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Stability of lyophilized human growth hormone

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ABSTRACT

To evaluate relationships between the extent of protein–excipient interactions, structural relaxation of an amorphous matrix, and the physico-chemical stability of a protein, human growth hormone (hGH) was lyophilized with sucrose and trehalose in a 1:2 weight ratio. The protein–excipient interactions were analyzed immediately after lyophilization with isoperibol solution calorimetry (ISC), water sorption analysis (WSA), differential scanning calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR). The physical and chemical stability of hGH during storage at 50 °C was monitored by reverse phase (RP)–HPLC, SEC–HPLC and UV absorption spectroscopy. The hGH formulation containing sucrose demonstrated greater protein–excipient interactions and faster initial relaxation times compared to the trehalose formulation. Although both formulations had similar chemical stability (rate of deamidation), physical stabilities (e.g. degree of aggregation) were different. The hGH/sucrose formulation manifested a higher rate and lower extent of insoluble aggregate formation. The decreased amount of aggregation in the sucrose formulation could be correlated with a greater extent of protein–excipient interactions and the presence of a more homogeneous mixture. In contrast, the higher rate of aggregation in the sucrose formulation could be directly correlated with the higher molecular mobility of the matrix.

1. Introduction

Studies of lyophilized protein formulations have suggested that excipients that are able to hydrogen bond (H-bond) to proteins can enhance their stability. Excipients are thought to H-bond to proteins during secondary drying which leads to the preservation of protein structure and ultimately enhancement of stability during storage (Liao et al., 2002). Although, studies have shown that some H-bonding excipients produce better protein stability than others (Allison et al., 1999), to the best of our knowledge, there are no systematic studies that establish direct relationships between the magnitude of protein–excipient interactions, structural relaxation of the dry amorphous matrix and the stability of a protein. A better understanding of the relationships between the physical characteristics of amorphous matrices and the stability of proteins in solid formulations may aid in development of more stable formulations and therefore be of significant interest.

To better understand the influence of the extent of protein–excipient interactions and molecular relaxation of any accompanying matrix on chemical (e.g. deamidation, oxidation, etc.) and physical (e.g. conformational integrity, formation of soluble and insoluble aggregates, etc.) protein stability, a model system was selected. The protein, human growth hormone (hGH), in the presence of two carbohydrates commonly used for lyophilization (sucrose and trehalose) was formulated in a 1:2 protein to excipient weight ratio. It has been shown previously that the 1:2 hGH to excipient weight ratio is effective in stabilizing the protein and prohibits crystallization of the excipients during storage (Costantino et al., 1998). Sucrose and trehalose are structural isomers (they possess the same molecular formula but different topological structures) and contain equal numbers of H-bond donors and acceptors. In addition, sucrose forms an amorphous matrix with a lower glass transition temperature (Tg) and has a tendency to form more homogeneous mixtures with polymers compared to trehalose (Taylor and Zografi, 1998). These similarities and differences make sucrose and trehalose ideal candidates for obtaining a better understanding of the role of protein–excipient interactions as they are related to amorphous/amorphous phase separations and structural relaxation of amorphous matrices.

The extent of interaction between hGH and excipient were measured using a number of different techniques including ISC, DSC, WSA and FTIR. The use of these methods to characterize protein solids is described in detail elsewhere (Allison et al., 1999; Taylor and Zografi, 1998; Lopez-Diez and Bone, 2000, 2004; Shamblin et al., 1998; Souillac et al., 2002a,b). The fragility of the amorphous matrix was determined from the dependence of the glass transition temperature on scanning rate (utilizing DSC) and was used to calculate initial relaxation times employing the

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Vogel–Tammann–Fulcher (VTF) equation (Qui et al., 2005). Additionally, solutions of hGH with and without sucrose and trehalose were characterized by a variety of techniques to see to what extent solution properties might be predictive of the protein in the lyophilized solid state.

2. Materials and methods

2.1. Materials

Human growth hormone was provided by Genentech Inc. (South San Francisco, CA) in highly purified form. All reagents were of analytical grade and were purchased from Sigma (St. Louis, MO). The protein was dialyzed into potassium phosphate buffer (5 mM) at pH 7.4 using sulfur and heavy metal free Spectra/Por dialysis membranes (Torres et al., 1996) with MWCO of 10 kDa at refrigerator temperature. Subsequently, concentration of the protein to 12 mg/ml resulted in a stock solution which was diluted with buffer containing the excipient (sucrose or trehalose) to obtain solutions with the desired protein concentration. As shown in previous studies of hGH in lyophilized formulations (Pikal et al., 1991), solutions at pH of 7.4 lead to adequate stability of the protein in the solid state and therefore were selected for these studies.

