Journal of Pharmaceutical Sciences xxx (2017) 1-8

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Mini Review

Arginine as an Excipient for Protein Freeze-Drying: A Mini Review

Peter Stärtzel*

Division of Pharmaceutics, Friedrich-Alexander University (FAU) Erlangen-Nürnberg, 91058 Erlangen, Germany

ARTICLE INFO

Article history:
Received 23 August 2017
Revised 26 October 2017
Accepted 16 November 2017

Keywords:
Freeze-drying
Lyophilization
Drying
Protein formulation
Solid-state stability
Protein aggregation
Arginine
Amino acids
Amorphous solids
Glass transition

ABSTRACT

Successful development of marketable freeze-dried protein formulations requires adequate stabilization of the active biopharmaceutical ingredient. The choice of a stabilizer must therefore be based on sound knowledge of the physical and chemical properties of the excipients and specific needs of the protein component. Amino acids, such as arginine, have exhibit cryo- and lyoprotective effects similar to those of sugars and polymers and may therefore be considered to be an alternative approach to these established formulation strategies. The chemical structure and physicochemical characteristics of arginine are unique among amino acids and can provide additional benefits to freeze-dried protein formulations with regard to liquid and solid-state stability. This mini review provides a brief summary of research focused on the application of arginine in freeze-dried protein pharmaceuticals, including a discussion of its basic physical and chemical attributes as well as thermal behavior in the frozen and solid states. Mechanisms contributing to solid-state stabilization by arginine are discussed in the context of available stability studies on arginine-containing protein formulations. This mini review seeks to deepen the understanding of the opportunities and challenges associated with arginine-based preparations for freeze-dried protein pharmaceuticals.

Introduction

Therapeutic protein formulations are becoming increasingly important on the pharmaceutical market, but their formulation often imposes significant challenges because many proteins are unstable in the liquid state and are prone to chemical and physical degradation.[1-3] Among the various degradation pathways, aggregation is the most common and is also the most challenging pathway given that low amounts of aggregated proteins can trigger immunogenic responses *in vivo*.[4-8] A relatively simple and efficient method to prevent aggregation with minimum loss in therapeutic activity is to transfer the protein into a solid form, which can be achieved using freeze-drying.[9,10] However, this process imposes several different stress vectors associated with freezing and dehydration. In addition to chemical degradation in solution and solid states arising from molecular modifications involving covalent bonds (e.g., hydrolysis, deamination, oxidation, and disulfide bond shuffling), physical protein degradation plays an important role.[4,11] During freezing, cold denaturation on temperature decreases, and phase separation due to ice crystallization challenges protein stability.[12,13] The continuous change in concentrations of the protein and other unfrozen solutes (freeze concentration) may result in pH changes or liquid phase separations that can also cause protein degradation.[14-16] During the subsequent drying phase, water molecules are removed from the protein surface.[17] The loss of its hydration shell may lead to destabilization of the native protein structure and hence its inactivation. All of these effects may compromise the therapeutic efficacy and shelf-life stability of proteins even in the freeze-dried state.[18]

To improve protein stability, stabilizing excipients (typically sugars or sugar/polymer mixtures) are often added to the formulation to prevent protein inactivation during freezing (cryoprotection), drying (lyoprotection), and storage.[19-23] The basic mechanisms of cryoprotective stabilization are comparable to thermodynamic protein stabilization in solution.[23] Stabilizing additives are preferentially excluded from the protein surface, because the interaction between the co-solute and the protein is thermodynamically unfavorable. Because unfolding of the protein structure would possess an even greater surface area, which would increase the systems' free energy, the folded (native) conformation is stabilized.[24] However, cryoprotectants need to remain amorphous during freeze concentration to effectively protect the protein structure.[22,25,26] Stabilization in the solid state is based on 2 major concepts: the water replacement theory (thermodynamic stabilization)[27-29] and the vitrification theory (kinetic stabilization).[19,20] Water replacement means that during

* *Correspondence to:* Peter Stärtzel (Telephone: +49-9131-852-9551; Fax: +49-9131-852-9545).
E-mail address: peter.staertzel@fau.de (P. Stärtzel).

https://doi.org/10.1016/j.xphs.2017.11.015
0022-3549/

drying the stabilizer molecules replace water molecules by hydrogen bonding to the protein.[30] This preserves the native structure of the protein despite removal of water during drying. The vitrification theory is based on mechanical immobilization (suppression of global motions, also referred to as α-relaxation) in an amorphous glassy matrix. α-relaxation occurs due to translational or rotational molecular motions, potentially leading to protein aggregation.[21] Restricting molecular mobility significantly slows down protein degradation. Given that the protein is well immobilized (glass transition temperature T_g of the amorphous glass 10°C-20°C above storage temperature), water replacement is the predominant mechanism of stabilization.[31,32] However, vitrification becomes limiting with storage temperature at or above T_g.[31] Such rigorous control of storage conditions is costly and can become a challenging task when it comes to worldwide drug product distribution including remote areas in developing, sometimes even tropical, countries.[32] This raises a continuous need to investigate new approaches and excipients for protein stabilization in liquid and solid state.

