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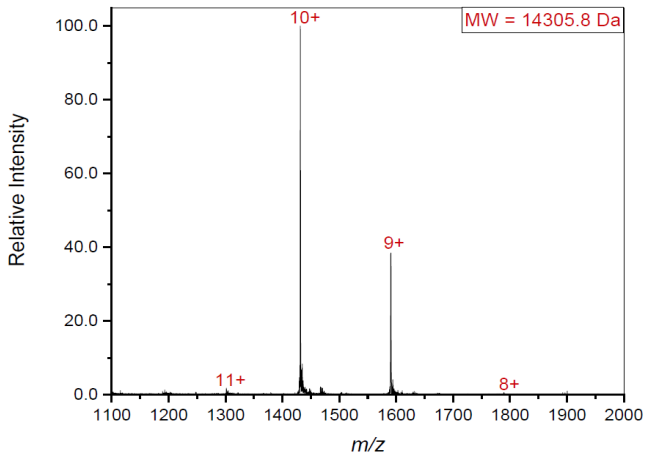

Figure 4: Positive nano-ESI mass spectrum of the sample tuned to the lower m/z range. Multiple charge states of a species with a molecular weight of 14.3 kDa are indicated.

Conclusions

The results from both SDS-PAGE and FTICR-MS of the synthesized sample align to indicate the successful synthesis of gelsolin 1-3. While this has previously been confirmed indirectly by the development of both fluorescence-based and electrochemistry-based biosensors for the detection of lysophosphatidic acid using gelsolin 1-3, this work serves to provide more concrete evidence and structural information about the protein. The total mass was similar to the expected mass of approximately 41 kDa, with small but expected amounts of dimerization being observed. In addition, there was dissociation of a fragment equivalent to the mass of one subdomain from the whole complex, the most likely place for fragmentation to occur. These results combine to support the successful synthesis of gelsolin 1-3, which can now be confidently put to use in future biosensors for the detection of lysophosphatidic acid.

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Data Availability Statement: The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author. The crystal structure depicted in Figure 1 was obtained from RCSB Protein Data Bank under PDB ID: 1RGI; http://doi.org/10.2210/pdb1rgi/pdb.

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Conflicts of Interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Dominguez R, Holmes KC. Actin Structure and Function. Annual Review of Biophysics 40 (2011): 169–186.
2. Patkowski A. Size, Shape Parameters, and Ca^{2+}-Induced Conformational Change of the Gelsolin Molecule: A Dynamic Light Scattering Study. Biopolymers 30 (3-4): 427–435.
3. Bryan J. Gelsolin Has Three Actin-Binding Sites. Journal of Cell Biology 106 (1988): 1553–1562.
4. Schoepper B, Wegner A. Rate Constants and Equilibrium Constants for Binding of Actin to the 1:1 Gelsolin-Actin Complex. European Journal of Biochemistry 202 (1991): 1127–1131.
5. Gremm D, Wegner A. Co-Operative Binding of Ca^{2+} Ions to the Regulatory Binding Sites of Gelsolin. European Journal of Biochemistry 262 (1999): 330–334.
6. McLaughlin PJ, Gooch JT, Mannherz H-G, Weeds AG. Structure of Gelsolin Segment 1-Actin Complex and the Mechanism of Filament Severing. Nature 364 (1993): 685–692.
7. Nag S, Ma Q, Wang H, Chumnarnsilpa S, Lee WL, Larsson M, et al. Ca^{2+} Binding by Domain 2 Plays a Critical Role in the Activation and Stabilization of Gelsolin. Proceedings of the National Academy of Sciences 106 (2009): 13713-13718.
8. Burtnick LD, Koepf EK, Grimes J, Jones EY, et al. The Crystal Structure of Plasma Gelsolin: Implications for Actin Severing, Capping, and Nucleation. Cell 90 (1997): 661–670.

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9. Kwiatkowski DJ, Stossel TP, Orkin SH, Mole JE, et al. Plasma and Cytoplasmic Gelsolins Are Encoded by a Single Gene and Contain a Duplicated Actin-Binding Domain. Nature 323 (1986): 455–458.

