Analysis of LNP-Encapsulated mRNA Using MultiNA™ II Microchip Electrophoresis System

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1. Introduction

Messenger RNA (mRNA)-based therapeutics have undergone rapid development in recent years, with mRNA-based vaccines for infectious diseases, vaccines for cancers, and treatments for genetic disorders attracting increasing interest. mRNA is a large, negatively charged molecule that exhibits very poor cell membrane permeability. For this reason, delivery technologies that can effectively transport mRNA molecules into cells are essential. At present, lipid nanoparticles (LNPs) are the most common drug delivery system (DDS) used in mRNA therapeutics. LNPs can protect fragile mRNA molecules from degradation in the extracellular environment while also effectively delivering the mRNA into cells.

While LNP-encapsulated mRNA (LNP-mRNA) shows promise for treating various diseases, quality control during production and storage remains a critical challenge. Quality control is essential for the safety, efficacy, and reliability of LNP-mRNA preparations. Therefore, developing suitable analytical methods for LNP-mRNA quality evaluation is essential to enhance product reliability and ensure regulatory compliance.

mRNA purity can be tested using agarose gel electrophoresis and capillary electrophoresis, but these techniques have some major drawbacks, such as time-consuming setup and analysis and insufficient resolution or sensitivity. This presentation demonstrates the simple and rapid analysis of LNP-encapsulated mRNA and extracted mRNA using the MultiNA™ II system (Fig. 1), followed by a comparison and evaluation of the obtained data.

