Solid state stabilization of proteins by sugars

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1 Introduction & Thesis outline

INTRODUCTION & THESIS OUTLINE

Over the past decades protein drugs have gone from being of little therapeutic relevance to being a cornerstone of modern day medicine. Since the introduction of the first recombinant protein drug (insulin) in 1982, the number of protein drugs on the market has increased drastically to well over 100 in 2016.[1–3] Protein drugs have provided immense improvements for various therapeutic areas like metabolic disorders, oncology, and inflammatory diseases (e.g. rheumatoid arthritis). This is mainly because of their unprecedented specificity, allowing for high effectivity with limited side-effects.[1]

A large part of the protein drugs which are currently on the market are liquid formulations. A major disadvantage of liquid protein formulations is their limited shelf life.[4] Proteins in solution often deteriorate rapidly if they are not refrigerated continuously, causing them to lose functionality and potentially become immunogenic.[4] To achieve an acceptable shelf life, protein solutions are therefore dependent on refrigerated handling and storage, the so-called cold chain. Maintaining this cold chain is expensive and basically not feasible in developing, often tropical regions.[5] Circumventing this cold chain would thus reduce costs of storage and transport, and improve availability of protein drugs in those areas. Moreover, it improves safety.

Drying proteins in the presence of sugars can provide stabilization and increase protein shelf life, even at elevated temperatures, eliminating the need for the cold chain.[6] Much research has focused on how sugars stabilize proteins in the solid state in a general sense.[7,8] How sugars protect against drying and other stress conditions in a general sense is discussed in chapter 8. However, less attention has gone into why some sugars are more suitable than others and therewith which sugar characteristics are desirable for protein stabilization. In this dissertation, this latter topic will be addressed in more depth.

First it is shown that size and molecular flexibility of a sugar influence their ability to stabilize proteins (chapter 2). This was investigated by freeze-drying (lyophilizing) several sugars of different size and molecular flexibility with 4 different model proteins and comparing the ability of the sugars to maintain protein functionality during storage at elevated temperature. To enable investigation of the role of molecular flexibility of the sugars, a polysaccharide (sugar polymer) with an exceptionally flexible backbone, inulin, was used. Because inulin is unique in its molecular flexibility and plays a key role in this thesis, the physicochemical characteristics of this sugar are reviewed (chapter 3) and related to its pharmaceutical applications, including stabilization of proteins (chapter 4).

In the second part of this dissertation, it is mechanistically discussed why size and molecular flexibility of sugars are important for protein stabilization. Using in-line near infrared spectroscopy during freeze-drying it is shown that smaller and molecularly more flexible sugars form more hydrogen bonds with a model protein during the last phase of freeze-

drying because they are less affected by steric hindrance, resulting in improved storage stability (chapter 5). Similar results are found in the solid state using terahertz time domain spectroscopy, where smaller sugars show better interactions with another model protein (chapter 6). Furthermore, it is shown by solid-state nuclear magnetic resonance spectroscopy that larger sugars have a higher tendency to phase-separate from the protein, resulting in reduced protein stabilization (chapter 7). Lastly, an overview is provided of the general theories regarding stabilization of proteins by sugars in combination with the new knowledge presented here (chapter 8). There, common degradation routes and stress factors are reviewed and related to how sugars protect against them.

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