International Journal of Pharmaceutics 523 (2017) 238–245

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Correlation between calculated molecular descriptors of excipient amino acids and experimentally observed thermal stability of lysozyme

Helena Meng-Lund[a,1], Natascha Friis[a,1], Marco van de Weert[a], Jukka Rantanen[a], Antti Poso[b,c], Holger Grohganz[a,*], Lene Jorgensen[a]

[a] Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark
[b] University of Eastern Finland, School of Pharmacy, Yliopistonranta 1, P.O. Box 1627, FI-70211 Kuopio, Finland
[c] University Hospital Tübingen, Dept. of Internal Medicine I, Division of Translational Gastrointestinal Oncology, Otfried-Müller-Strasse 10, 72076 Tübingen, Germany

ARTICLE INFO

Article history:
Received 7 December 2016
Received in revised form 8 February 2017
Accepted 19 March 2017
Available online 21 March 2017

Keywords:
Lysozyme
Amino acids
Thermal stability
Molecular descriptors
QSPR
PLS regression

ABSTRACT

A quantitative structure-property relationship (QSPR) between protein stability and the physicochemical properties of excipients was investigated to enable a more rational choice of stabilizing excipients than prior knowledge. The thermal transition temperature and aggregation time were determined for lysozyme in combination with 13 different amino acids using high throughput fluorescence spectroscopy and kinetic static light scattering measurements. On the theoretical side, around 200 2D and 3D molecular descriptors were calculated based on the amino acids' chemical structure. Multivariate data analysis was applied to correlate the descriptors with the experimental results. It was possible to identify descriptors, *i.e.* amino acids properties, with a positive influence on either transition temperature or aggregation onset time, or both. A high number of hydrogen bond acceptor moieties was the most prominent stabilizing factor for both responses, whereas hydrophilic surface properties and high molecular mass density mostly had a positive influence on the unfolding temperature. A high partition coefficient (logP(o/w)) was identified as the most prominent destabilizing factor for both responses. The QSPR shows good correlation between calculated molecular descriptors and the measured stabilizing effect of amino acids on lysozyme.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Proteins are sensitive to chemical and physical degradation during manufacturing and storage. Excipients with stabilizing functionality are usually chosen based on experience and prior knowledge, but this knowledge cannot be easily generalized for all proteins and the mechanisms by which the excipients stabilize are not always well understood. Thus it would be desirable to establish a correlation between general physicochemical parameters of the excipients calculated *in silico* and their effect on experimentally observed protein stability. This may also help to find new suitable excipients that have not been considered previously. One category of excipients is amino acids, which are well-known stabilizers for both liquid and solid-state protein formulations (Arakawa et al., 2007b; Forney-Stevens et al., 2015; Jorgensen et al., 2009a; Soenderkaer et al., 2005; Wang, 1999). L-Arginine, L-glycine, L-glutamate and L-histidine are examples of amino acid excipients in approved protein pharmaceuticals such as human tissue plasminogen activator, human growth hormone, streptokinase and coagulation factor IX, respectively (Arakawa et al., 2007b). However, little is known about which physicochemical properties of stabilizing amino acids are responsible for the observed positive effect on proteins. In a recent study using human serum albumin and α-chymotrypsin (Forney-Stevens et al., 2015), weak correlations were found between the stabilizing effect of amino acids and their side chain charge and the molar volume. It would therefore be of interest to further explore the possibility to establish a relationship between protein stability and the physicochemical properties of the amino acids calculated based on their chemical structure, *i.e.*, establish a quantitative structure-property relationship (QSPR) (Grover et al., 2000). Of course one size does not fit all, and the stabilizing effect of amino acids can be expected to contain a generic property (thus protein-independent) and a protein-specific component. If the latter dominates, it is likely difficult to

* Corresponding author.
E-mail address: holger.grohganz@sund.ku.dk (H. Grohganz).
[1] Contributed equally.

http://dx.doi.org/10.1016/j.ijpharm.2017.03.043
0378-5173/© 2017 Elsevier B.V. All rights reserved.

prepare a QSPR, whereas in the former case it should be more feasible. A more elaborate model including the properties of both the amino acids and proteins is possible but would require a very large data set. If a predictive model can be generated based on molecular descriptors, good stabilizing excipients for therapeutic protein formulation may be predicted from the chemical structure alone.

Quantitative structure-activity relationships (QSAR) and QSPR have been used in drug discovery and development for many decades. The possibility to predict certain desired properties of *e.g.* new potential drug compounds can prevent synthesis of compounds with suboptimal structures (Grover et al., 2000). Within pharmaceutical research the QSAR/QSPR approach has been used for a wide variety of applications. Besides their use in modeling/optimization of potential lead compounds (Cruciani et al., 2000a, b), molecular descriptors have been correlated with, *e.g.*, selection of solvents for polymorph screening (Allesø et al., 2008), prediction of solubilizers effect on partitioning (Hoest et al., 2007), permeability across the blood brain barrier (Clark, 2003; Hakkarainen et al., 2012), toxicity (Altenburger et al., 2003; Dearden, 2003), and biopharmaceutics drug disposition classification system class (Broccatelli et al., 2012). Prior work has also been done on correlating molecular descriptors with glass transition temperature and predicting structural properties of polymer plasticizers for optimal plasticization efficiency (Tarvainen et al., 2001), and for predicting glass transition temperatures, crystallization tendency, and phase separation in amorphous drugs or drug-systems (Alhalaweh et al., 2014; Alzghoul et al., 2014; Pajula et al., 2014).

