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Structural Response of Bovine Growth Hormone to Dead-Ended Ultrafiltration

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ABSTRACT

Bovine growth hormone (bGH) was used as a model pharmaceutical protein to assess protein structural stability during dynamic ultrafiltration. Two popular membrane materials of different wettabilities, poly(ether sulfone) (PES) and regenerated cellulose (RC), are exposed to acidic and basic bGH solutions in a dead-ended filtration. After a three-fold concentration run, samples of the feed, permeate, and retentate are subjected to a series of spectroscopic and physical analytical techniques, permitting quantitative characterization of the secondary and tertiary structure of bGH as well as the aggregation state of the bGH in these streams. At pH 4, where the monomeric form predominates in the feed,

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bGH loses helix and gains sheet content as it passes through the membranes. At pH 9, where the feed contains mainly dimers, bGH in the permeate is mainly monomeric and also gains sheet content at the apparent expense of helix content. Tertiary structure analyses suggest that passage through the membranes induces bGH to adopt a more compact form than the bGH in either the feed or retentate streams. At both pHs 4 and 9, the less wettable PES membrane induces a greater structural change in the permeating bGH than does the more wettable RC membrane. Alkaline pH also tends to preserve the structure of bGH in the retentate streams, perhaps reflecting the extensive dimerization. Our results suggest that the choice of filtration conditions is critical in preserving protein structure and, hence, bioactivity.

Key Words: Bioprocessing; Protein denaturation.

INTRODUCTION

Membrane processes are growing in popularity for the manufacture of proteins, with applications in upstream harvesting, buffer exchange, fractional protein separations, virus removal, and formulation.[1] However, protein adsorption and deposition on membranes, interactions between the protein and the internal surface of the membrane pores as proteins transit the membrane, and exposure to high shear rates within the membrane pores can degrade performance both in terms of yield, purity, and throughput of active target protein and of membrane longevity.

Despite this growing use, there have been few studies of the effect of solution conditions and membrane properties on protein structure during dynamic filtration. However, it is well known that adsorption and, in some instances, shear can have adverse effects on the structure and bioactivity of proteins. Charm and Lai[2] studied the effect of shear during the ultrafiltration of protein solutions. Two different membrane filtration systems, a recycle system and a vibrational filtration system, were tested using catalase as a model protein. They found activity losses of up to 50% that are attributed to adverse shear effects in the device. However, it is unclear from their experimental setup as to whether the shear was higher in the membrane device or in the tubing that connected the protein solution reservoir with the membrane cell. Tirrell and Middleman[3,4] performed a controlled study to determine the effect of shear on the catalytic activity and denaturation of lactic dehydrogenase (LDH) and urease. The mechanical stress was generated in a concentric viscometer. The investigators found that stress levels up to 2.1 MPa do not have any effect on

LDH activity. However, increasing the shear stress by roughly a factor of five while reducing the shear rate produced significant irreversible inactivation. Their conclusion is that hydrodynamic shear stress, as opposed to shear rate, is the controlling factor in the shear inactivation of LDH. It is not clear if the air–water interface in the viscometer played a role in the observed denaturation; air–water interfaces are known to be denaturing. They also performed circular dichroism studies that indicated that the secondary structure of LDH was significantly perturbed due to shear. In the case of urease, it was shown that a small quantity of metal ions may catalyze the oxidation of the surface sulfhydryl groups leading to the formation of covalently linked aggregates, reducing the activity of the enzyme. Kelly and Zydney[5,6] and Tracy and Davis[7] have shown that during microfiltration, bovine serum albumin (bSA) behaves in a similar fashion to urease: membrane fouling is enhanced due to an intermolecular thiol-disulfide exchange reaction between bSA adsorbed on the membrane and that in the adjacent fluid layer.