2.2. Lyophilization procedure

Lyophilization vials (5cc Fisher brand amber glass with linerless screw tops) were filled with 1 ml of solution containing hGH and sucrose or trehalose in a 1:2 weight ratio (each vial contained 1 mg/ml of protein and 2 mg/ml of excipient). Protein or sugar alone was lyophilized under the same conditions (e.g. concentration and volume) to obtain a lyophilized solid with one component. Lyophilization was performed in a Virtis Advantage Lyophilizer (Gardiner, NY). The vials were loaded at ambient temperature and a shelf temperature of −40 °C was maintained for 2 h. A chamber pressure of 60 mTorr was applied and the shelf temperature was maintained at −40 °C for additional 5 h. The temperature was ramped to −35 °C over 5 h and was held at −35 °C for 2 h, ramped to −10 °C over 25 min and held at −10 °C for 10 h. The initial steps of primary drying were performed at temperatures below Tg' (−28.5 °C ± 0.3 for hGH/sucrose and −28.0 °C ± 0.4 for hGH/trehalose). The Tg' values (onsets of the thermal transition) were measured with a Q100-DSC (TA instruments Inc., New Castle, DE). Approximately 20 μl samples in hermetically sealed aluminum pans were frozen to −60 °C and subsequently heated to 0 °C at 10 °C/min. Secondary drying was performed by increasing the shelf temperature to 15 °C over 30 min, holding it for 3 h with a subsequent ramp to 30 °C over 5 min and a hold at 30 °C for 6 h. This lyophilization cycle resulted in visually elegant amorphous lyo-cakes with less than 1% residual moisture. The absence of crystallization was confirmed with powder X-ray diffraction (D8 Advance, Bruker AXS) and the moisture content was measured by a Karl–Fischer coulometric titration method (May et al., 1982) (DL36 KF Coulometer, Columbus, OH). Vials with lyophilized solids were placed in a glove box (Labconco), purged with Argon for 2 h, parafilm caped and stored in desiccators to assure absence of oxygen in the headspace to minimize oxidative degradation of hGH.

2.3. Extent of protein–excipient interactions

2.3.1. Isoperibol solution calorimetry (ISC)

The enthalpies of dissolution of hGH/excipient physical mixtures and colyophilized samples were measured utilizing a Hart Scientific isoperibol solution calorimeter (Model 4285). Measurements of dissolution enthalpies and calculation of the protein–excipient enthalpy of interaction are described in detail elsewhere (Souillac et al., 2002a,b; Souillac, 2000).

2.3.2. Water sorption analysis (WSA)

Hydration isotherms of the samples were monitored with a SGA-100 symmetric vapor sorption analyzer (VTI Corporation, Hialeah, FL). Prior to each measurement, the samples were dried in vacuum for 24 h. Water uptake by the lyophilized hGH/sugar mixture and the single component (protein or sugar) solids was measured during a stepwise increase of water partial pressure by 3% over a hydration range of 10–90% at 25 °C. The humidity level was equilibrated for 5 min at each step. Interaction parameters were calculated over the range of 10–40% relative humidity (RH) based on theoretical and observed hydration isotherms as described previously (Lopez-Diez and Bone, 2000, 2004).

2.3.3. Differential scanning calorimetry (DSC)

The Tg values (onsets of the thermal transition) were measured with a Q100-DSC (TA instruments Inc.). Samples (2–5 mg) were sealed in aluminum pans (TA instruments) and were equilibrated for 5 min at −20 °C. The temperature was modulated by ± 0.5 °C every 100 s while ramped to 200 °C at a rate of 1 °C/min. The theoretical Tg values for hGH/excipient (sucrose or trehalose) formulations were predicted employing the Gordon–Taylor equation (Taylor and Zografi, 1998; Shamblin et al., 1998).

$$\mathrm{Tg}_{12} = \frac{w_1 \mathrm{Tg}_1 + K w_2 \mathrm{Tg}_2}{w_1 + K w_2}$$

where Tg_{12} is a Tg of a mixture, w_1 and w_2 are the mass fractions of each component, and Tg_1 and Tg_2 are the respective glass transition temperatures. Calculation of the constant K was performed according to the Simha–Boyer rule with an assumption that the density of each component (ρ) is 1 g/cm^3.

