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Thermodynamic stability and formation of aggregates of human immunoglobulin G characterised by differential scanning calorimetry and dynamic light scattering

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Abstract

The final process step of polyclonal human immunoglobulin G is formulation with agents such as sugars, polyols, amino acid and salts. Often the most stable formulations were empirically identified. Physicochemical methods, such as differential scanning calorimetry and dynamic light scattering, provide a deeper insight on the biophysical properties of such a protein solution. The combination of these methods proved to be sensitive enough to detect fine differences in the properties relevant for the development of stable protein solutions. The influence of additives, such as maltose and glycine in combination with water or low concentrations of salts, on human immunoglobulin preparations was analysed. Differential scanning calorimetry illustrated that 0.2 M glycine had better stabilising effects compared to 10% maltose. Dynamic light scattering and differential scanning calorimetry revealed that solutions preventing aggregation were not optimal in terms of thermodynamic stability. Aggregation was minimised with increasing ionic strength, shown by dynamic light scattering, whereas thermodynamic stability for heat sensitive parts of human immunoglobulin G, analysed with differential scanning calorimetry, was decreased.

Keywords: Differential scanning calorimetry; Light scattering; Stability; Aggregation; Immunoglobulin

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1. Introduction

A crucial problem of protein-based biopharmaceuticals is their long-term stability or so-called shelf-life. Comprehensive knowledge about prediction of protein stability would facilitate the development of optimal formulations. Factors influencing stability are especially important concerning formulation developments [1]. Desired are conditions, where conformational stability of the final product is given during delivery and storage. At the same time it is often favoured to provide an isotonic solution in case of an intravenous applied therapeutic. Concerns about stability are not only important for formulation development but also during the whole processing of the therapeutic protein to prevent process-induced aggregation.

Two types of stability have to be considered, thermodynamic and the kinetic stability, respectively [2,3]. Both influence the shelf-life of a protein based therapeutic and the integrity of the conformation during processing. The simplest model for describing the thermodynamic stability is the two-state model, where the native state N of a protein is in equilibrium with the denatured state D:

$$N \underset{k_2}{\overset{k_1}{\rightleftharpoons}} D \tag{1}$$

where a low denaturation rate constant k_1 indicates a high thermodynamic stability. The kinetic stability can be described by extending Eq. (1), in which the reversible unfolding is followed by a kinetically controlled irreversible denaturation:

$$N \underset{k_2}{\overset{k_1}{\rightleftharpoons}} D \xrightarrow{k_3} A \tag{2}$$

where A stands for irreversible aggregates. A protein with a high aggregation rate constant k_3 has a low kinetic stability, but at the same time it can possess a low k_1, which results in a high thermodynamic stability.

Aggregation is one of the major problems in pharmaceutical industry since proteins are not infinitely stable in solution [4]. Generation of aggregates in solution can lead to irreversible or reversible precipitation [1], which can cause a loss of activity and reduced solubility. Irreversible aggregation is attributed to the covalent or non-covalent interaction of denatured protein molecules and can often not be dissolved without major changes of the solvent conditions, such as adding chaotropic and/or reducing agents. Reversible aggregation is often considered to be caused by the self-association of protein molecules, which is caused by ionic strength [5–7] or pH changes [8]. Dissociation can be normally achieved by readjusting pH [9,10] or salt concentration [11]. Shire et al. [12] pointed out that reversible protein association is less studied compared to irreversible aggregation and is often overlooked due to poor analytical methods. Since a lot of analytical methods require dilution of the sample the equilibrium between the native state of a protein and reversible aggregates may shift compared to the equilibrium in higher concentrated protein solutions. These aggregates may subsequently denature with time due to the solvent environment, which causes protein–protein interaction.

When dealing with a therapeutic protein knowledge of stability in solution is normally achieved with time consuming long term stability trials. Another possibility is to determine the thermodynamic stability with the help of differential scanning calorimetry (DSC) or fluorescence measurements and therefore a reduction of time consuming trials can be achieved. DSC is one powerful tool, which can be used for optimising the stability of proteins