10. Burtnick LD, Urosev D, Irobi E, Narayan K, Robinson RC. Structure of the N-Terminal Half of Gelsolin Bound to Actin: Roles in Severing, Apoptosis and FAF. The EMBO Journal 23 (2004): 2713–2722.

11. Goetzl EJ, Lee H, Azuma T, Stossel TP, et al. Gelsolin Binding and Cellular Presentation of Lysophosphatidic Acid. Journal of Biological Chemistry 275 (2000): 14573–14578.

12. Xu Y, Shen Z, Wiper DW, Morton RE, Elson P, Kennedy AW, et al. Lysophosphatidic Acid as a Potential Biomarker for Ovarian and Other Gynecologic Cancers. Journal of the American Medical Association 280 (1998): 719–723.

13. Sedláková I, Vávrová J, Tošner J, Hanousek L. Lysophosphatidic Acid (LPA)-a Perspective Marker in Ovarian Cancer. Tumor Biology 32 (2011): 311–316.

14. SEER*Explorer: An Interactive Website for SEER Cancer Statistics [Internet]. Surveillance Research Program, National Cancer Institute, United States of America; 2024 Apr 17. [updated: 2024 Jun 27].

15. De La Franier B, Thompson M. Detection of the Ovarian Cancer Biomarker Lysophosphatidic Acid in Serum. Biosensors 10 (2020): 13.

16. Ahmadi S, Lotay N, Thompson M. Affinity-Based Electrochemical Biosensor with Antifouling Properties for Detection of Lysophosphatidic Acid, a Promising Early-Stage Ovarian Cancer Biomarker. Bioelectrochemistry 153 (2023): 108466.

17. Davoudian K, Spagnolo S, Lotay N, Satkauskas M, Mészáros G, Hianik T, et al. Design and Characterization of a Dual-Protein Strategy for an Early-Stage Assay of Ovarian Cancer Biomarker Lysophosphatidic Acid. Biosensors 14 (2024): 287.

18. Li H, Nguyen HH, Ogorzalek Loo RR, Campuzano IDG, Loo JA. An Integrated Native Mass Spectrometry and Top-Down Proteomics Method That Connects Sequence to Structure and Function of Macromolecular Complexes. Nature Chemistry 10 (2018): 139–148.

19. Jensen N, Tomer K, Gross M. Fast Atom Bombardment and Tandem Mass Spectrometry of Phosphatidylserine and Phosphatidylcholine. Lipids 21 (1986): 580–588.

20. Rademaker GJ, Heerma W, Haverkamp J. The Fast Atom Bombardment Mass Spectrum and Fragmentation Pathway of N-[2-(Acetamido)-2-Deoxy-β-D-Glucopyranosyl]-L-Asparagine. Biological Mass Spectrometry 21 (1992): 667–671.

21. Hearn JD, Smith GD. A Chemical Ionization Mass Spectrometry Method for the Online Analysis of Organic Aerosols. Analytical Chemistry 76 (2004): 2820–2826.

22. Byrdwell WC. Atmospheric Pressure Chemical Ionization Mass Spectrometry for Analysis of Lipids. Lipids 36 (2001): 327–346.

23. Leney AC, Heck AJR. Native Mass Spectrometry: What Is in the Name? Journal of the American Society for Mass Spectrometry 28 (2017): 5–13.

24. Tamara S, den Boer MA, Heck AJR. High-Resolution Native Mass Spectrometry. Chemical Reviews 122 (2022): 7269–7326.

25. Rose RJ, Labrijn AF, van den Bremer ETJ, Loverix S, Lasters I, et al. Quantitative Analysis of the Interaction Strength and Dynamics of Human IgG4 Half Molecules by Native Mass Spectrometry. Structure 19 (2011): 1274-1282.

26. Pottiez G, Haverland N, Ciborowski P. Mass Spectrometric Characterization of Gelsolin Isoforms. Rapid Communications in Mass Spectrometry 24 (2010): 2620–2624.

27. Sethi S, Dasari S, Amin MS, Vrana JA, Theis JD, et al. Clinical, Biopsy, and Mass Spectrometry Findings of Renal Gelsolin Amyloidosis. Kidney International 91 (2017): 964–971.