$$K \approx \frac{\mathrm{Tg}_1 \rho_1}{\mathrm{Tg}_2 \rho_2}$$

2.3.4. Fourier Transform Infrared Spectroscopy (FTIR)

Deuterium oxide exchanged samples were prepared as reported previously (Allison et al., 1999) employing the lyophilization cycle described above. Approximately 0.5 mg of solid sample was used in diffuse reflectance (DRIFT) infrared measurement (Souillac et al., 2002a,b). A diffuse reflectance accessory Graseby Specac Minidiff™ PN 4500 (Graseby Specac Inc., Faifield) was utilized in all measurements. Infrared spectra were recorded with a Nicolet Magna 560 ESP spectrometer (Nicolet Instrument, Madison, WI). For each spectrum, a 256-scan interferogram was collected in single beam mode with 4 cm^{-1} resolution. For the aqueous samples, native hGH (20 mg/ml in D_2O solution) was placed onto a ZnSe attenuated total reflectance (ATR) crystal (Spectra-Tech, Shelton, CT). The exchange into D_2O was performed by lyophilization of the protein and resuspension in D_2O.

The analysis of the spectra and calculation of the predicted moisture content is described in detail elsewhere (Allison et al., 1999). Briefly, each spectrum was corrected employing the Kubelka–Munk correction utilizing GRAMS/AI (7.0) software (Thermo Galactic). The amide I second derivative spectrum was area normalized. The area of the carboxylate peak at 1580 cm^{-1} was expressed as the fraction of the carboxylate band area measured for the native protein in solution and was utilized to calculate the predicted moisture content. The assumption that the moisture content of a sample with a relative carboxylate band area between 0 and 1 would fall between 0 and 12% moisture was utilized.

2.4. Conformational characterization of freeze-dried protein

Infrared spectra were recorded using a Nicolet Magna 560 ESP spectrometer (Nicolet Instrument, Madison, WI) in a DRIFT mode (Souillac et al., 2002a,b). A diffuse reflectance accessory Graseby Specac Minidiff™ PN 4500 (Graseby Specac Inc., Faifield) was utilized in the measurement of a solid sample. For each spectrum, a 256-scan interferogram was collected in the single beam mode with 4 cm^{-1} resolution. The analysis of spectra and the calculation of the correlation coefficient is described in detail elsewhere (Dong et al., 1995, 1998, 2000; Nielsen et al., 2001). Briefly, the amide I region of each spectrum was corrected using the Kubelka–Munk relationship and the area normalized second derivative spectra were utilized to calculate a correlation coefficient (r).

$$r = \frac{\sum(x_i y_i)}{\sqrt{\sum x_i^2 \sum y_i^2}}$$

where x_i is the intensity of the spectrum for the solid sample and y_i is the intensity of the spectrum of protein in solution at a wavenumber (i).

An aqueous sample (20 mg/ml in H_2O solution) was placed onto a ZnSe attenuated total reflectance crystal (Spectra-Tech, Shelton) and the spectra were recorded as described above.

2.5. Structural mobility measurements

The Tg values (onsets of the thermal transition) were measured with the Q100-DSC (TA instruments Inc.) at heating rates of 2, 5, 10, 15 and 20 °C/min. Indium was used to calibrate the temperature and cell constant. The average onset temperature of three measurements was reported as the glass transition temperature. The slope of the glass transition temperature changes as a function of scanning rate was used to calculate a fragility factor (m), which was further utilized for calculation of the parameters D and T_0. The parameters were utilized to estimate molecular relaxation time (τ) employing the Vogel–Tammann–Fulcher equation (Qiu et al., 2005).

$$\tau = \tau_0 \exp\left(\frac{DT_0}{T - T_0}\right)$$

where τ_0 ($\sim 10^{-14}$ s) is the relaxation time at the high temperature, D is a parameter related to the fragility of material, and T_0 is the temperature at which the relaxation time tends towards infinity.

