapsulation in an Anionic Reverse Micellar Solution of Dioctyl Sodium Sulfosuccinate

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Abstract As nanometric systems, reverse micelles have attracted a great deal of interest especially from the biomedical sciences and widely used in the processes such as the separation and synthesis of biomembranes. We carried out the DNA encapsulation in an anionic reverse micellar solution (RMS) of dioctyl sodium sulfosuccinate (DSS), and studied the interactions through the circular dichroism (CD) and ultraviolet (UV) spectroscopy techniques. The results indicate that the DSS reverse micellar nanosystems provide a suitable medium for encapsulating DNA.

Keywords CD Spectroscopy, DNA Encapsulation, DSS, RMS, UV Spectroscopy

1. Introduction

In structural terms, a reverse micellar solution contains clots immediately formed due to the self-assembling of the surfactant molecules in the organic solvents. After 1910, McBain et al. reported the formation of colloidal clots by fatty acid soaps[1-4]. Concerning the quantitative properties and molar conductivity of the soap solutions, McBain came to the conclusion that a part of the soap forms large clots in the aqueous solution called micelle. He found that the micelle formation should be due to the reversible association of positively charged ions. However, this model's details were revised later. Over the decade 1910s, this idea was considered rather a bold assumption since most researchers at that time believed that the colloidal systems are never thermodynamically stable and their properties depend on the synthesis technique. In 1913, Reychler showed that the sodium cetyl sulfate behaves like the fatty acid soaps and guessed that the molecules are arranged in the micelle such that the hydrophilic core is surrounded by the surfactant hydrophobic end groups[5]. Nowadays, it has been accepted that the clots formed in the ternary mixtures of organic solvent, surfactant, and a very little amount of water are usually spherical with the radius 0.5-5 nm, called the reverse micelle. As evident in Figure 1, such nanostructures are able to solubilize the hydrophilic molecules in the organic phase mass, owing to their aqueous core.

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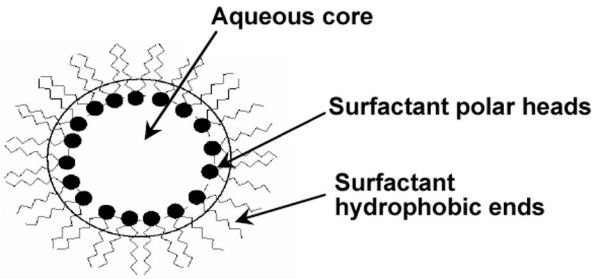

Figure 1. A schematic view of a reverse micelle structure

In the past two decades, the reverse micellar systems have been used in various highly selective separation processes like protein separation from the mixtures[6], enzyme continuous separation from the growth medium[7], and protein isolation in a bacterial cell[8]. Different experiments have indicated that not only does the biological activity of the enzymes decline in such nanosystems but also it increases in specific conditions. Hence, the reverse micellar systems can also be used for the enzymatic reactions. Increasing attention of researchers to develop the nanotechnology in the recent decade, many investigations have been carried out to synthesize nanomaterials such as cadmium, copper, gold, and carbon nanotubes in the RMSs as the nano-reactor[9-13]. According to the recent studies, the RMSs can be an appropriate candidate for gene synthesis in the gene therapy process[14, 15]. Another applicational aspect of RMSs is to use them as biological models in vivo conditions. The empirical results have shown that the reverse micelles can also exist in the double-layer biomembrane walls[16-18]. In this state, the reverse micelles are sandwiched between the membrane layers. Figure 2 shows a schematic view of these intramembrane reverse micelles.

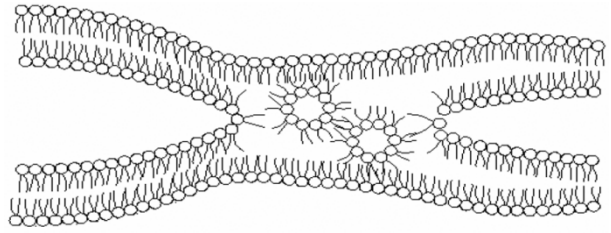

Figure 2. A schematic view of reverse micelles sandwiched into biomembranes and the intermembrane exchanges

The membrane and the condensed reverse micelle are interacted extremely fast[19] such that the researchers think that protein can be transferred via these reverse micelles through the biomembrane[20].

