European Journal of Pharmaceutical Sciences 97 (2017) 151–157

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Diversity selection, screening and quantitative structure–activity relationships of osmolyte-like additive effects on the thermal stability of a monoclonal antibody

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ARTICLE INFO

Article history:
Received 28 July 2016
Received in revised form 11 November 2016
Accepted 14 November 2016
Available online 16 November 2016

Chemical compounds studied in this article:
Mannitol (PubChem CID: 6251)
Glutamine (PubChem CID: 5961)
Tricine (PubChem CID: 79,784)
L-Phenylalanine methyl ester hydrochloride (PubChem CID: 75,736)
Sarcosine ethyl ester hydrochloride (PubChem CID: 171,173)
1,4-Anhydroerythritol (PubChem CID: 641,773)

Keywords:
Osmolyte
Monoclonal antibody
Thermal stability
Protein stabilization
Differential scanning fluorimetry
Diversity selection
QSAR

ABSTRACT

Solvents used for therapeutic proteins in downstream processing and in formulations often contain stabilizing additives that inhibit denaturation and aggregation. Such additives are mostly selected based on their positive effect on thermal stability of the protein, and are often derived from naturally occuring osmolytes. To better understand the structural basis underlying the effect of additives, we selected a diverse library of compounds comprising 79 compounds of the polyol, amino acid and methylamine chemical classes and determined the effect of each compound on thermal stability of a monoclonal antibody as a function of compound concentration. Thermal stabilization of the antibody was influenced by solution pH. Quantitative structure–activity relationships (QSAR) were derived by partial least squares regression for individual compound classes and globally. The global model suggests that ligands with a phenyl ring will decrease the T_m, while highly soluble, polar compounds with at least two hydrogen bond donors will increase the T_m. This approach may be beneficial for further studies on the influence of other solution conditions like ionic strength and buffer species on additive-mediated protein stabilization.

1. Introduction

Osmolytes are small molecule compounds that stabilize the native structure of proteins. They occur naturally in organisms exposed to high temperature, extremes of ionic strength or pH, where intracellular proteins would otherwise unfold and aggregate (Macchi et al., 2012; Harries and Rösgen, 2008; Street et al., 2006). Some of these compounds are used as additives in the therapeutic protein production process and in formulations to stabilize the protein native state and to prevent aggregation (Macchi et al., 2012; Vagenende et al., 2009). Osmolytes were also used with specific aims of facilitating protein crystallization or preventing protein aggregation during production and in the life cycle of the finished product (Vedadi et al., 2006).

However, as osmolytes belong to different compound classes, the key molecular properties responsible for their protein-stabilizing effect are probably not uniform and overall only partially understood. In a situation, where only a small number of stabilizing additives identified by trial and error have made it into biopharmaceutical applications, the design of more potent stabilizers would strongly benefit from a better insight into the underlying principles of osmolyte-mediated protein stabilization, which is hence also the objective of the current study.

Abbreviations: mAb, Monoclonal antibody; MOE, Molecular Operating Environment; MDS, Multi-dimensional scaling; QSAR, Quantitative structure–activity relationship; DSC, Differential scanning calorimetry; DSF, Differential scanning fluorimetry; DMSO, Dimethyl sulfoxide; NaOH, Sodium hydroxide; HCl, Hydrochloric acid; LOO-CV, Leave-one-out cross-validation; PCA, Principal components analysis; PLS, Partial least squares; TMAO, Trimethylamine N-oxide; T_m, Melting temperature; TS potency, Thermal stabilization potency; VIP, Variable importance in projection; vs., Versus; RMSE, Root mean square error; Adj. R-squared, Adjusted R-squared.

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http://dx.doi.org/10.1016/j.ejps.2016.11.016
0928-0987/

Osmolytes can interact non-specifically or non-covalently with a solvated protein via hydrogen bonding, electrostatic interactions and indirectly by altering the water structure (Kamerzell et al., 2011). While more focus had been on non-specific interactions, protein-specific interactions with osmolytes have also been reported (Falconer et al., 2011). Different indirect measures of osmolyte–protein interactions include osmolality (Harries and Rösgen, 2008), the preferential interacting parameter (Arakawa and Timasheff, 1985), thermal conductivity (Park et al., 2011) and melting temperature (Kamerzell et al., 2011). However, a single osmolyte might interact with a solvated protein in multiple ways, and various biophysical methods must be applied to detect all of those interactions as each method has its inherent strengths and weaknesses (Kamerzell et al., 2011). Protein stabilization by osmolytes could then be achieved via various mechanisms such steric exclusion, cohesive force or surface tension effects, and the widely accepted preferential surface exclusion (Ohtake et al., 2011; Kumar et al., 2012)., However, it is not always clear to what extent these interactions or mechanisms contribute to the overall observed effect on the thermal stability of the protein.