2.6. Stability studies

Lyophilized samples were placed in an incubator (Precision scientific, Chicago, IL) at 50 ± 1 °C for 7 months. At different storage times, the samples were reconstituted with 1 ml of water. To assure reproducibility, 2 samples were assayed at each time point. Deamidation of the hGH was monitored by reverse phase (RP)–HPLC using a method developed previously (Riggin et al., 1987). A Shimadzu LC instrument (Kyoto, Japan) was equipped with a LC6A pump, SPD-6A UV detector, SCL-613 system controller and SIL-10AXL autoinjector. A Vydac C4 column with a mobile phase of 29% n-propanol and 71% 0.005 M Tris buffer at pH 7.5 was utilized for isocratic separation with UV detection at 220 nm. The stability data were fitted to a first order rate equation to obtain the rate constants (Chang et al., 2005a,b). Formation of hGH soluble aggregates was monitored by SEC-HPLC as reported earlier (Riggin et al., 1988; Pikal et al., 1991) and an equation describing "square root of time" kinetics (Chang et al., 2005a,b) was used to fit the data. A TSK G3000SW column (Toyo Soda) with mobile phase containing 0.025 M ammonium bicarbonate at a flow rate of 1 ml/min was utilized for size exclusion separation and UV detection at 220 nm. Formation of hGH insoluble aggregates was studied by measuring the differences in concentration before and after filtration through a 0.22 μm filter[3] and the data were fitted to "square root of time" kinetics (Chang et al., 2005a,b).

2.7. Studies of solution protein formulations before lyophilization

2.7.1. High-resolution UV absorbance spectroscopy

Aggregation of hGH alone and in solution containing sucrose or trehalose was studied using an Agilent 8453 UV–visible spectrophotometer by monitoring optical density at 350 nm (OD 350 nm) every 2.5 °C over the temperature range of 10–85 °C. A 5 min incubation period (sufficient for equilibrium to be reached) was employed at each temperature point.

2.7.2. Dynamic light scattering (DLS)

The mean hydrodynamic diameter of the hGH in solution with and without excipient was analyzed with a dynamic light scattering instrument (Brookhaven Instrument Corp., Holtzille, NY). The instrument was equipped with a 50 mW diode-pumped laser (λ = 532 nm) and the scattered light was monitored at 90° to the incident beam. Autocorrelation functions were generated using a digital auto-correlator (BI-9000AT). The hydrodynamic diameter was calculated from the diffusion coefficient by the Stokes–Einstein equation using the method of cumulants (lognormal number based).

2.7.3. Solution differential scanning calorimetry

Solution DSC was performed using a MicroCal VP-DSC with autosampler (MicroCal, LLC; Northampton, MA). Thermograms of hGH (1 mg/ml) alone and in the presence of excipient were obtained from 10 to 90 °C using a scan rate of 60 °C/h. The filled cells were equilibrated for 15 min at 10 °C before beginning each scan. Thermograms of the buffer alone were subtracted from each protein scan prior to analysis.

3. Results and discussion

3.1. Extent of protein–excipient interactions

The extent of interaction between hGH and sucrose or trehalose was studied with ISC, DSC, WSA and FTIR measurements. Comparison of the theoretical and measured Tg values for the hGH/sugar formulations is shown in Fig. 1. The measured Tg (371.5 ± 0.4 K for hGH/sucrose and 400.1 ± 0.4 K for hGH/trehalose) was significantly lower than the theoretical value (398 K for GH/sucrose and 422 K for GH/trehalose) in both formulations. The larger difference between the theoretical and observed Tg in the case of hGH/sucrose formulation suggests that this mixture is more homogeneous and has a greater extent of interactions between the protein and sugar.

ISC studies showed that both hGH/sucrose and hGH/trehalose mixtures had exothermic enthalpies of dissolution (Fig. 2). The dissolution of the hGH/excipient colyophilized mixture was less exothermic than the physical mixture in both formulations. The enthalpy of interaction (the difference of the enthalpy of dissolution between the colyophilized and physical mixtures) was almost twice as much in the case of hGH/sucrose (2.1 ± 0.1 kcal/g) compared to the hGH/trehalose (1.3 ± 0.1 kcal/g) formulation. This suggests that both sugar containing formulations had detectable levels of intermolecular interactions and that the hGH/sucrose formulation had a greater extent of such interactions in agreement with the DSC results.

