Advanced Drug Delivery Reviews 93 (2015) 2–13

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Chemical degradation of proteins in the solid state with a focus on photochemical reactions☆

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ARTICLE INFO

Available online 4 December 2014

Keywords:
Protein
Solid
Formulation
Stress
Light
Photochemistry
Stability

ABSTRACT

Protein pharmaceuticals comprise an increasing fraction of marketed products but the limited solution stability of proteins requires considerable research effort to prepare stable formulations. An alternative is solid formulation, as proteins in the solid state are thermodynamically less susceptible to degradation. Nevertheless, within the time of storage a large panel of kinetically controlled degradation reactions can occur such as, e.g., hydrolysis reactions, the formation of diketopiperazine, condensation and aggregation reactions. These mechanisms of degradation in protein solids are relatively well covered by the literature. Considerably less is known about oxidative and photochemical reactions of solid proteins. This review will provide an overview over photolytic and non-photolytic degradation reactions, and specially emphasize mechanistic details on how solid structure may affect the interaction of protein solids with light.

Contents

1. Introduction

In the future, pharmaceutical development will be increasingly dominated by biologics produced in organisms. Biologics developed by the biotechnology industry encompass a wide range of products

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Protein stability in drug delivery applications".

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http://dx.doi.org/10.1016/j.addr.2014.11.016
0169-409X/

including monoclonal antibodies, blood products, and vaccines [1]. Most of the biologics currently under development target the treatment of cancer, followed by cardiovascular diseases, autoimmune and hormone disorders [2]. The development cost for a biologic is estimated to $1–2 billion but this cost does not discourage pharmaceutical companies to expect profits from biologics currently in late-stage development. In the long-term, the development of biosimilars may save costs, and their production will be guided by regulatory agencies, and will depend on the degree to which cost saving measures are required by national health systems and medical insurers. Economic studies show that biologics should drive the market's growth for the next few years [3]. The fragile nature of protein structure represents a major limiting factor for the development of protein therapeutics. The instability of proteins renders them susceptible to multiple degradation routes during manufacturing, storage, and handling. A prerequisite for the production of safe protein drugs is to avoid any chemical and physical degradation processes that may reduce potency, limit shelf life, and could increase the potential for immunogenic side effects. In a first approximation, the internal energy of a protein in a solid, in comparison to that in solution, is close to zero, making lyophilized solids products of high interest to prevent the chemical and physical degradation of proteins.

The physico-chemical properties of protein solids are essentially determined by their thermal history [4]. Drying conditions (e.g. temperature, vacuum, time) affect the thermal history of the solid. Because the shell of water around a protein affects its structure, the removal of water can irreversibly change protein structure. Therefore, making the freeze-drying process of proteins is a determinant step which controls the nature of the solid, and ultimately its final physico-chemical properties, that will limit the long-term stability of the final product [5]. Mechanistic studies of protein degradation in solids need to take into account the types of solids eventually produced during the drying process. Because of the importance of thermal processes during the transformation of a liquid protein formulation to its equivalent in the solid state, most stability studies of protein drugs in the solid state have essentially focused on the impact of variation of temperature, pH, and the presence of surfactants or excipients during the drying process and the subsequent storage of the products. Mechanistic studies of degradation reactions such as deamidation [6–8], the Maillard reaction [9,10], hydrolysis [6,11,12], diketopiperazine formation [13–15], and β-elimination at cysteine (Cys) [16], were extensively performed and reviewed. Costantino's thesis presents an extensive description of the most important oxidation reactions [17]. In the present review, we will cover degradation reactions in general and specifically emphasize photochemical reactions encountered by proteins in solids. For many years the photostability of proteins has not been of great concern. However, the number of photo-labile drugs, and protein drugs, is increasing, resulting in guidance from the European Pharmacopeia to protect more than 250 different drugs from light [18,19]. In this regard, the underlying photochemical processes must be clearly identified to develop better guidelines for the testing of protein photostability [20,21]. More importantly, photochemical stability studies offer a versatile toolbox to investigate the effects of formulation on protein degradation: photochemical reactions can be initiated *after* preparation of the respective solid, the duration of primary photochemical processes can be controlled through the duration of light exposure, and quantum yields for solid state photochemical reactions can be determined by means of an integrating sphere.

