Characterization of the Self-Association of Human Interferon-α2b, Albinterferon-α2b, and Pegasys

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ABSTRACT: The self-association of human interferon-α2b (hIFN-α2b), albinterferon-α2b (a recombinant protein with human serum albumin and hIFN-α2b peptides fused together in a single polypeptide chain), and Pegasys (PEGylated hIFN-α2a) was characterized by analytical ultracentrifugation analyses. By examining the apparent sedimentation coefficient distribution profiles of each protein at different concentrations, it was concluded that the above three proteins are self-associating in albinterferon-α2b formulation buffer. By model fitting of sedimentation data using SEDANAL software, the stoichiometry and equilibrium constants of the self-association of these proteins were characterized. The self-association of hIFN-α2b results in the formation of stable dimers, fast-reversible tetramers, octamers, and hexadecamers. In contrast, although both albinterferon-α2b and Pegasys are self-associated, their self-association stoichiometries are significantly different from that of hIFN-α2b. The self-association of albinterferon-α2b results in the formation of reversible dimers and trimers, whereas the self-association of Pegasys gives only reversible dimers. The self-association behaviors of hIFN-α2b and albinterferon-α2b involves attractive electrostatic forces, which can be suppressed to a negligible level in low pH (pH 4.0–4.5) and high salt concentration (400 mM NaCl) buffer, allowing quantification of their size variant contents by sedimentation velocity analysis. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:68–80, 2012

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INTRODUCTION

Interferons (IFNs) are a family of pleiotropic cytokines with antiviral, antiproliferation, antitumor, and immunomodulatory properties.[1] Recombinant human interferon (hIFN)-α2a and hIFN-α2b were the first two IFNs licensed by the US Food and Drug Administration for treatment of hairy cell leukemia. Subsequently, these IFNs were approved for clinical use for a variety of viral and cancer indications.[2] Owing to their rapid clearance from the body, frequent dosing (daily or three times weekly) of these two IFNs over an extended period (6–12 months or more) is necessary for some indications such as hepatitis B and C.[3] To prolong the serum half-life, PEGylated forms of these two IFNs were developed. PEGylated hIFN-α2a (Pegasys), manufactured by Hoffmann La Roche, Inc. (Basel, Switzerland), is a covalent conjugate of hIFN-α2a with a single 40 kDa branched bis-monomethoxy-polyethylene glycol (PEG) chain.[4–6] PEGylated hIFN-α2b (Pegintron), manufactured by Schering Corporation (now Merck & Co.) (Kenilworth, New Jersey), is a covalent conjugate of hIFN-α2b with a linear 12 kDa PEG chain.[7] Both products have a half-life much longer than their unmodified counterparts.[8] A different approach to improve the pharmacokinetic properties of IFNs was adopted by Human Genome Sciences, Inc. This approach is based on the expression of the recombinant "gene" formed by fusing DNA sequences encoding human serum albumin (HSA) and hIFN-α2b. The fusion protein manufactured in this manner is referred to as albinterferon-α2b. The clinical trial data showed that albinterferon-α2b has a half-life significantly longer than PEGylated IFNs.[9–11]

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Self-association of a protein pharmaceutical may impact its stability, biological function, and bioavailability.[12–16] Moreover, there is a concern that self-association may favor the formation of stable, long lived aggregates, which might cause immunogenicity.[17] Therefore, characterizing the self-association of a drug protein is important for drug development. Although the structures and biological functions of various IFN-α species have been extensively studied, the self-association of various forms of IFN-α in aqueous environment has not been characterized. In this paper, the work on characterizing the self-association of albinterferon-α2b in comparison with hIFN-α2b and Pegasys is reported.

EXPERIMENTAL

Materials

Albinterferon-α2b, hIFN-α2b, HSA, albinterferon-α2b formulation buffer, and Pegasys formulation buffer were manufactured in-house in Human Genome Sciences, Inc. Pegasys was from Hoffmann La Roche, Inc.

Albinterferon-α2b formulation buffer composition: 10 mM sodium phosphate, 200 mM mannitol, 60 mM trehalose, 0.01% polysorbate 80, pH 7.2.