The WSA based interaction parameter was also much higher in case of hGH/sucrose compared to the trehalose containing formula-

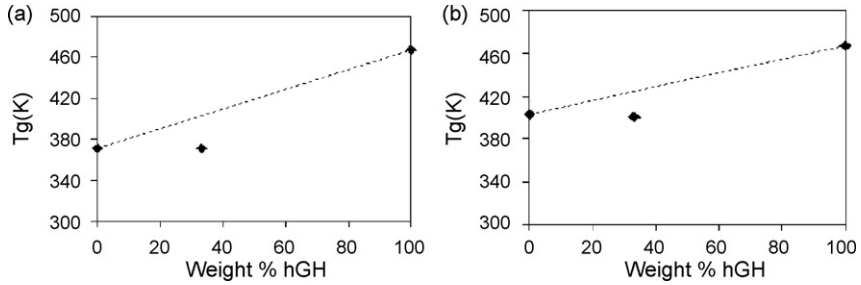

Fig. 1. Tg values of colyophilized (a) hGH/sucrose, and (b) hGH/trehalose formulations as a function of composition. Measured Tg values are represented as symbols and the prediction by Gordon–Taylor equation is represented by the dotted line. Each measurement was conducted in duplicate and has a S.D. of 0.5.

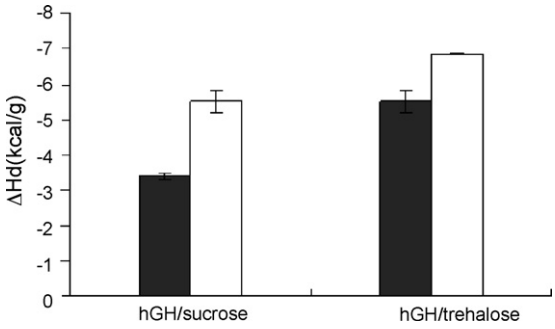

Fig. 2. Enthalpy of dissolution for colyophilized (dark bars) and physical (white bars) mixtures of hGH in the presence of sugars.

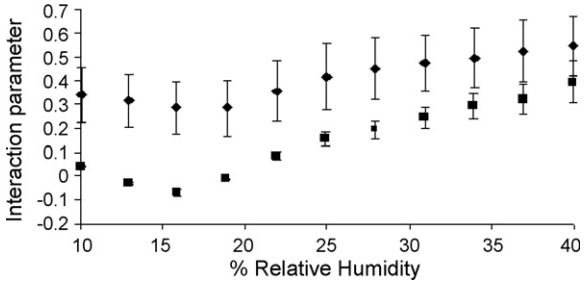

Fig. 3. WSA-based interaction parameters for colyophilized hGH/sucrose (♦) and hGH/trehalose (■) mixtures as a function of RH.

tion over the range of 10–40% RH (Fig. 3). Surprisingly, the moisture content based on the FTIR carboxylate band area was lower than the actual moisture content in both formulations (Table 1). Thus, the FTIR based approach does not appear to be appropriate to measure the extent of the protein–excipient interactions in the case studied here.

DSC, ISC and WSA based evaluations of the extent of protein–excipient interactions demonstrate that hGH formulations containing sucrose and trehalose possess detectable extents of interaction between sugar and protein. In addition, the hGH/sucrose formulation displays a greater extent of interactions compared to the hGH/trehalose mixture. The latter observation probably is related to the presence of a more chemically homogeneous mixture and/or more favorable spatial arrangement of the

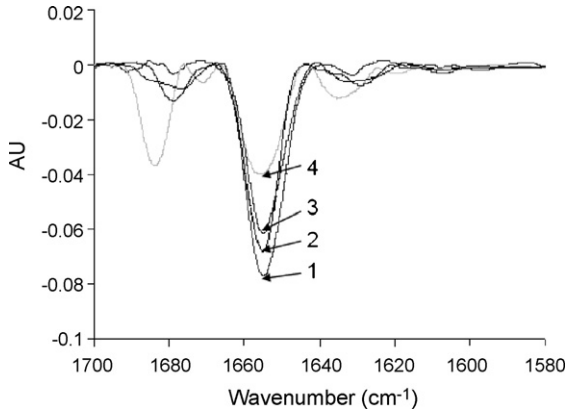

Fig. 4. Second derivative amide I FTIR spectra of native hGH in solution (1), hGH in a lyophilized formulation with sucrose (2), trehalose (3) and dried protein alone (4).

H-bonding groups in the case of sucrose. It has been suggested that partial phase separation in protein/excipient formulations can lead to physical separation of the components (Pikal, 1994).