Fig. 1 MultiNA™ II MCE-301 Microchip Electrophoresis System

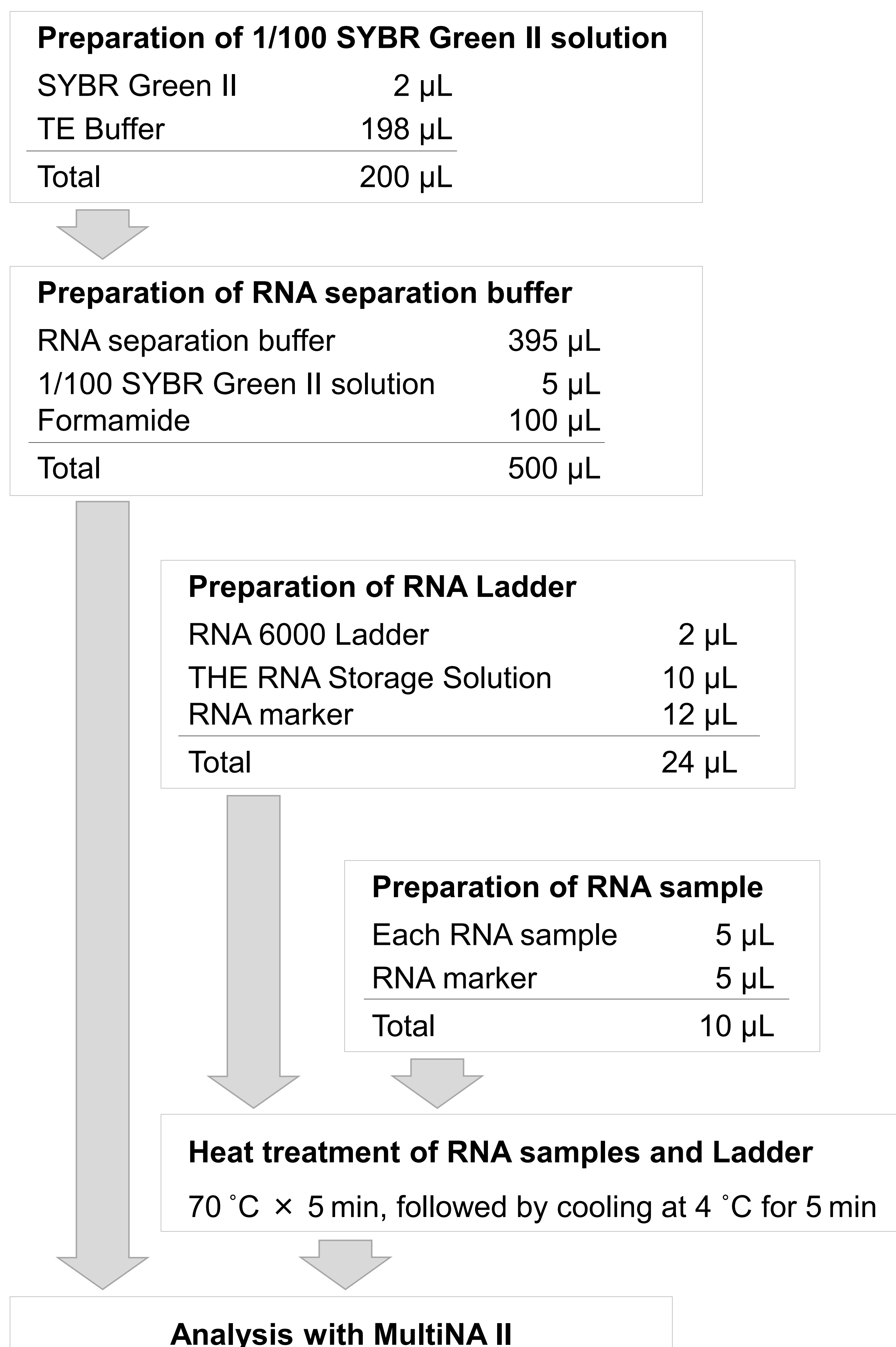

Fig. 2 MultiNA II Analysis Procedure for mRNA-LNP (for 6 Analyses)

2. Methods

Electrophoresis was performed using reagents from an RNA kit (P/N: 292-27913-91). An RNA marker solution from the RNA kit was combined in a 1:1 ratio with the samples, and according to the draft guidelines published by the US Pharmacopeia (Analytical Procedures for Quality of mRNA Vaccines and Therapeutics, Draft Guidelines: 3rd Edition)[1)], the sample mixture was heated at 70 ˚C for 5 minutes then cooled at 4 ˚C for 5 minutes. The procedure used for analysis is shown in Fig. 2.

SYBR Green II (Thermo Fisher Scientific, P/N: S-7564) was used as a fluorescent dye and RNA 6000 Ladder (Thermo Fisher Scientific, P/N: AM7152) was used as a size standard (size marker). The size standard was diluted 6-fold with THE RNA Storage Solution (Thermo Fisher Scientific, P/N: AM7001), mixed 1:1 with the RNA marker solution from the RNA kit, heated at 70 ˚C for 5 minutes, and then cooled at 4 ˚C for 5 minutes (similar to the samples).

3. Results and Discussion

Microchip electrophoresis was performed on the mRNA sample and LNP-encapsulated mRNA (mRNA-LNP). The resulting electropherograms are shown in Fig. 3. The blue triangles indicate the peaks corresponding to mRNA. From top to bottom, Fig. 3 shows electrophoresis results for: (a) mRNA-LNP solution after heat treatment (70 ˚C for 5 min); (b) mRNA-LNP solution with Triton X-100 (final conc.: 0.1 %) followed by heat treatment; (c) mRNA sample solution; and (d) 0.1 % Triton X-100 solution. The samples analyzed in (a) to (c) were prepared to a final mRNA concentration of 10 ng/µL.

Electrophoresis of mRNA-LNP solution without surfactant (a) produced a very small peak that could not easily be used to determine concentration. This demonstrated that the heat treatment alone released almost no mRNA from the mRNA-LNP preparation. Triton X-100 was added to the mRNA-LNP sample and, following heat treatment, electrophoresis was performed according to the draft guidelines published by the USP for mRNA quality evaluation (b). The RNA fragment peak is clearly visible in this electropherogram, and its estimated size is equivalent to that of the model mRNA sample.