Also, osmolyte effects had been linked to measured physical properties such as pKa (Falconer et al., 2011), viscosity (He et al., 2011a) and surface tension (Kaushik and Bhat, 1998). Many of these reports seek to identify general stabilization mechanisms of certain osmolyte classes irrespective of the protein sequence or source. The ubiquitous nature of the peptide backbone in all proteins is often exploited. However, there are mentions of protein-specific effects due to the presence of side groups (Falconer et al., 2011; Street et al., 2006; Harries and Rösgen, 2008). Computational and experimental studies aimed at rationalizing additive effects on protein stability either focus on a few selected additives like trehalose (Jain and Roy, 2009), proline (Ignatova and Gierasch, 2006), glycerol (Gekko and Timasheff, 1981) and TMAO (Ma et al., 2014) or on specific chemical moieties such as the guanidino group (Zarrine-Afsar et al., 2006). Other related studies are limited to either a class of osmolyte, e.g. polyols (Roughton et al., 2012; London et al., 1979), amino acids (Falconer et al., 2011; Taneja and Ahmad, 1994) methylamines (Arakawa and Timasheff, 1985) or to a limited number of the three classes (Macchi et al., 2012; Street et al., 2006).

In the present study, we present a systematic approach that focuses entirely on molecular properties of additives and does not postulate a specific mechanism of interaction. To that end, we selected a comprehensive compound library comprising amino acids, methylamines and polyols, and measured their effects on the thermal stability of a monoclonal antibody (mAb). The library was not restricted with respect to toxicity, compound stability or GMP compliance at the present stage, as we were primarily interested in identifying the molecular properties responsible for effects on protein stability. Such properties can be included in a later step, when the results will be used for the design of improved stabilizing additives. Measurement techniques for studying the thermal unfolding of proteins include circular dichroism, differential scanning calorimetry (DSC), nuclear magnetic resonance and differential scanning fluorimetry (DSF) using intrinsic protein fluorescence or extrinsic fluorescent probes (Kamerzell et al., 2011). We chose DSF as a measurement technique of thermal stability because of its high throughput capacity and the well-established correlation of DSF results with those of DSC (Ericsson et al., 2006; Niesen et al., 2007; Menzen and Friess, 2013). Drawbacks with respect to equilibrium methods such as DSC are subtle influences of the extrinsic fluorescent probe on T_m and the inability to measure the reversibility of unfolding transitions, resulting in apparent T_m values that do not necessarily reflect the equilibrium state. However, shifts of apparent T_m have been useful to detect changes in protein stability in many reports (He et al., 2010, 2011b). Since pH can affect protein conformation, experiments were carried out at two (three for polyols) pH values.

In a quantitative structure–activity relationship (QSAR) approach, the concentration-dependent effect of additives on the apparent T_m was correlated with physicochemical and structural molecular properties of compounds, coded as numerical descriptors. Roughton et al. described the utilization of QSARs in the design of carbohydrate excipients as aids for lyophilization (Roughton et al., 2012), thereby demonstrating the plausibility of this method. PLS regression yielded local and global models with satisfactory predictive statistics for interpolation. Variable importance in projection (VIP) (Chong and Jun, 2005) assessment of the global model revealed a strong dependence of observed effects on the polarity and charge of the compounds which are in accordance with most of previous findings, thereby demonstrating the capability of this approach. The results are encouraging for more extended studies including a variety of proteins, a broader formulation space and automated data requisition. This approach may also be explored to investigate osmolyte effects on colloidal stability since the conformational stability of proteins does not guarantee colloidal stability under the same conditions.

2. Experimental section

2.1. Materials

Disodium hydrogen phosphate (Na_2HPO_4), sodium chloride and citric acid monohydrate were purchased from Carl Roth GmbH (Karlsruhe, Germany). Sypro Orange at a concentration of 5000× in DMSO was purchased from Invitrogen GmbH (Darmstadt, Germany). Taurine, serine, proline and beta-alanine were purchased from Applichem (Darmstadt, Germany), other screening compounds were purchased from Sigma-Aldrich (Taufkirchen, Germany). All other reagents were of analytical grade.

2.2. Diversity selection of screening compounds

All amino acids with a molecular weight <300 $gmol^{-1}$ from the eMolecules database (James, 2011) were selected and sarcosine and mannitol were used as queries for the methylamine and polyol class, respectively. A molecular weight cutoff of <500 $gmol^{-1}$ and a Tanimoto similarity (Jaccard, 1912) of 0.5 was applied for the polyols. Molecular Access System (MACCS) keys were calculated with the Molecular Operating Environment (MOE) (ChemicalComputingGroupInc, 2004) and used to rank all the compounds in the master dataset based on molecular similarity and by Jarvis–Patrick clustering. Afterward, the most diverse compounds were selected; further pruning was done to exclude reactive and toxic compounds as indicated on the material safety data sheets. Compounds that were not soluble at or above 0.1 M in water were excluded; their solubility was assessed by visual observation. The final lists of the selected compounds can be found in the supporting information.

3. Methods

3.1. Buffer preparation

Citrate-phosphate buffers were prepared from 1 M citric acid monohydrate and 0.5 M disodium hydrogen phosphate based on a formula from the reference buffer table (Dawson et al., 1986). The buffer system was selected because of its constant buffering capacity, which spans from pH 2.6 to 7.6, and which allowed for the utilization of a single buffer system, thereby limiting the influence of differing buffer ions on the results obtained.

3.2. mAb preparation

A recombinant human monoclonal antibody of the IgG1 subclass (mAb1) was produced in-house in Chinese hamster ovary cells. The cell-producing line was obtained from Rentschler Biotechnologie GmbH (Laupheim, Germany). Purification was carried out with protein A affinity chromatography and subsequent sterile filtration. Before use,