3.2. Structural mobility measurements

The slope from the dependence of the glass transition temperature on scanning rate was utilized to calculate initial relaxation times employing the Vogel–Tammann–Fulcher equation (Qui et al., 2005). The initial relaxation time at 50 °C was 4×10^{10} s for hGH/trehalose and 6.4×10^{5} s for the hGH/sucrose formulation. The much smaller relaxation time seen in the sucrose formulation suggests a correspondingly rapid structural relaxation. Thus, the hGH/trehalose seems to possess a more rigid matrix and slower molecular mobility at 50 °C. Similar observations that trehalose/protein mixtures display slower structural relaxation than sucrose/protein formulations have been reported previously (Duddu et al., 1997).

3.3. Conformational characterization of freeze-dried protein

Lyophilization of hGH in the absence of sugars led to a significant perturbation of its secondary structure as shown in Fig. 4. The correlation coefficient describes structural differences between the native protein in solution and the protein in the lyophilized state.

Table 1
Predicted and actual moisture content for hGH/excipient formulations

Formulation	Sample moisture (%)	Carboxylate band area	Relative carboxylate band area	Predicted moisture (%)
hGH/sucrose	1.76 ± 0.14	0.00455	0.095	1.14
hGH/trehalose	2.37 ± 0.23	0.0066	0.138	1.66

The prediction of moisture content was based on the area of the carboxilate band at 1580 cm^{-1} from FTIR measurements.

Table 2
Degradation rate constants for lyophilized hGH formulations

Formulation	Deamidation rate constant (mg/ml-week)	Rate of soluble aggregates formation (%dimer/√week)	Rate of insoluble aggregate formation (% insoluble aggregate/√week)
hGH/sucrose	0.019 ± 0.01	0.4 ± 0.2	2.38 ± 0.01
hGH/trehalose	0.021 ± 0.01	0.7 ± 0.1	1.97 ± 0.06
hGH	–	2.1 ± 0.1	–

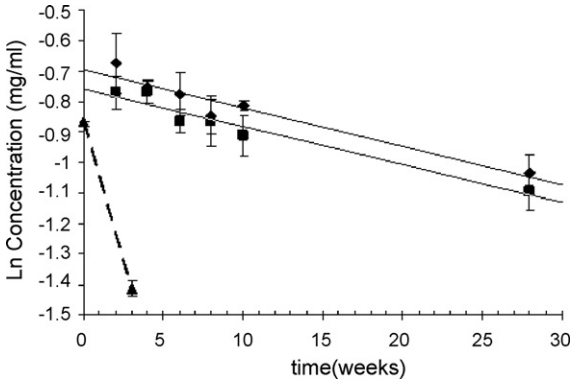

Fig. 5. The kinetics of hGH deamidation in lyophilized formulations containing hGH alone (▲ and dotted line), hGH with sucrose (♦ and solid black line) and hGH with trehalose (■ and solid grey line).

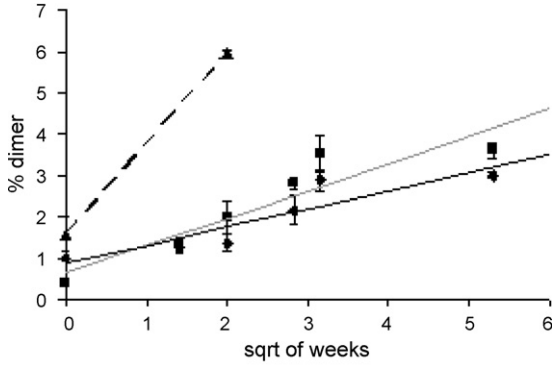

Fig. 6. The kinetics of soluble aggregate formation in lyophilized formulations containing hGH alone (▲ and dotted line), hGH with sucrose (♦ and solid black line) and hGH with trehalose (■ and solid grey line).

A correlation coefficient close to 1.00 corresponds to a protein with unperturbed by lyophilization secondary structure. The correlation coefficient for the lyophilized sugarless hGH was 0.77, whereas addition of an excipient (sucrose or trehalose) led to preservation of secondary structure and a high correlation coefficient (∼0.98). Both the hGH/sucrose and hGH/trehalose formulations had similar correlation coefficients.

