POLITECNICO DI TORINO
Repository ISTITUZIONALE

Rational design of freeze-drying formulations: A Molecular Dynamics approach

Original
Rational design of freeze-drying formulations: A Molecular Dynamics approach / Arsiccio, A., Pisano, R.. - STAMPA. - (2019), pp. 55-56. (9th International Symposium on Lyophilization of Pharmaceuticals Ghent September 2-6, 2019).

Availability:
This version is available at: 11583/2939700 since: 2021-11-23T15:23:29Z

Publisher:
International Society of Lyophilization/Freeze-Drying

Published
DOI:

Terms of use:

This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

Publisher copyright

(Article begins on next page)

08 September 2026

ISLFD 2019 – 9th International Symposium on Lyophilization of Pharmaceutials
Ghent, Belgium, 2-6 September 2019
DOI: http://dx.doi.org/XXX

Rational design of freeze-drying formulations: A Molecular Dynamics approach

Arsiccio, A.[a]; Pisano, R.[a*]

[a] Department of Applied Science and Technology, Politecnico di Torino, Torino, Italy

*E-mail of the corresponding author: roberto.pisano@polito.it

Abstract

Even though the freeze-drying process is often applied to biopharmaceuticals, it may result in protein unfolding or aggregation, and suitable excipients should therefore be added to avoid loss of activity. However, the choice of the formulation is, at present, mostly empirical, due to a lack of knowledge about the phenomena involved. Here, molecular dynamics is used to understand the molecular mechanisms at the basis of protein stabilization, and guide the choice of suitable excipients.

Introduction

Freeze-drying is the most common technique for the storage of protein-based drugs in the solid state. However, several stresses could arise during the process, that may result in loss of therapeutic activity. Some excipients should therefore be added to stabilize the protein and prevent its denaturation.[1] Here we propose an approach, based on molecular dynamics (MD) simulations, capable of identifying the formulation that mostly preserves the biological activity of proteins to be freeze dried. We will show how this in silico approach could be used to clarify the mechanisms of protein stabilization by typical pharmaceutical excipients, including sugars, polyols and amino acids.

Materials and Methods

Molecular dynamics is used to study the interaction between some model proteins, namely, human growth hormon (hGH) and lactate dehydrogenase (LDH) (Fig. 1a), and typical pharmaceutical excipients. A typical simulation box is shown in Fig. 1b. During freezing, the preferential exclusion of excipient molecules from the protein surface [2], which should stabilize the protein against unfolding, can be quantified from the MD trajectory. The strength of the hydrogen-bonding network formed during drying can also be computed, making it possible to evaluate the degree of protein stabilization in the solid state.[3]

Results and Discussion

We observed that the mechanisms of protein stabilization change significantly during the freeze-drying process, mostly as a result of the increase in excipient concentration. Preferential exclusion prevails during freezing, while MD simulations suggest that the mechanisms of lyoprotection should be related to the formation of a dense, compact hydrogen bonding network between the formulation components. It was also observed that not all the excipients are equally effective; for instance, the disaccharides should be better

than polyols and amino acids both during freezing and in the dried state (see Fig. 1c). According to our simulations, small differences may exist among the disaccharides, as well, with sucrose and lactose being extremely good cryoprotectants, and trehalose and cellobiose being slightly better for lyoprotection. Some molecular properties seemed to correlate with the protective effect of stabilizers. The higher the molecular volume was, the more the osmolyte was excluded from the protein surface. By contrast, a high hydrogen bonding propensity was linked to the efficiency as a lyoprotectant.

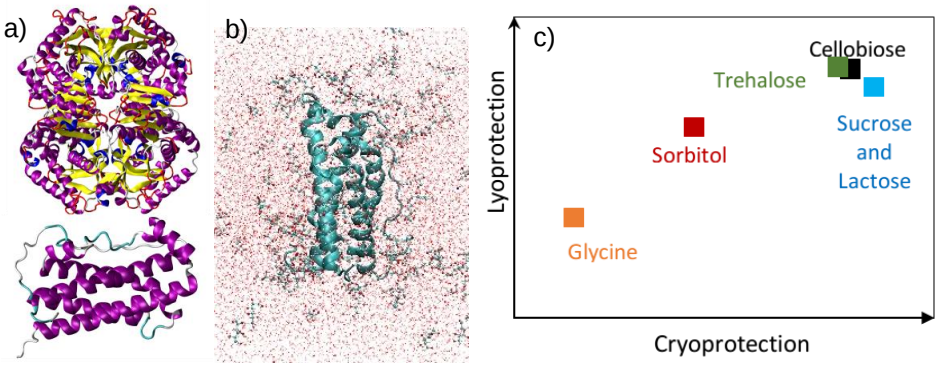

Fig. 1 *a) Cartoon structures of LDH (top) and hGH (bottom). b) Example of an MD simulation box. c) Graphic representation of the efficiency of common pharmaceutical excipients as lyo- (y-axis) or cryo- (x-axis) protectants.*

Conclusions

Our study shows that MD simulations allow the identification of the molecular properties at the basis of protein stabilization, thus simplifying the choice of the formulation. This approach could be combined with experimental techniques to improve the stability of freeze-dried biopharmaceuticals.

References

[1] W. Wang, Lyophilization and development of solid protein pharmaceuticals, Int. J. Pharm. 203 (2000) 1-60.

[2] T. Arakawa, S.N. Timasheff, Stabilization of protein structure by sugars, Biochemistry 21 (1982) 6536-6544.

[3] A. Arsiccio, R. Pisano, Water entrapment and structure ordering as protection mechanisms for protein structural preservation, J. Chem. Phys. 148 (2018) 055102.