International Journal of Pharmaceutics 524 (2017) 215–225

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Rational approach for design and evaluation of anti-aggregation agents for protein stabilization: A case study of trehalose phenylalaninate

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ARTICLE INFO

Article history:
Received 12 December 2016
Received in revised form 25 March 2017
Accepted 27 March 2017
Available online 30 March 2017

Keywords:
Protein aggregation
Anti-aggregation agent
Trehalose phenylalaninate
Protein stabilization
Bovine serum albumin
Molecular docking

ABSTRACT

The present work introduces new anti-aggregation agent (AAA) derived through our new approach for design and evaluation of anti-aggregation agent as a multi-purpose excipient to combat protein aggregation. Therapeutic proteins undergo aggregation due to even minor changes in environmental conditions like temperature, pH, shear and stress. Excipients play a vital role in prevention of aggregation. To stabilize a protein formulation different classes of excipients are used in combination after carefully selecting through laborious and time consuming trial and error experiments. To resolve these concerns, we have developed a rational approach based on molecular docking analysis and have designed, synthesized AAAs, and validated the approach by experimental studies. Trehalose phenylalaninate (TPA) has been synthesized and evaluated for stabilization of Bovine serum albumin (BSA). TPA was found to be non-toxic with a LC50 of >80 μg/ml. BSA solutions with and without TPA were subjected to thermal and agitation stress and aggregation was monitored using sophisticated analytical techniques. The helical structure of BSA was completely retained in stressed samples at 0.1% concentration of TPA. SEC-HPLC clearly demonstrated the absence of aggregates in presence of TPA. Although aggregation was not seen in fluorescence spectra but quenching due to TPA was evident. Moreover, molecular dynamics study on BSA-TPA complex showed lower RMSD.

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1. INTRODUCTION

Unlike, small molecular drugs, protein drugs present several challenges due to marginal stability and different dimensionality to the structure. Aggregation is perhaps the most common manifestation of protein instability encountered during all the steps of protein drug development. Excipients are crucial for stabilization of protein pharmaceuticals (Jorgensen et al., 2009; Wang, 2005). During pre-formulation studies, different classes of excipients need to be evaluated by a number of trial and error methods which demand highly sophisticated analytical techniques and as a result becomes a cumbersome and expensive task (Kamerzell et al., 2011 Chang and Hershenson, 2002). In this regard, two approaches have been described in literature. Fan et al. have screened a large array of excipients by constructing an empirical phase diagram, where the effect on protein structure and stability in presence of several excipients in solution is evaluated and boundaries are established (Fan et al., 2005; Fan et al., 2007). It is considered a rapid and rational approach for protein formulation, and it is even compatible with high-throughput instrumentation that can reduce the time for a formulation study to days or weeks. Shulda et al. (2008) and Roughton et al. (2012) have employed computational studies for selection of excipients based on maximal protein-excipient interactions at hot spots of protein (Shulda et al., 2008; Roughton et al., 2012). The empirical phase diagram approach requires extensive experimental studies and the computational studies have been employed only for selection and not explored for designing novel excipients.

In view of the expectation that an increasing number of pharmaceuticals will be based on therapeutic proteins in the future, there is a substantial need for the development and approval of new excipients (Challener, 2015). On the whole, selection of appropriate stabilization excipients, limited choice of excipients and lack of new multipurpose excipients are the problem areas which need to be addressed.

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http://dx.doi.org/10.1016/j.ijpharm.2017.03.075
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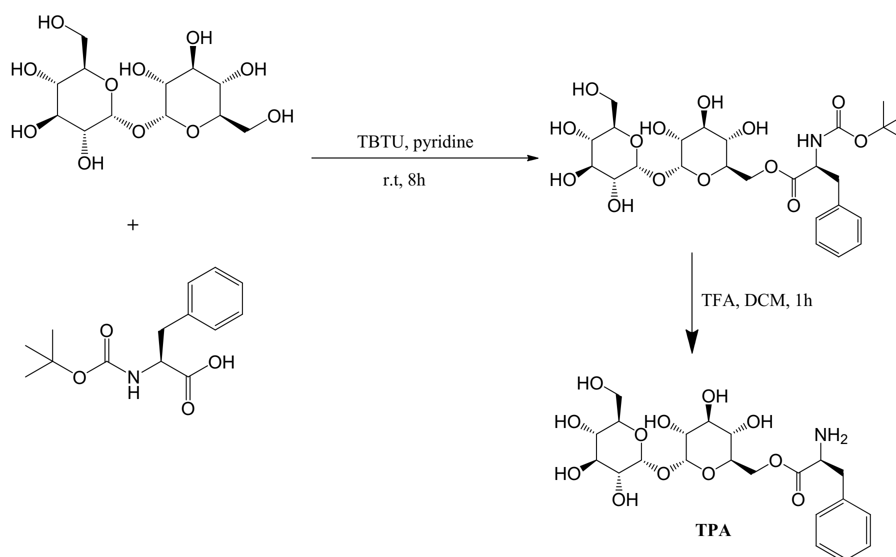

Scheme 1. Synthesis of TPA.