Recent studies have reported lyo- and cryoprotective effects of different types of amino acids and their salts with mechanisms similar to those established for disaccharides, polymers, or polyols.[33-36] The most frequently used amino acid in protein manufacturing, purification and formulation over the past 2 decades is arginine.[37-39] Arginine is preferred among amino acids due to its unique chemical structure. Its application in protein formulations available for freeze-drying has been subject to a number of patents using arginine as a stabilizer,[40-43] buffer,[44,45] solubilizer,[46,47] or tonicity-modifying excipient.[48] Arginine is a natural metabolite in organisms and cells and part of the human diet with an inherently low toxicity.[49,50] According to available safety information, arginine is considered "practically nontoxic" when taken appropriately and administered by injection.[50,51] The physicochemical properties of arginine in the presence of different counter-ions improve the solid-state stability of freeze-dried protein pharmaceuticals.[34,52,53] However, long before the discovery of cryo- and lyoprotective effects, arginine was shown to increase the solubility of proteins and to minimize aggregation in solution. Its unique effect on protein aggregation was discovered almost serendipitously in the late 1980s by Rudolph and coworkers[37,54] seeking for a way to prevent autocatalytic digestion of refolded tissue-type plasminogen activator. The increase in the refolding yield of tissue-type plasminogen activator with the addition of arginine was associated with an increased solubility of aggregate-prone folding intermediates, without a significant effect on conformational stability of the native protein structure itself.[55,56] This is in contrast to other additives like sugars or polyols which stabilize the most compactly folded protein species (in most cases the native state) by preferential exclusion.[57,58] Arginine weakly binds to the protein surface by interacting with amino acid side chains and the peptide backbone but is simultaneously repelled due to an increase in surface tension and volume exclusion effects.[39,49] The net effect of these opposing forces is concentration dependent and ends with exclusion of the arginine molecules at a concentration above 0.5 M.[59] In addition, the arginine counter-ion was found to affect protein stabilization in the liquid state, basically following the empirical principles of the Hofmeister series.[38] Although a generally accepted understanding of the molecular mechanism has yet to be determined,[38,56,60-62] the positive effect of arginine on protein refolding has already been demonstrated for a series of protein systems.[37,54,55,63,64] Moreover, arginine (≥0.15 M) has been reported to reduce viscosity of highly concentrated antibody formulations, which should improve the applicability of these often highly viscous liquids.[24] These properties in the liquid state may provide additional benefits for the manufacturing of freeze-dried protein drugs because protein aggregation during storage and handling of the mostly aqueous product solutions before filling into the vial, syringe, or cartridge and after reconstitution may be significantly reduced. This feature renders arginine particularly interesting as an additive to formulate labile proteins for commercial freeze-drying on a large scale with significant hold times throughout the fill to finish process.[34,62]

In this mini review, a summary of research focused on the application of arginine in freeze-dried protein formulations is provided. Its potential as a successful stabilizer is discussed by reviewing general physical and chemical characteristics of the arginine molecule and its thermal properties in the frozen and solid state that are relevant to the freeze-drying process. An overview of available stability studies of protein systems containing arginine is presented to illustrate examples of applications in formulation design. The current knowledge about mechanisms that likely contribute to protein stability in arginine-based systems is also taken into account. The goal of this mini review is to increase insight into the opportunities and challenges associated with arginine-based preparations for freeze-dried protein pharmaceuticals.

General Chemical and Physical Properties of Arginine

Generally, arginine belongs to the chemical group of amino acids that are defined as molecules that possess a carboxyl group, an amino group, and a specific side chain bound to the same carbon atom. The pKa difference between the basic amino group (pKa 6.5-10.6)[65] and the acidic carboxyl-function (pKa 1.8-2.6)[65] of amino acids implies that amino acids are zwitterionic in aqueous solution, which makes them useful natural buffer systems.[65] However, most of the physical and chemical properties of these molecules are defined based on side chain properties.[66] This is why amino acids are often classified according to these side chain characteristics, typically including the major classes of (a) charged, (b) hydrophobic, or (c) polar amino acids.[66]

The molecular structure of arginine can be divided into 3 main segments: the polar terminal (consisting of the amine and carboxylic group), the aliphatic segment (with the 3 methylene groups), and the guanidinium terminal (Fig. 1). With a pKa of 13.8,[68] the guanidinium group is positively charged in acidic, neutral and even most basic environments. Thus, arginine is typically classified as a "positively charged" amino acid. The positive charge is delocalized due to conjugation between the double bonds and the lone pairs of the nitrogen atoms, facilitating the formation of multiple hydrogen bonds. Arginine is the most hydrophilic among the protein building amino acids and exhibits the potential to form up to 7 hydrogen bonds per molecule.[69] The potential to form hydrogen bonds between molecules is critical to protein stabilization prior to freeze-drying in solution, during drying, and in freeze-dried solids.[19] With an isoionic point of pH 10.76,[70] the unadjusted pH of arginine-base in aqueous solution is close to pH 11.[68] This is an unfavorable environment to formulate the majority of parenteral administered proteins, which typically show optimum stability at weak acidic to neutral conditions.[34] Without pH adjustment or buffering, pharmaceutical activity of these molecules will be lost under such extreme pH-conditions. Therefore, arginine is rarely used as the free base in protein formulations, but in most cases in a particular salt form (most often as the hydrochloride salt). Within the following sections of this article, the arginine-base is referred to as "arginine", whereas the use of a specific arginine salts is specified wherever applicable.

Another aspect relevant to the physical properties of arginine is its rather small molecular weight (174.2 g/mol).[67] Smaller than typical disaccharides, such as sucrose (342.3 g/mol),[71] arginine is best to be compared with excipients, such as polyols (e.g., sorbitol = 182.2 g/mol or glycerol = 92.1 g/mol).[72,73] Small molecular weight components (e.g., glycerol) can effectively decrease the free volume