An early study on the effect of membrane filtration on protein conformation was conducted by Truskey and coworkers.[8] This group investigated the extent of conformational change that occurs when proteins undergo dead-ended microfiltration. Three different membrane materials, polyvinylidine difluoride, a hydrophilized polyvinylidine difluoride, and nylon, were investigated in their study along with three different model pore sizes, 0.1, 0.22, and 0.45 microns, respectively. Conformational perturbations in three different proteins, human immunoglobulin G, bovine insulin, and bovine alkaline phosphatase, were qualitatively examined using circular dichroism spectroscopy. Their overall conclusion was that protein structural changes were greater over hydrophobic membranes than over the hydrophilized PVDF membranes. They also found that the rated pore size did not have any effect on the extent of structural perturbation. The limited quality of the circular dichroism spectra leaves their conclusions open for further investigation. Meireles and coworkers[9] discovered that when extensive concentration polarization occurs during filtration, albumin denatures and the protein–protein interactions result in the formation of a gel or cake on the membrane surface. Bowen and Gan[10] investigated the effect of shear and adsorption on the activity of yeast alcohol dehydrogenase. They concluded that prolonged adsorptive contact leads to losses in activity even though the enzyme is stable in solution. If the enzyme is relatively unstable in solution, a very low mass-average shear rate is enough for the enzyme to lose activity. Increasing feed concentrations, while performing crossflow microfiltration of streptokinase from a streptococcal fermentation broth, result in larger losses in the protein activity.[11,12] In an attempt to demonstrate the extent to which temperature affects protein conformation and

fouling of ultrafiltration membranes, Campbell et al.[13] subjected α-amylase to crossflow ultrafiltration with a polysulfone membrane at different feed temperatures. Their results indicate that the specific activity of the enzyme is reduced by over 50% as the feed temperature increases from 25°C to 60°C. The activity loss corresponded with an observed red shift in the tryptophan emission maximum of a control sample held at the operating temperature for the duration of the experiment.

In this work, we characterized the structural stability of recombinant bovine growth hormone (bGH) during dynamic ultrafiltration in order to screen for appropriate filtration conditions. Bovine growth hormone is a 22 kDa, 191 amino acid, four helix-bundle motif protein used in the management of the milk production and feed efficiency of dairy cows[14]; bGH also shares high structural homology with other pharmaceutically relevant, four-helix bundle proteins including human growth hormone, granulocyte colony-stimulating factor, granulocyte macrophage colony stimulating factor, and erythropoietin.[15] Protein samples from the feed, retentate, and permeate pools are analyzed after three-fold concentration runs via dead-end ultrafiltration using regenerated cellulose and poly(ether sulfone) membranes. The secondary structure contents of the protein samples are estimated by circular dichroism spectroscopy and the perturbations in tertiary structure are quantified by second derivative UV spectroscopy and fluorescence spectroscopy. Tertiary structure analyses were facilitated by the lone tryptophan residue, Trp^{86}, that resides within the hydrophobic core formed by the four-helix bundle of bGH. The presence of aggregates in the feed, retentate, and permeate pools was assessed by size exclusion chromatography.

MATERIALS AND METHODS

Materials

Acetic acid, sodium acetate, sodium bicarbonate, Ponceau S, sulfosalicylic acid, trichloroacetic acid, sodium chloride, and potassium chloride were obtained from Sigma Chemical Co. (St. Louis, MO); sodium hydroxide was obtained from Fisher Scientific (Pittsburgh, PA). Purified bovine growth hormone (bGH) was a gift from the Protiva Division of Monsanto Agricultural Company, now Pharmacia (Lot No. M9011-004, St. Louis, MO). bGH has a nominal molecular weight of 22 kDa and contains 191 amino acids[16]; bGH typically comprises three isoforms with isoelectric points of 6.3, 7.3, and 8.3.[17] Regenerated cellulose (RC, PTGC 032097 AGC 2B) and poly(ether sulfone) (PES, NOVA No. 7167E) membranes were gifts from Pall Filtron

(East Hills, NY). Each membrane had a 10 kDa molecular weight cut off (MWCO). Prior to dynamic filtration studies, each membrane was thoroughly rinsed by at least 20 L/m^2 of DI water. The PES membrane was further rinsed by a mixture of 50% (v/v) ethanol in DI water.