Pegasys formulation buffer composition: 8 g/L of NaCl, 0.05 g/L of polysorbate 80, 10 g/L of benzyl alcohol, 2.62 g/L of sodium acetate trihdrate, 0.0462 g/L acetic acid, pH 6.0.

Preparation of Pegasys

Pegasys sample (8 × 0.6 mL) in its own formulation buffer (containing polysorbate 80) was subjected to cation-exchange chromatography as given below. The sample (8 × 0.6 mL) was buffer exchanged into 20 mM sodium acetate (pH 4.5) and concentrated to approximately 950 μL by ultrafiltration with an Amicon Ultra-4 30 kDa centrifugal filter device (Millipore, Billerica, Massachusetts). Chromatography of the buffer-exchanged Pegasys was performed using a Gold HPLC System (Beckman Coulter, Fullerton, California) with a 4.0 × 250 mm Propac WCX-10 column (Dionex, Sunnyvale, California). The chromatography was performed at a flow rate of 1 mL/min. For each of the four injections, the column was equilibrated with mobile phase A (25 mM sodium acetate, pH 4.5). After sample application, the column was washed 10 min with mobile phase A. Pegasys was eluted from the column with a 30 mL gradient of 0%–90% mobile phase B (mobile phase A containing 400 mM NaCl). The eluted Pegasys was pooled, concentrated, and buffer exchanged to the buffer for ultracentrifugation.

Sample Preparation for Ultracentrifugation

All four proteins, HSA, IFN-α2b, albinterferon-α2b, and Pegasys (purified by cation-exchange chromatography), were dialyzed against the buffers used for ultracentrifugation. Concentrations of the dialyzed proteins were determined by measuring sample absorbance at 280 nm using an 8453 diode-array spectrophotometer (Agilent, Santa Clara, California). After protein concentration determination, the sample proteins were diluted to desired concentrations with dialysate.

Analytical Ultracentrifugation

Sedimentation velocity (SV) and sedimentation equilibrium (SE) were performed in a ProteomeLab™-XLA protein characterization system analytical ultracentrifuge (Beckman Coulter, Brea, CA). Except for the SV run of Pegasys in a low concentration range (0.019, 0.056, and 0.167 mg/mL), the SV runs of all the proteins were monitored with absorbance scans at 280 nm. The SV run of Pegasys in low concentrations was monitored with absorbance scans at 220 nm for higher signal/noise ratio. To keep the loading absorbance values within the suitable range for SV run, a cell assembled with a 3-mm centerpiece was used for the samples with high protein concentrations. These samples include HSA at 3 mg/mL, albinterferon-α2b at 3 and 2.7 mg/mL, hIFN-α2b at 1.2 mg/mL, and Pegasys at 0.167 mg/mL (for run monitored by 220 nm scans) and at 1.5 mg/mL (for run monitored by 280 nm scans). All other samples were loaded into cells with a 12-mm centerpiece. For cells with a 12-mm centerpiece, 440 μL of reference buffer was loaded into the reference sectors and 430–435 μL of sample was loaded into the sample sectors. For cells with a 3-mm centerpiece, 110 μL of reference buffer was loaded into the reference sector and 108 μL of sample was loaded into the sample sector. Test scans at a rotor speed of 3000 rpm were performed to ensure proper loading without leaking before an SV run was performed. All SV runs were performed at 20°C. The rotor speed and protein concentrations for SV analysis were varied for different proteins and will be given in the *Results and Discussion* section.

Sedimentation equilibrium runs of albinterferon-α2b and hIFN-α2b were performed at 20°C, at three loading concentrations (0.3, 0.9, and 2.7 mg/mL for albinterferon-α2b; 0.2, 0.6, and 1.2 mg/mL for IFN-α2b) and three rotor speeds (8000, 12,000, and 16,000 rpm).

Determination of the Physicochemical Parameters for Data Analysis and Model Fitting

The densities of the buffers were measured using a DMA 5000 density meter (Anton Paar, Graz, Austria). The viscosities of the buffers at 20°C were measured using an Ostwald viscosmeter (VWR, West Chester, Pennsylvania). The ε280 (absorbance extinction coefficient at 280 nm) value of albinterferon-α2b was

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