3.4. Stability studies

Both the hGH/sucrose and hGH/trehalose formulations had similar rates of deamidation and insoluble aggregate formation

Table 3
Percent insoluble aggregate formed immediately after lyophilization and after 28 weeks of storage at 50 °C

Formulation	% insoluble aggregate	
	Initial	After 28 weeks
hGH/sucrose	0.35 ± 0.04	12.0 ± 0.2
hGH/trehalose	5.0 ± 0.2	15.0 ± 0.2

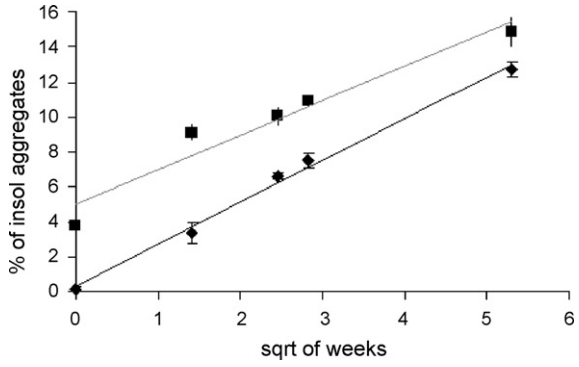

Fig. 7. The kinetics of insoluble aggregate formation in lyophilized mixtures containing hGH with sucrose (♦ and solid black line) and hGH with trehalose (■ and solid grey line).

(Figs. 5 and 6, and Table 2). It is important to note, however, that the stability of the lyophilized hGH in the absence of any lyo-protectants was significantly decreased. The hGH/sucrose displayed significantly less insoluble aggregates immediately after lyophilization (Fig. 7, Tables 2 and 3). This suggests that sucrose serves as an efficient lyo- and cryo-protectant against lyophilization induced stresses that lead to formation of the insoluble protein. Moreover, the greater extent of protein–excipient interactions seen in hGH/sucrose formulations supports the hypothesis that the presence of sucrose leads to the formation of a more homogeneous mixture with a decreased extent of aggregation. In contrast, the rate of insoluble aggregate formation was much slower in the hGH/trehalose formulation (Table 2). This decreased rate of aggregation may be related to the slower structural relaxation (molecular mobility) of the trehalose containing matrix.

3.5. Studies of protein formulations before lyophilization

hGH was also characterized in solution with and without trehalose or sucrose. The mean hydrodynamic diameter of hGH (∼5 nm) remained unchanged despite the presence or absence of the sugars. Thermally induced unfolding of hGH was monitored with solution DSC and did not reveal any differences in behavior (Table 4). The thermally induced aggregation of hGH had the same onset temperature in all formulations. The midpoint temperature of thermally induced aggregation, however, was delayed by 2–6 °C in the presence of the sugars. Sucrose was more effective in delay-

Table 4
Midpoint transition temperature (T_m) for hGH in solutions with and without sugar

Formulation	T_m	
	DSC	OD at 350 nm
hGH	79.0 ± 0.1	74.5 ± 0.6
hGH/sucrose	79.1 ± 0.1	80.4 ± 1.1
hGH/trehalose	79.0 ± 0.2	76.2 ± 0.1

The T_m was monitored with solution DSC. The midpoint of the temperature of thermal aggregation was monitored by the OD at 350 nm.

ing the midpoint temperature of the observed aggregation. The tendency of sucrose to alter the pattern of the thermally induced aggregation of hGH may be related to its ability to serve as a potent cryo- and lyo-protectant as seen in reduced insoluble aggregate formation upon lyophilization.

4. Summary and conclusions

hGH formulations containing sucrose demonstrated greater protein–excipient interactions and faster initial relaxation times than formulations containing trehalose. Although both formulations had similar rates of deamidation and soluble aggregate formation, the extent and rate of insoluble aggregate formation was different. The hGH/sucrose formulation demonstrated a higher rate and lower extent of insoluble aggregate formation. The decreased amount of higher order aggregate seen in the sucrose formulations can be correlated with the greater extent of the hGH–excipient interactions and the presence of a more homogeneous matrix. In contrast, the higher rate of insoluble aggregate formation in the sucrose formulation may be related to the greater molecular mobility of its amorphous matrix. Additionally, the characteristics of the protein in the presence of the sugar(s) may be related to the cryo- and lyo-protective properties of the excipient(s). Relationships between the extent of protein–excipient interactions, structural relaxation of the matrix and protein stability are evident and can potentially serve as a basis for the development of stable lyophilized formulations.

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