Molecular descriptors of amino acids have previously been used for the evaluation of the physicochemical parameters and biological activity of peptides (Sandberg et al., 1998; Tyunina and Badelin, 2009) and to correlate to the stabilizing effect of amino acids on human growth hormone and human insulin (Soenderkaer et al., 2005). However, the study by Soenderkaer et al. investigated a smaller selection of amino acids (6 in total) with a limited number of pre-chosen descriptors very close to the number of investigated amino acids (namely 3–5), which is not best practice for PLS calibration. Furthermore, it differed in its experimental method where stability was measured by determining the presence of larger aggregates (Soenderkaer et al., 2005). In the present study, another approach to monitor the stability of a protein is used, namely to monitor its unfolding as a function of temperature using intrinsic fluorescence to determine the melting temperature (T_m) and to use static light scattering (SLS) to determine the aggregation onset time (t_{agg}) (Bhambhani et al., 2012; Kheddo et al., 2014). Generally, protein aggregation can be described by the Lumry-Eyring model (Sanchez-Ruiz, 1992), stating that the initial step is an unfolding of the protein, which is followed by the actual aggregation step. The first step is thus describing the thermodynamical stability of the protein which is related with T_m. In our model, an increase in the thermodynamical stability by various excipient amino acids is thus modeled by an increase in T_m. Another stability indicator is the aggregation onset temperature, which is related to the second step of the aggregation process. As this temperature could not be found for a fraction of the amino acids in our study, the aggregation onset time was used as an alternative. This aggregation onset time estimates the likelihood of unfolded proteins to interact, which means it is related to the colloidal stability of the unfolded protein. Summarizing, an excipient amino acid may stabilize lysozyme by slowing down aggregation either by increasing T_m (more energy required for unfolding) or by increasing t_{agg} (more difficult for unfolded proteins to interact), or both.

Fluorescence is very sensitive to conformational changes in the protein as the fluorophores, in particular tryptophan, become exposed to the solvent during unfolding resulting in a change in the fluorescence signal (Eftink, 1994; Wang, 1999). As unfolded proteins are likely to lose their biological function/activity an increase in unfolding temperature is most often related to better stability of the protein. Light scattering can be used to determine the aggregation onset because the scattered light signal changes when the protein aggregates and thus forms larger particles (Tsai et al., 1998).

The aim of this study was to investigate the (de)stabilizing effect of a selection of amino acids on lysozyme and to correlate the findings with theoretical physicochemical characteristics (molecular descriptors) of the amino acids. Thirteen amino acids, which were soluble in the concentration range of interest, were chosen for the study.

2. Materials and methods

2.1. Materials

Lysozyme from hen egg white and the L-amino acids alanine (Ala), arginine (Arg), asparagine (Asn), glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), methionine (Met), proline (Pro), serine (Ser), threonine (Thr), and valine (Val) were obtained from Sigma-Aldrich (St. Louis, MO). Sodium chloride was obtained from Merck (Kenilworth, NJ) and sodium dihydrogen phosphate from VWR International (Søborg, Denmark). All reagents were of analytical grade and used without further purification unless otherwise stated.

2.2. Methods

2.2.1. Preparation of samples

Stock solutions of the varying amino acids were prepared in 10 mM phosphate buffer, yielding a concentration of 150 ± 5 mM amino acid. A stock solution of 75 ± 5 mM NaCl was prepared in the same way and added to a solution without amino acids in order to obtain the same osmolality. The pH of the stock solution was adjusted to pH 7 ± 0.05, and the osmolality was measured on a Gonotec Osmometer (Berlin, Germany). On the same day the measurements were to be carried out, an excess amount of lysozyme was dissolved in the amino acid stock solution and the pH was adjusted again if required. Concentration was measured by UV–vis spectrophotometry using an extinction coefficient of $A_{0.1\%} = 2.64\ L/g\ cm^{-1}$ at 280 nm (Sophianopoulos et al., 1962) using a NanoDrop 2000c (Thermo Scientific, Waltham, MA), and the solutions were diluted to 5 ± 0.5 mg lysozyme pr. mL using the amino acid stock solution. The pH was controlled right before filling of the Optim 9 μL micro-cuvette arrays (*vide infra*).

2.2.2. Intrinsic fluorescence

An Optim 1000 (Avacta Analytical Ltd., Wetherby, England) was used to follow the unfolding process of lysozyme as a function of temperature using intrinsic fluorescence. The lysozyme fluorophores were excited at 266 nm using a laser and the intrinsic fluorescence was recorded in the range from 300 nm to 450 nm using a CCD camera. The thermal range was 20–90 °C at a scan rate of 0.3 °C/min. For each measurement one micro-cuvette array, containing 16 samples of 9 μL and placed in the middle slot, was used. Each sample was measured in triplicate. The barycentric mean fluorescence (BCM) was plotted against the temperature, yielding an unfolding curve for lysozyme. T_m was determined using the second derivative calculated by the Optim Analysis software (Avacta Analytical Ltd., England).

Download English Version:

https://daneshyari.com/en/article/5550518

Download Persian Version:

https://daneshyari.com/article/5550518

Daneshyari.com