Methods

Dynamic filtration experiments were performed with both the regenerated cellulose and poly(ether sulfone) membranes under two different buffer conditions: a 25 mM acetate buffer at pH 4.0 and a 25 mM bicarbonate buffer at pH 9.0. The pHs of the bGH-containing feed and permeate streams were measured and corresponded to that of the composing buffer; the pHs of the retentate streams were assumed to match the composing buffer. At pH 4.0, bGH exists primarily as a monomer and at pH 9.0, it is primarily dimeric[18]; bGH solutions are minimally soluble at neutral pHs.[17] The ultrafiltration experiments are carried out in a 50 mL, stirred, dead-ended ultrafiltration test cell (Model 8050, Amicon Division, Millipore, MA) at 24°C. The membrane area was 12.57 cm^2. A schematic of the experimental setup is shown in Fig. 1. The membranes are precompacted for 30 min at a transmembrane pressure of 170 kPa (25 psi). The transmembrane pressure was then reduced to 70 kPa (10 psi) and the buffer flux was monitored using

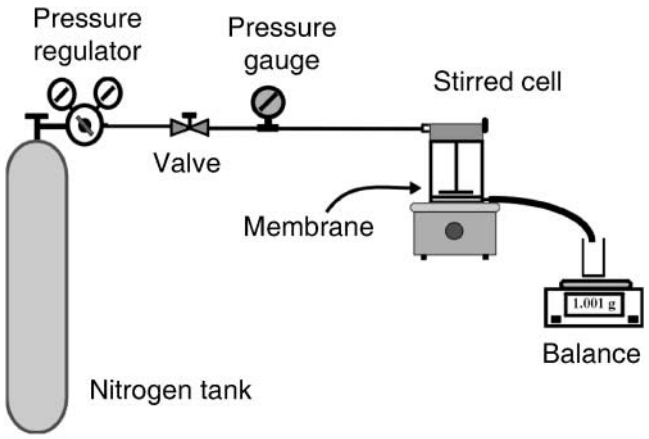

Figure 1. Schematic of the dead-ended stirred cell ultrafiltration system.

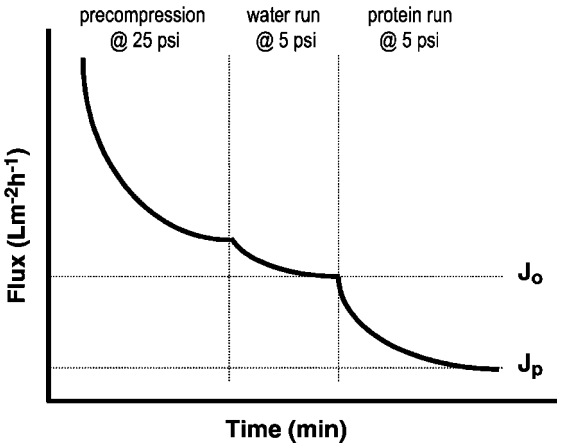

Figure 2. Standard filtration procedure for evaluating membrane performance using permeate flux as a measure of fouling: steady-state precompression, followed by pure buffer steady-state flux determination, followed by protein solution steady-state permeate flux determination.

a balance (Mettler BasBal2400, Mettler Instruments Corporation, NJ) until steady state was achieved. Steady state was assumed when the flux remained constant for three successive readings; the flux at this point is termed J_o. J_o was typically achieved after 20 to 30 minutes of operation at 70 kPa. Next, the bGH solution was filtered at a bulk concentration of 2 mg/mL. This solution was filtered until the final concentration in the retentate was 6 mg/mL. At this point the permeate flux is termed J_p. The concentration by a factor of three typically occurred after 10 to 20 minutes of filtration. Figure 2 shows a schematic of a typical flux vs. time profile for an ultrafiltration experiment. The ratio of J_p to J_o is an indication of the propensity of the membrane to foul.[19] The closer the ratio is to 1, the more resistant the membrane is to fouling. Fluxes are reported in terms of LMH, or liters of permeate per square meter of membrane area per hour. Samples from the feed, permeate, and retentate pools are collected for bGH structure and size analyses.