2. Photochemical reactions

Most drug substances are formulated as white powders, minimizing the absorption of visible light by these formulations. However, all lamps, even incandescent ones, emit some radiation in the ultraviolet (UV) region of the spectrum. Scheidegger et al. observed that proteins in whole and skim milk underwent severe oxidative damage (e.g., formation of dityrosine, N-formylkynurenine, fragmentation) after the exposure to fluorescent light [22]. Thus, the effect of UV-light exposure needs to be evaluated. From production to delivery, a protein is exposed to light from various light sources [19]. The photochemistry of tryptophan (Trp) [23,24], tyrosine (Tyr) [24–26], phenylalanine (Phe) [24,27], and cystine [24,28] has been well documented, predominantly for the individual amino acids but also for these amino acid residues located in peptides and proteins [29–33]. Protein conformation plays an important role in protein photo-degradation processes [19,34]. Variation of protein conformation can be achieved by modification of pH, ionic strength, and the presence of ligands, which may change sites for energy transfer(s), which ultimately generate reactive species [35,36]. For example, the structural properties of α-crystalline are modified when the protein is subjected to UV-C irradiation. The primary degradation reactions, which correspond to the oxidation of the methionine residue (Met1) and the racemization of aspartate (Asp151), contribute to the alteration of secondary structure of α-crystalline [37].

While the photo-degradation of a protein is initiated by the exposure to light, final product formation can depend on a series of complex processes such as the formation and reaction of excited states, radical species, and energy transfer [38]. The respective extents of these processes depend on the wavelengths, the intensity of light, the time of light exposure, as well as the geometry of the photo-irradiated sample (e.g. the nature of the container, the distance between the light source and the sample, and the orientation of the sample towards the flux of light). All these parameters ultimately control the dose of photo-irradiation, which can be determined by actinometry [39]. However, at any given dose the processes taking place in the solid upon light exposure need to be defined. To address this issue, we will briefly introduce the photophysics of proteins in solids, and we will review the nature of photoproducts observed during light exposure of solid protein formulations, together with a mechanistic rationale for product formation. A comparison of the photochemical behavior of proteins in solution and in the solid state needs to take into account multiple variables. In solution, the temperature range for the thermal degradation of a protein is limited to the physical properties of the solvent itself, and, as we will see later, the thermal processes are essentially but not exclusively represented by hydrolysis and deamidation reactions. During photo-degradation the energy of the photons usually exceeds the thermal activation energy [39,40]. Therefore, primary photochemical reactions are rather independent of temperature. However, secondary processes may depend on temperature. The rates of photo-degradation in solution and in the solid state are different since the probability of photon absorption by solid matter is lower than in solution. The latter is essentially rationalized by the radiative nature of light and the lack of transparency of most solids, which is, in part, related to the reflection of photons at the surface of the solid. According to the Beer-Lambert law, the intensity of an electromagnetic wave inside a material (I) decreases exponentially from the surface as described in Eq. 1, where I_0 is the intensity of the incident light, z the depth of the material, and κ a constant relative to the nature of the material. That is why the crystal structures of solids can be identified solely from the diffraction [41] of a wave front and light scattering [41] of incident X-ray photons, for which the penetration depth is optimal. The latter raises the question of how does penetration depth vary with the wavelength of incident light? For X-rays, in a first approximation, the penetration depth and the absorption of the radiation increase as the wavelength of incident light decreases. The latter is only true if the material has a constant conductivity over a given bandwidth. In such case the absorption of the radiation within the material is exponentially dependent of the penetration depth in terms of wavelengths. X-ray penetration increases with decreasing wavelength because the cross-section of the material increases by λ^3. Now, if photons are absorbed and not refracted or diffracted by the solid, the atomic lattice starts to vibrate, leading to a net displacement of electric charges. However, it is impossible for the positive charge lattice (the nuclei) to vibrate in unison with the negative charges (the electrons). Thus, layers of charge density will appear along with the vibrations. The displacement of charges generates a local electric