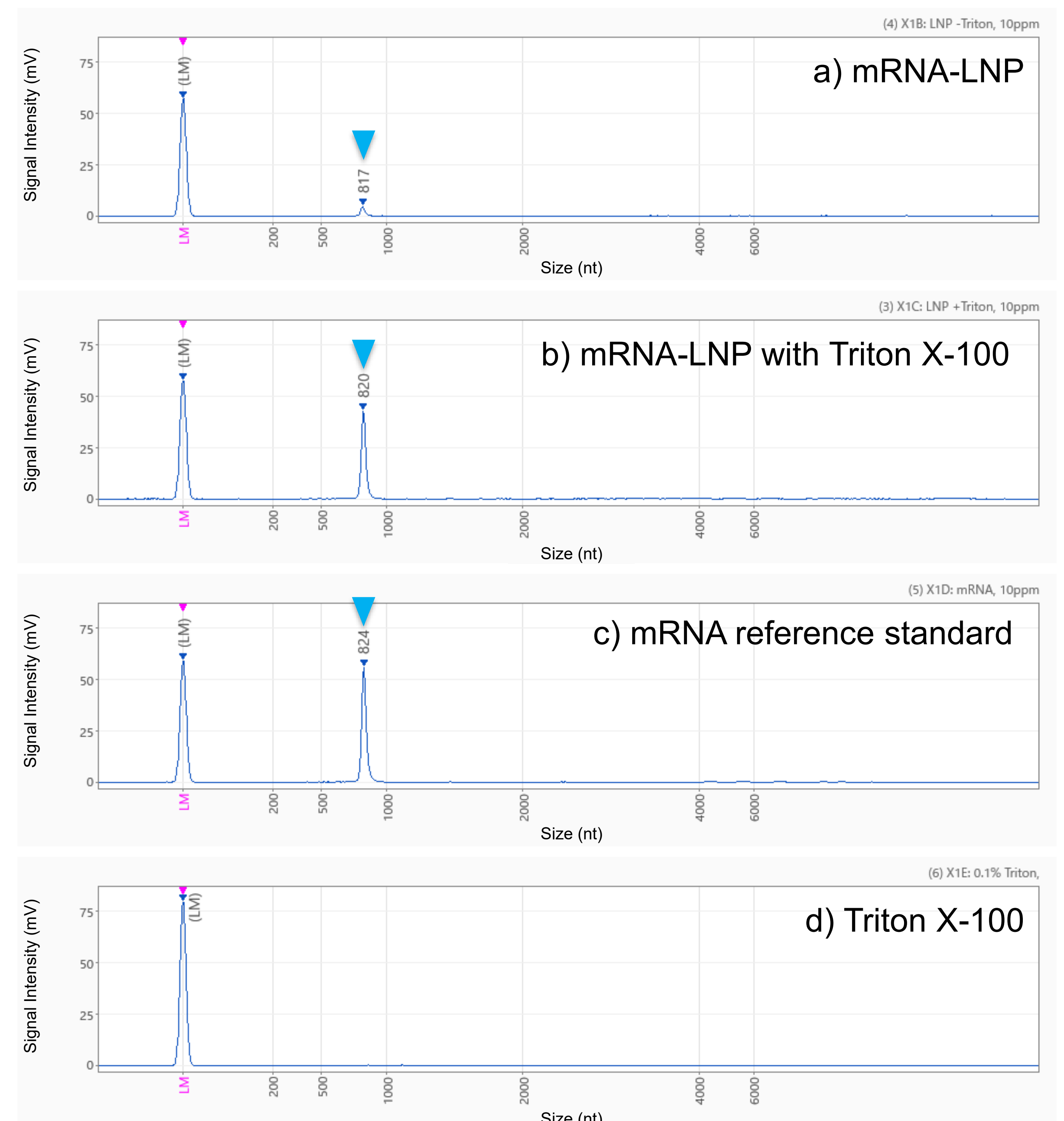

Fig. 3 Electrophoresis Results for mRNA-LNP

4. Conclusion

Adding a surfactant (Triton X-100) to the sample before heat treatment, as described in the USP-published guidelines, allowed the detection of mRNA from an mRNA-LNP preparation using the MultiNA II system. This analysis offers a simple and rapid method of evaluating mRNA-LNP that can potentially help to streamline the development of mRNA therapeutics.

Acknowledgments

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References

Analytical Procedures for Quality of mRNA Vaccines and Therapeutics, Draft Guidelines: 3rd Edition