Membrane Characterization

Contact Angle

The static contact angle of each membrane was determined by the inverted captive bubble technique.[20–22] Small diameter air bubbles

(approximately 2–3 mm) are released from the tip of a syringe into a glass chamber containing DI water and allowed to float upward to the surface of an inverted membrane.[23] Images of the membrane surfaces with bubbles attached are projected onto a video screen using a SIT camera (SIT66, Dage-MTI Inc., Michigan City, IN) equipped with a focusing lens; contact angles are estimated directly from these images. Contact angle values are reported as the average for bubbles on four different spots on each membrane. The static contact angles for the RC and PES membranes are listed in Table 1; lower contact angles indicate greater water wettability.

Protein Characterization

Circular Dichroism Spectroscopy

The secondary structure of bovine growth hormone in the feed, retentate, and permeate pools of the dynamic filtration experiments was characterized via far-UV circular dichroism spectroscopy. Spectra are recorded on a Jasco (Japan Spectroscopic, Tokyo, Japan) J-715 spectrometer under constant nitrogen purge with 0.01 cm or 0.1 cm cells (Starna Cells Inc., CA). Four scans, recorded between 260 nm and 190 nm with a resolution of 0.1 nm at a scan speed of 20 nm/min, 1 nm bandwidth, and a response time of 1 s, are averaged and smoothed. Secondary structure estimates are made using software known as CDSSTR developed by Johnson's group.[24]

Ultraviolet Absorption Spectroscopy

The concentrations of bGH in solutions from the dynamic filtration experiments are measured by the optical absorbance at 278 nm using a Cary 300 Bio UV–Visible spectrophotometer (Varian Instruments, Palo Alto, CA). An extinction coefficient of $15270\,M^{-1}\,cm^{-1}$ was used.[25] The tertiary structure of bGH was qualitatively evaluated in terms of perturbations in the molecule's core structure via second derivative UV spectroscopy, using Trp^{86} as a reporter group. The near-UV absorption properties of aromatic amino acids in proteins are often different from those in corresponding aqueous solutions, reflecting the relative hydrophobicity of the local environments as well as quenching effects of nearby residues. The trough to crest distance between 291 nm and 295 nm is a function of the polarity of the local environment of Trp^{86}.[26,27]

Table 1. Summary of filtration flux measurements. Uncertainties are reported as the mean ± one standard deviation for measured values (θ, measured in quintuplicate; J_o and R, measured in triplicate) and the mean ± the propaged standard error for derived values.

Membrane	θ[a] (°)	cos θ[b]	pH[c]	J_o[d] (L/m^2/h)	J_p/J_o[e]	R[f]	J_s[g] (g/m^2/h)
Regenerated cellulose	20 ± 2	0.94 ± 0.01	4.0	24.8 ± 4.9	0.86 ± 0.09	0.97 ± 0.02	1.26 ± 0.27
Poly(ether sulfone)	64 ± 3	0.44 ± 0.05	4.0	92.6 ± 8.3	0.46 ± 0.08	0.99 ± 0.02	0.42 ± 0.12
Regenerated cellulose	20 ± 2	0.94 ± 0.01	9.0	26.5 ± 5.2	0.89 ± 0.12	0.97 ± 0.04	1.30 ± 0.29
Poly(ether sulfone)	64 ± 3	0.44 ± 0.05	9.0	93.0 ± 7.8	0.50 ± 0.08	0.99 ± 0.02	0.29 ± 0.07