2. Experimental

A stochastic ratio of DSS, Sigma Tris-Hydrochloride, lionfish's DNA, isooctane, ethylenediaminetetraacetic acid (EDTA) was used. The aqueous phase contains 4 mg/mL of DNA and the reverse micellar phase contains 100 mM of DSS. The impurities in the initial RMSs (solid and dust-like suspended particles) were filtered using sterilization membranes (Millex 0.22 μm, Millipore, Bedford, MA, USA). The aqueous phase was directly injected at 25 ℃ to solubilize DNA in the RMS. The concentration of DNA in the RMSs was measured through a Varian Cary 1E UV spectrophotometer at the wavelength 260 nm using a quartz cell with 1 cm path length. The system did not contain DNA in the reference state. Also, the molar ratio of water to surfactant was chosen 18.5 in the reverse micellar phase.

The interaction between the reverse micelles and DNA molecules should be investigated after the determination of DNA presence in the reverse micellar phase. In this regard, a circular dichroism (CD) device was employed. This device is an ideal technology to study the structure of the optically active molecules. This optical method is only sensitive to asymmetric molecules and its application in biosystems is of great interest. The CD measurement principles are: adsorption difference (A), levorotatory and dextrorotatory polarized lights that given by the molecules as

$$CD = A_l - A_r \quad (1)$$

where l and r respectively denote the levorotatory and dextrorotatory adsorption states. Figure 3 shows a simple view of the CD operation.

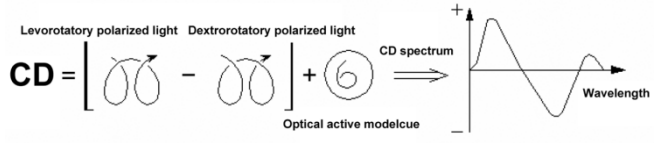

Figure 3. Circular dichasium (CD) spectroscopy process

The CD spectroscopy can be used to determine the protein secondary structure, compare the different-source identical proteins' structures, study the interactions in the solution, and investigate the stability of proteins under pressure and temperature. A JASCO J-600 spectrometer was used to study the DNA structure in the reverse micellar and aqueous solutions at the constant temperature. A double beam of levorotatory and dextrorotatory polarized lights was usedin the CD device. This double beam was passed first through a Pockels cell crystal and then the solution. The main property of this crystal is that the double beam of levorotatory and dextrorotatory lights passes selectively with respect to the voltage applied from an electric field. Therefore, the light passed through this cell periodically becomes levorotatory and dextrorotatory. After this light passes through the solution, it enters the photomultiplier and the adsorption difference of this double beam to the wavelength is measured[21].

3. Results and Discussion

Since DNA is a hydrophilic macromolecule, it can only be placed in the aqueous core of the reverse micellar systems. In other words, DNA encapsulation makes its solubilization possible in the organic phase. The UV spectroscopy was utilized to prove DNA solubilization in the reverse micellar phase. Figure 4 shows the UV adsorption of DNA macromolecules in the aqueous and reverse micellar phases. The appearance of the culminating point around the wavelength 260 nm indicates the presence of DNA in these phases. If the conditions are changed and DNA is not encapsulated and directly added to the organic solvent, its natural shape will be immediately changed and the UV spectroscopy shows a relatively horizontal line instead of curve, implying the absorption of the scattered lights.

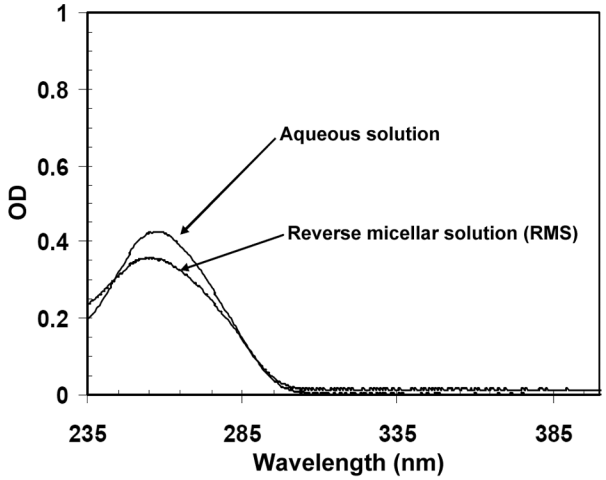

Figure 4. UV spectrum of DNA macromolecules in the aqueous and reverse micellar phases