It is widely accepted that proteins are stabilized by various non-covalent interactions like hydrophobic interactions and hydrogen bonding. (Rainer, 2000). The protein interaction with small ligands often takes place with an increase in protein thermostability due to the coupling of binding with unfolding equilibrium (Barreca et al., 2010). In order to understand binding and non covalent interactions of excipients; molecular docking was carried out specifically at aggregation prone regions (APRs). After the docking analysis, AAAs were designed to encompass the characteristics of two or three stabilizers. Previously, Trehalose monooleate as AAA has been successfully designed and validated (Kale and Akamanchi, 2016). The same molecular docking approach has been extended to design yet another AAA trehalose phenylalaninate (TPA). Further, Molecular dynamics study has been carried out to comprehend the behavior of BSA- TPA complex during simulation. Chemicaly TPA is a sugar amino acid ester (SAE). A literature search indicated that TPA is a known compound and has been synthesized as potential monomers for biodegradable polymer synthesis (Park et al., 1999). However, it has never been considered as an excipient for protein stabilization. We have synthesized TPA by chemical synthesis route unlike reported enzymatic synthesis and evaluated its anti-aggregation potential using various analytical techniques like UV spectroscopy, CD spectroscopy, fluorescence spectroscopy, size-exclusion chromatography and Dynamic light scattering (DLS).

2. Materials and methods

2.1. Materials

Trehalose was purchased from Sigma-Aldrich (Sigma-Aldrich Chemicals Pvt. Ltd., Mumbai, India). *N*-Boc-L-phenylalanine was procured from Avra Synthesis Pvt. Ltd, India. 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) was obtained from Spectrochem, India. Dry pyridine and all other chemicals used in the study were of analytical reagent grade and were purchased from S D Fine Chemicals, India. BSA with a purity of >96% was obtained from Hi-Media, India. High-purity Milli-Q water (Millipore, Billerica, USA) was used for all solutions. BSA solutions were freshly prepared by dissolving the protein in the 0.1 M phosphate buffer of pH 7.4 prior to use. TLC was performed on precoated aluminum plates of silica gel 60 F254 (0.25 mm, E. Merck).

2.2. Instrumentation

FT-IR spectra were recorded using Perkin Elmer spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent NMR spectrometer at 400 MHz.

2.3. Molecular modeling

Docking studies were performed using GLIDE, version 5.6, Schrodinger suite, LLC, New York, NY, 2010 (Friesner et al., 2004; Halgren et al., 2004). AGGRESCAN (http://bioinf.uab.es/aggrescan/) was used to predict aggregation prone regions (APRs) on BSA (pdb-3v03). The structures of the excipients and designed AAAs were drawn and using LigPrep (2.3) module (Ligprep, Version 2.3, 2009), the drawn ligands geometry was optimized by means of Optimized Potentials for Liquid Simulations-2005 (OPLS-2005) force field. Finally, the ligand conformations with minimum energy were chosen. Protein preparation wizard of Schrodinger suite has been used to prepare protein. The prepared protein was employed to build energy grids using the default value of protein atom scaling (1.0 Å) within a cubic box, centered around the centroid of the selected APRs. After Grid generation, the ligands were docked with the protein by using GLIDE 5.6 module in extra precision mode

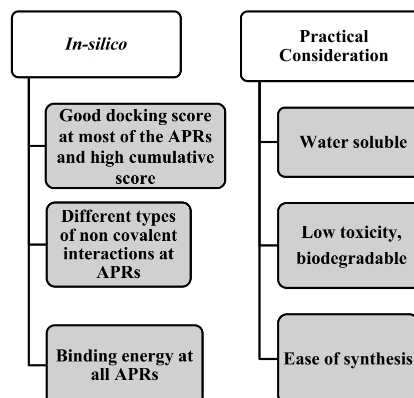

Fig. 1. Criteria for selection of AAAs.

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