[a] θ is the static, captive bubble, air-in-water contact angle.
[b] The quantity cos θ is also called the wettability.
[c] Acetate buffer at pH 4.0 or bicarbonate buffer at pH 9.0.
[d] J_o is the ultimate pure buffer volume flux; see Fig. 2.
[e] J_p/J_o is the ratio of the ultimate permeate volume flux during protein ultrafiltration to the pure buffer volume flux; see Fig. 2.
[f] R is the solute (protein) retention computed as the ratio of the mass of protein in the retentate to that in the feed.
[g] J_s is the solute (protein) mass flux; $J_s = J_p (1 - R)$.

Fluorescence Spectroscopy

The emission behavior of Trp^{86} can also be exploited to characterize tertiary structure perturbations in terms of alterations in the core structure of bGH. Changes in the wavelength of the emission maximum reflect changes in the polarity of the Trp^{86} environment and changes in the intensity of the emission reflect changes in exposure to the quenching effects of nearby residues or of penetrating solvent molecules.[28] Steady-state fluorescence measurements are performed on an LS-5B fluorimeter (Perkin Elmer, Norwalk, CT). The samples are excited at 295 nm, to minimize the excitation of the six tyrosine residues within bGH,[28] and five emission spectra, recorded between 310 and 360 nm with a 0.5 nm bandwidth, are collected and averaged.

Size Exclusion Chromatography

The extent of protein aggregation was determined by size exclusion chromatography. The stationary phase was a 30 cm × 7.8 mm ID TSK-GEL $G2000SW_{XL}$ column (TosoHaas, Malvern, PA), the mobile phase consisted of bGH buffer, the sample size was 50 μl, and the mobile phase flow rate was 1 mL/min. The column was used with an Äkta Explorer chromatography system (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Relative bovine growth hormone concentrations are determined by peak integration using the Äkta Explorer Unicorn software package.

RESULTS

Table 1 summarizes the results of flux and retention measurements for the two different ultrafiltration membranes. As has been observed by several investigators, poly(ether sulfone) membranes have a higher initial flux or pure buffer flux when compared to regenerated cellulose membranes, but have an undesirably large susceptibility to fouling as indicated by the ratio of the J_p to the J_o. This is attributed to the low wettability of poly(ether sulfone) membranes as compared with regenerated cellulose membranes. Poly(ether sulfone) membranes have a higher retention for bGH, possibly because of their higher degree of concentration and fouling, acting as a secondary membrane. An objective of membrane research has been to substantially decrease membrane fouling while maintaining high protein solution flux so as to reduce the process time and extend membrane lifetime.

Samples collected after each experimental run are analyzed to ascertain structural state and aggregation state of bovine growth hormone in the feed, permeate and retentate streams. Figure 3 shows typical circular dichroism

spectra exhibited by bovine growth hormone in these samples. Qualitative differences in the spectra from these streams are readily apparent, indicating varying degrees of structural perturbation.

Secondary structure contents estimated from circular dichroism spectra of bovine growth hormone samples from the feed, permeate and retentate streams are displayed in Table 2. A decrease in α-helix content with a corresponding increase in β-sheet, turn and unordered structure contents is observed in the retentate and permeate pools relative to the feed pool for both membranes challenged with bGH in acetate buffer. The type and relative extents of secondary structure perturbation found for bGH with the polysulfone and regenerated cellulose membranes are consistent with those found in a static adsorption study of adsorbed lysozyme on samples of the same membranes using infrared spectroscopy.[29]

For the retentate samples, the structural perturbation was noted to be greater with the PES membrane as compared with the RC membrane. The retentate stream samples represent the protein that has had the longest exposure to the membrane surface during filtration and that has experienced the highest protein concentration. The observed perturbations should reflect

