Stabilization of a Hydrophobic Recombinant Cytokine by Human Serum Albumin

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ABSTRACT: The objective was to evaluate the impact of pH and NaCl content on aggregation, particle formation, and solubility of a hydrophobic recombinant human cytokine in formulations with human serum albumin (HSA) as stabilizing excipient. While cytokine-HSA formulations were stable at physiological pH, a tremendous increase in turbidity at pH 5.0, close to the isoelectric point of HSA was caused by a partially irreversible precipitation. By dynamic light scattering (DLS), disc centrifugation, atomic force microscopy (AFM), and light obscuration it could be shown that the turbidity was mainly caused by particles larger than 120 nm. SDS–PAGE provided evidence that the precipitation at pH 5.0 was mainly caused by the cytokine. The HSA-stabilizers Na-octanoate and Na-N-acetyltryptophante were less effective in preventing the turbidity increase of unstabilized-HSA compared to NaCl. The interactions between HSA and cytokine were weakened by NaCl, as determined by fluorescence spectroscopy. The positive effect of NaCl on the formulation could be attributed to a direct stabilization of HSA and weaker interactions between HSA and the cytokine, which in consequence provided an overall stabilization of the cytokine. J Pharm Sci 96:2987–2999, 2007

Keywords: albumin; fluorescence spectroscopy; light scattering (dynamic); nephelometry; protein aggregation; protein formulation; interaction

INTRODUCTION

The limited solubility of hydrophobic cytokines, which can range below 0.05 mg/mL at physiological pH,[1] associated with a strong aggregation and adsorption tendency are the major challenges during formulation development. One possible approach is the use of the physiologically well tolerated human serum albumin (HSA) as stabilizer for the cytokine. Numerous examples of commercial formulations with HSA as stabilizer for therapeutic proteins are available, for example, for factor VIII, interferons, urokinase, streptokinase, immunoglobulins, and others.[2] The stabilizing effect of HSA on a second protein is often explained by the stabilizing properties a polymer can provide.[3] There is also evidence that direct interactions between HSA and the active proteins are responsible for the stabilization.[4] Generally, HSA is extracted from human plasma and therefore a pasteurization process is required to eliminate potential blood born pathogens. According to the US Food and Drug Administration the pasteurization process has to be conducted for 10 h above 60°C.[5] The FDA requires the addition of 0.16 mM stabilizer per gram HSA, either as combination of 0.08 mM Na-octanoate and 0.08 mM Na-N-acetyltryptophanate or 0.16 mM

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Na-octanoate as single substance.[5] Na-octanoate and Na-N-acetyltryptophanate increase the denaturation temperature of HSA and thereby, prevent HSA from aggregation and denaturation.[6,7] In addition, HSA is stabilized by NaCl, which also inhibits the heat-induced aggregation.[8] These additives brought into the formulations indirectly via HSA may induce stability problems, for example, for proteins which are sensitive to ionic strength. Furthermore, they can impact the physico-chemical properties of other excipients, for example, crystallization behavior or glass transitions which can become critical for lyophilized formulations.[9] Therefore, it is important to study the impact of these compounds on the formulations.

In the presented study, a hydrophobic cytokine was formulated in combination with stabilized-HSA and mannitol in analogy to commercially available products.[2] The goal was to elucidate the stabilizing properties of HSA for the hydrophobic cytokine. The physical stability of cytokine-HSA formulations was characterized with special focus on aggregation under different pH and ionic strength conditions. To get comprehensive insight into the aggregation phenomena in cytokine-HSA formulations, turbiditimetry, DLS, disc centrifugation, AFM, and light obscuration were performed. We further wanted to measure the interactions between the cytokine and HSA by fluorescence spectroscopy to understand the properties of the cytokine–HSA formulation under different pH and ionic strength conditions.

MATERIALS AND METHODS

Materials

A formulation with 0.25 mg/mL cytokine, 12.5 mg/mL mannitol, and 12.5 mg/mL stabilized-HSA was used. This formulation further contained between 0.08 and 0.1% NaCl as analyzed by ICP-OES, deriving from HSA and pH-adjustment. Unstabilized-HSA (fraction V, 96–99% purity) from Sigma-Chemicals (Steinheim, Germany) was solid and contained no further excipients. Stabilized-HSA from Grifols (Langen, Germany) was used as 20% solution and contained 16 mmol Na-octanoate, 16 mmol Na-N-acetyltryptophanate, and 130–160 mmol/L sodium. As HSA-free cytokine material a bulk with 1.2 mg/mL cytokine in 20 mM glycine at pH 3.0 was used. Sodium chloride, Na-octanoate, and N-acetyl-DL-tryptophanate were purchased from Sigma. Potassium chloride, lithium chloride, sodium acetate, ammonium chloride, potassium thiocyanate, and potassium iodide were purchased from Merck (Darmstadt, Germany). All salts were of reagent grade and used without further purification.

Turbidity Measurement

Turbidity measurement was performed with a NEPHLA turbidimeter (Dr. Lange, Düsseldorf, Germany). Light at $\lambda = 860$ nm was sent through the samples and the scattered light was detected at a 90° angle. The system was calibrated with formazine as standard and the results were given in formazine nephelometric units (FNU).

Light Obscuration

Particles from 1 to 200 μm were determined by light obscuration measurement using a PAMAS—SVSS-C Sensor HCB-LD-25/25 (Partikelmess- und Analysensysteme GmbH, Rutesheim, Germany). Five aliquots of 0.3 mL were analyzed of each sample.

Zetapotential

The zetapotential was determined with a Zetasizer Nano (Malvern, Herrenberg, Germany). The measurements were performed in the automatic measurement mode using disposable capillary cells (Malvern DTS 1060).

Dynamic Light Scattering (DLS)

DLS performed on a Zetasizer Nano (Malvern, Herrenberg, Germany) was used to characterize protein molecules and particles in the range from 1 to 1500 nm. The Zetasizer Nano is operating with a 4 mW He–Ne-Laser at 633 nm and noninvasive back-scatter technique. The size distribution by intensity and volume was calculated from the correlation function using the multiple narrow mode of the Dispersion Technology Software from Malvern (version 4.00).

Disc Centrifugation

The CPS disc centrifugation system (LOT-Oriel GmbH, Damstadt, Germany) was used to determine