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Probing the Environmental Fortitude of mRNA Lipid Nanoparticles: A TIMS-TOF Insight into Stress-Induced Degradation

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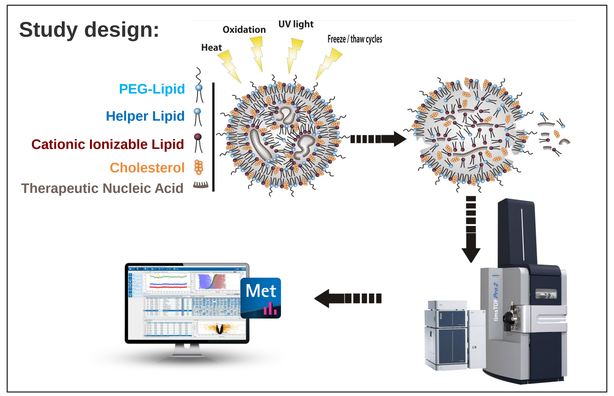
Introduction



Environmental stressors significantly impact the stability and effectiveness of mRNA lipid nanoparticles (LNPs). Key stressors include temperature fluctuations, which affect lipid and mRNA integrity, necessitating cold storage; humidity and moisture, potentially destabilizing the lipid bilayer; light exposure, leading to photo-oxidative damage; mechanical stress from physical handling; and oxidative stress from reactive oxygen species. These factors can alter the LNPs' structural and functional properties, posing challenges in storage, transportation, and maintaining therapeutic efficacy. In this study we employed Ultra High Pressure Liquid Chromatography (UHPLC) coupled with Trapped Ion Mobility Time-Of-Flight (TIMS-TOF) Mass Spectrometry (MS) technology to scrutinize the dynamic responses to stress, including heat, UV light, oxidation, and alternating freeze/thaw cycles for 72 hours. Addressing these environmental impacts is vital for the successful development, distribution, and application of mRNA LNP-based therapies, especially in regions lacking advanced infrastructure.

*Authors BW, MA, XP, and EF are employees of Bruker Corporation or one of its subsidiaries ("Bruker"). Bruker manufactures and sell sanalytical instruments including mass spectrometers and software. Bruker mass spectrometers and software were used in this study.

Methods

- Luciferase-encoding mRNA LNPs of three structurally distinct ionizable lipids (MC3, SM-102, and ALC-0315) were formulated independently using Spark NanoAssemblrTM (Precision NanoSystems) with a standard formulation technique.
- An equal amount of LNPs was exposed to an individual stress condition for 72 hours. These conditions were heating at 28 °C, exposure to UV light (wavelength = 360 nm), mixing with hydrogen peroxide to a final concentration of 100 mM, or freeze/thaw cycles of -20 °C for 12 hours followed by 22 °C for 12 hours (ambient temperature).
- Lipids were extracted with a mixture of chloroform and methanol (in the ratio of 2:1) v/v and transferred to LCMS vials for MS analysis.
- Samples were acquired on a Bruker TIMS-TOF Pro 2 supplemented with a Bruker Elute UHPLC and a VIP-HESI source. A full scan MS and MS/MS (PASEF) were implemented in both positive and negative modes. Data were analyzed by MetaboScape® 2024 and DataAnalysis 6.1.

Results

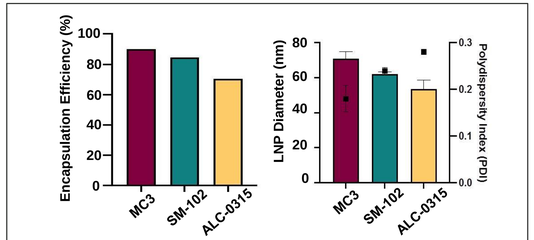


Fig. 1 Encapsulation efficiency using three ionizable lipids used in this study (left) and the nanoparticle diameter (right)

- The encapsulation efficiency was determined by the Quant-IT Ribogreen assay to be 70-90%. The nanoparticle average diameter was 55-70 nm as determined by dynamic light scattering using a Zetasizer Nano ZS. (Fig. 1).
- MC3, SM-102, and ALC-0315 demonstrated extensive degradation upon exposure to UV light and hydrogen peroxide (Fig. 2).

1. Carrasco MJ, et al. Commun Biol. 2021 Aug 11;4(1):956.
2. Schoenmaker L, et al.Int J Pharm. 2021 May 15;601:120586.
3. Oude Blenke E, et al. J Pharm Sci. 2023 Feb;112(2):386-403.

