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Review article

How sugars protect proteins in the solid state and during drying (review): Mechanisms of stabilization in relation to stress conditions

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ABSTRACT

This review aims to provide an overview of current knowledge on stabilization of proteins by sugars in the solid state in relation to stress conditions commonly encountered during drying and storage. First protein degradation mechanisms in the solid state (i.e. physical and chemical degradation routes) and traditional theories regarding protein stabilization (vitrification and water replacement hypotheses) will be briefly discussed. Secondly, refinements to these theories, such as theories focusing on local mobility and protein-sugar packing density, are reviewed in relationship to the traditional theories and their analogies are discussed. The last section relates these mechanistic insights to the stress conditions against which these sugars are used to provide protection (i.e. drying, temperature, and moisture). In summary sugars should be able to adequately form interactions with the protein during drying, thereby maintaining it in its native conformation and reducing both local and global mobility during storage. Generally smaller sugars (disaccharides) are better at forming these interactions and reducing local mobility as they are less inhibited by steric hindrance, whilst larger sugars can reduce global mobility more efficiently. The principles outlined here can aid in choosing a suitable sugar as stabilizer depending on the protein, formulation and storage condition-specific dominant route of degradation.

Contents

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1. Introduction

Over the past decades, the importance of protein therapeutics for the pharmaceutical industry has grown from a nearly negligible role to being a primary focus. As proteins are generally not stable for prolonged periods of time, formulation scientists faced many challenges in achieving sufficient shelf life for these protein therapeutics [1,2]. A lot of these challenges have been overcome, as is illustrated by the fact that in 2015 nearly 30% of drugs newly registered at the United States Food and Drug Administration (FDA) were protein drugs [3]. However, all but 1 of these protein drugs are liquid formulations which require refrigerated (2–8 °C) storage and transportation, the so-called cold chain, whereas the remaining dry powder formulation (mepoluzimab, Nucala®) must be stored and transported below 25 °C, see Table 1.

Maintaining the cold chain regime is costly and particularly provides difficulties in remote areas of developing, often tropical, countries [4]. One of the proven strategies to overcome this, is to dry proteins in the presence of stabilizers like sugars [5–7]. The number of licensed lyophilized protein drugs has also steadily grown, Table 2 provides an overview of lyophilized protein drugs which received a biological license approval by the FDA since 2011. A plethora of research on the topic of drying proteins with sugars has been published by scientists from food and pharmaceutical sciences, describing various aspects of how these sugars stabilize proteins. This review aims to provide an overview of the current knowledge regarding the mechanisms behind stabilization of proteins by sugars in the solid state in relationship to stress conditions commonly encountered during production and storage. First protein degradation mechanisms and traditional theories regarding protein stabilization will be briefly discussed; secondly refinements to these theories and how they come together will be reviewed. The last section will relate stress conditions to how sugars protect against them.

2. Degradation

Degradation of proteins commonly leads to a loss of functionality and formation of potentially immunogenic products [8]. To understand stabilization of proteins an understanding of how proteins can degrade is important. Therefore, the main mechanisms of degradation of proteins, classified as either physical or chemical degradation, will be addressed here briefly. For more in-depth information the reader is directed to several extensive reviews on this topic [2,9–11].

The most common physical degradation mechanisms are denaturation and noncovalent aggregation. Denaturation is the unfolding of the three-dimensional structure of the protein. This can be caused by various stresses such as heat, shear stress, exposure to interfaces, or chemical factors [2,9]. Denaturation can occur in the solid state but is more likely to happen when the protein is dissolved in a liquid and during drying [5,6,9,12,13]. Generally in the native conformation hydrophobic parts of the protein are folded inward and unfolding/denaturation results in these groups being exposed on the outside of the protein's three-dimensional structure [9]. The increased surface area and exposed hydrophobic groups of unfolded or partially refolded proteins increase the risk of adsorption and non-covalent aggregation [1,14]. Therefore, non-native proteins have a higher tendency to aggregate than native proteins [15,16]. Aggregation is in most cases irreversible [1]. Furthermore, aggregates in liquid formulations can be qualified as either soluble or insoluble and when aggregate size increases, sedimentation (or floating) will eventually occur [12].

Important chemical degradation mechanisms include covalent aggregation, deamidation, oxidation, and Maillard browning. Chemical covalent aggregation, rather than physical non-covalent aggregation, is the predominant route of aggregation in the solid state [17,18]. Chemical aggregation is in most cases linked to a thiol-disulfide interchange in the protein, and is accelerated by residual moisture or exposure to atmospheric water. Many other chemical degradation mechanisms (i.e. oxidation, deamidation, Maillard browning) are also dependent on moisture content (see Section 4.3). Other factors affecting these chemical degradation reactions include storage temperature, excipients, the physical state of the excipients (e.g. liquid, amorphous, crystalline), and obviously the chemical composition of the protein [11]. In the context of stabilizing proteins with sugars, Maillard browning is of particular interest as it involves reducing sugars. Maillard browning starts with a reaction between the aldehyde or ketone group of the reducing sugar and the amino group of the protein forming a Schiff's base and is followed by a cascade of reactions eventually leading to the formation of covalent aggregates [19].

3. Theories on stabilization by sugars

Two theories on the mechanism of stabilization of sugars on proteins in the solid state, the vitrification theory and water replacement theory, have been around for several decades and have been widely discussed in literature [20,21]. More recently, refinements and new theories focusing on global and local mobility of the protein, molecular flexibility of the sugar, and protein-sugar miscibility on a molecular level have been published.

3.1. Classic theories: vitrification and water replacement

Stabilization of bioactive proteins is traditionally based on two approaches: the vitrification theory which describes alterations in reaction kinetics and the water replacement theory which is based

Table 1
Overview of protein drugs newly registered at the United States Food and Drug Administration (FDA) in 2015, their type, physical form, and storage temperatures [3].

Protein	Trade name	Type	Form	Storage temperature
Alirocumab	Praluent	Monoclonal antibody	Liquid	2–8 °C
Asfotase alfa	Strensiq	Enzyme	Liquid	2–8 °C
Daratumumab	Darzalex	Monoclonal antibody	Liquid	2–8 °C
Dinutuximab	Unituxin	Monoclonal antibody	Liquid	2–8 °C
Elotuzumab	Empliciti	Monoclonal antibody	Liquid	2–8 °C
Evolocumab	Repatha	Monoclonal antibody	Liquid	2–8 °C
Idarucizumab	Praxbind	Monoclonal antibody	Liquid	2–8 °C
Insulin degludec	Tresiba	Hormone	Liquid	2–8 °C
Mepolizumab	Nucala	Monoclonal antibody	Lyophilized powder	>0 °C; <25 °C
Necitumumab	Portrazza	Monoclonal antibody	Liquid	2–8 °C
Recombinant human parathyroid hormone	Natpara	Hormone	Liquid	2–8 °C
Sebelipase alfa	Kanuma	Enzyme	Liquid	2–8 °C
Secukinumab	Cosentyx	Monoclonal antibody	Liquid	2–8 °C