The adsorption at the wavelengths longer than 290 nm is due to the light dispersion resulting from DNA condensation. After the determination of DNA encapsulation, the CD spectrum was used to investigate the interactions between these macromolecules and the anionic reverse micelle DSS. To study the CD spectrum of the encapsulated DNA, it is first necessary to obtain the spectrum of DNA in the aqueous

solution and the conditions with the same concentration as the reverse micellar organic phase has. For this purpose, in parallel with the injection of the organic phase containing DNA into the reverse micellar phase, the same amount should be injected into the aqueous phase, and its spectrum was investigated. Figure 5 shows the CD spectrum of DNA in the aqueous and reverse micellar phases.

A DNA macromolecule can have different formations like *A*, *B*, and so forth. The most common formation of these macromolecules that is also considered the most thermodynamically stable is B-conservation. As can be seen in Figure 5, there are a peak and a dip respectively around 275 and 245 nm. The CD spectrum of the encapsulated DNA suggests a resonance created in the normal spectrum, leading to the displacement of peaks and dips. This resonance that has been previously reported by some researchers indicates the condensation of the DNA macromolecules[14]. It is obvious that the molecular condensation depends on the resonance magnitude. Note that, to gain a better accuracy, the experiments were repeated nine times and the average was plotted in Figure 5.

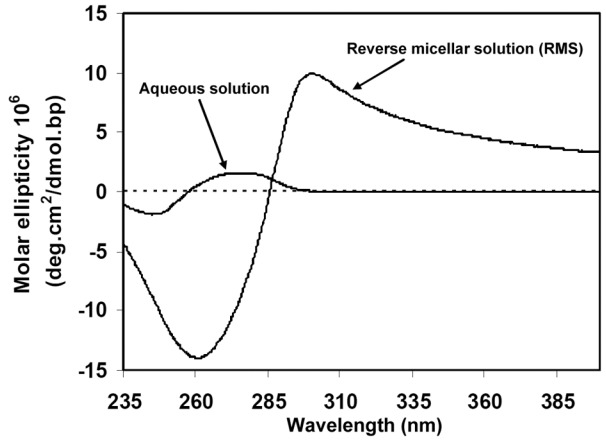

Figure 5. CD spectrum of DNA macromolecules in the aqueous and reverse micellar phases

4. Conclusions

In general, it can be concluded that the reverse micellar system DSS is able to solubilize and condense DNA in the DNA encapsulation process, which is expected to happen naturally in the body.

Here in fact a multidisciplinary approach is adopted to produce reverse micellar solution as an in vitro medium to encapsulate DNA for gene delivery. Biophotonics techniques such as CD spectroscopy were employed to optically monitor intracellular trafficking and gene transfection. Generally speaking, the micellar nanochemistry can produce encapsulated DNA molecules, highly mono-dispersed aqueous suspensions of RMS, and surface functionalized by cationic-amino groups.

Gel-electrophoresis investigations suggest that the particles can efficiently form a complex with DNA macromolecules, and protect them from enzymatic digestion of DNAse-1 by forming a membrane. DNA and RMS membrane, formed by DSS nanoparticles, are electrostatic bound thanks to the positively charged amino groups, which is detected by appropriately encapsulating DNA and monitoring the surfactant resonance energy transfer between intermembrane and intramembrane DNA macromolecules. CD and UV spectroscopy data clearly show that DNA macromolecules efficiently take up the DSS nanoparticles in vitro, and the DSS nanoparticles surround DNA. CD spectroscopy enabled us to describe the mechanism of DNA encapsulation and gene transfection in RMS media. This investigation suggests that this nanomedicine approach with DSS nanoparticles acting as a drug-delivery platform offers a promising horizon for the targeted therapy techniques and real-time drug reaction monitoring, a part which we aim to further study on in the near future.

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