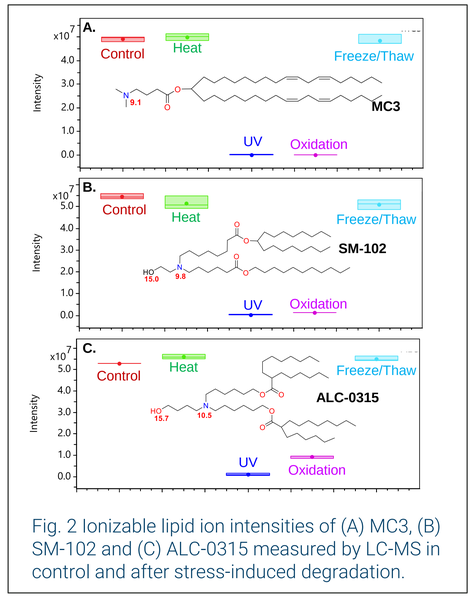


Fig. 2 Ionizable lipid ion intensities of (A) MC3, (B) SM-102 and (C) ALC-0315 measured by LC-MS in control and after stress-induced degradation.

- MC3 was heavily impacted by the oxidizing condition as well as the UV light exposure with MC3+1O being the dominating oxidation product (Fig. 3). Based on the fragmentation pattern, the additional oxygen was located between the tertiary nitrogen and the ester group leaving the tail end of the lipid surprisingly intact.
- Hydrogen peroxide also resulted in oxidation of DSPC between the quaternary head and the phosphate group (Fig. 4).
- While oxidizing conditions resulted in formation of the carboxylate adduct of SM-102, UV light only caused addition of oxygen in the form of a hydroxyl group on one of the carbons close to the hydroxyl terminus probably formed via a free radical mechanism. This was confirmed by the fragmentation pattern of m/z=726.6613. Three levels of oxidation were observed the SM102+1O (the carboxylate adduct), SM102+2O, and SM102+3O. (Fig. 5)
- In addition to the multiple levels of oxidation like ALC+1O and ALC+2O, ALC lipid demonstrated extensive alph cleavage and numerous C-C cleavages near the hydroxyl terminus. (Results not shown)

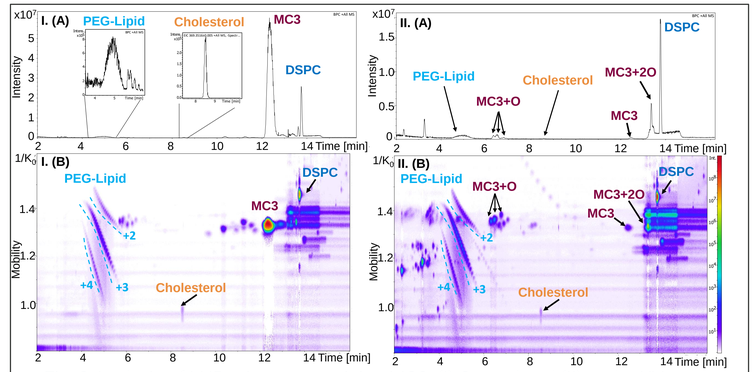


Fig. 3 Analysis of LNP mixture containing MC3: (I) Control and (II) under UV stress. (A) Base Peak Chromatogram of the mixture, and (B) Heat map of Retention time – Ion mobility

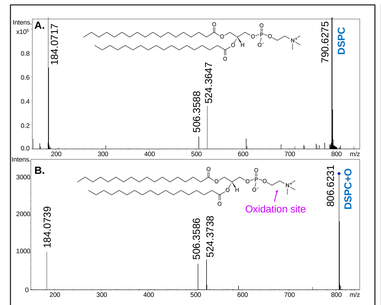


Fig. 4 MS/MS spectra of (A) DSPC and (B) oxidized DSPC after H_2O_2 treatment

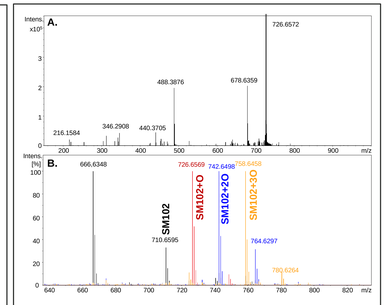


Fig. 5 (A) MS/MS spectra of oxidized SM-102 at m/z 726 and (B) overlaid MS spectra of SM102 and three levels of oxidation forms

Summary

The study highlights the stability of various ionizable lipids in mRNA LNPs under different conditions. These lipids showed significant degradation when exposed to UV light and hydrogen peroxide, suggesting a free radical mechanism initiated by reactive oxygen species, and with minimal impact from heat and freeze/thaw cycles. This indicates that oxidative and UV conditions significantly impact the integrity of ionizable lipids, emphasizing the need for protective strategies in mRNA LNP formulations.

Conclusion

- LNP stressed-induced degradation is investigated using LC-TIMS-PASEF on timsTOF Pro 2 and degradation products identified with MetaboScape
- Most severe degradation occurred under UV light and oxygen

timsTOF Pro 2 and MetaboScape®