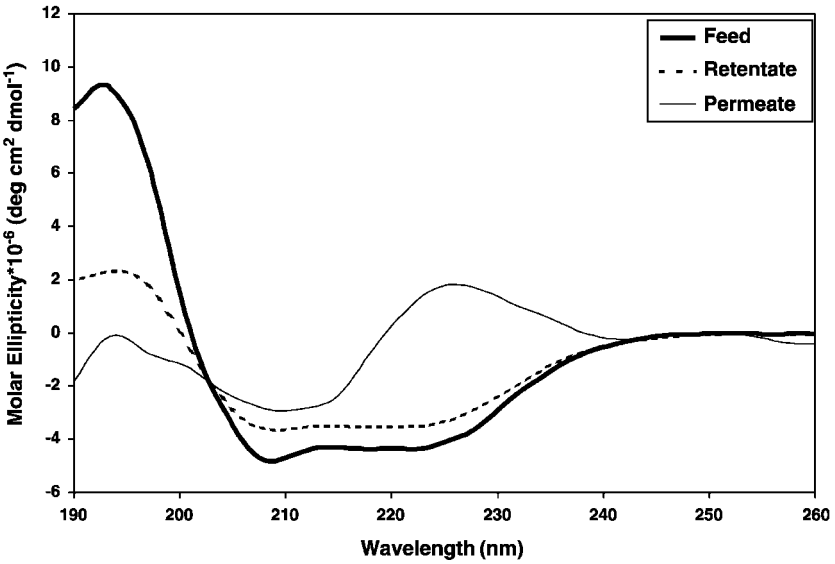

Figure 3. Typical circular dichroism spectra displayed by bGH in the feed, retentate, and permeate stream pools. Filtration conditions: poly(ether sulfone) membrane and acetate buffer, pH 4.0.

Table 2. Summary of spectroscopic and chromatographic characterization of bGH in feed, retentate, and permeate streams.

	Acetate feed, pH 4.0					Bicarbonate feed, pH 9.0				
Streams	Feed	RC retentate	RC permeate	PES retentate	PES permeate	Feed	RC retentate	RC permeate	PES retentate	PES permeate
Secondary structure content via circular dichroism spectroscopy[a]										
Helix (%)	58	50	32	26	41	65	70	25	71	17
Sheet (%)	13	18	26	34	22	06	05	31	03	27
Turns (%)	11	12	18	17	15	15	11	16	06	14
Unord. (%)	18	21	25	25	23	14	13	29	20	41
Tertiary structure via Trp^{86}*second-derivative UV spectroscopy*[b]										
291–295 nm	0.023	0.030	0.022	0.032	0.019	0.027	0.026	0.022	0.0286	0.016
Tertiary structure via Trp^{86}*emission spectroscopy*[c]										
λ_{max} (nm)	328	329	325	331	325	328	327	324	327	324
Aggregation state via size exclusion chromatography (only monomer and dimer observed)[d]										
Dimer (%)	0	0	4	1	ND[e]	91	100	14	100	ND[e]

[a] The x-ray secondary structure estimate for bGH is 56% helix, 0% sheet, 22% turns, and 22% random structure. The absolute error in % secondary structure estimation using CD is approximately ±3%.

the structural consequences of these more extensive protein–surface and protein–protein interactions.

The permeate pool for the regenerated cellulose membrane was somewhat more perturbed than that for the poly(ether sulfone) membrane. The observed perturbations should be a combination of surface exposure, transient shear, and concentration effects. The shear environments experienced by protein passing through each of these membranes should be similar due to their matching nominal molecular weight cutoffs. Differences in pore size distributions and protein residence times between the two membranes, reflecting the differing J_o and J_p/J_o values, should not be significant enough to cause observable differences in the extent of any structural perturbation due to shear effects, in our estimation. Interestingly, the regenerated cellulose permeate pool had a larger structural perturbation for bGH than that for the corresponding retentate stream. That for the poly(ether sulfone) permeate stream was less perturbed than that for the corresponding retentate stream. The poly(ether sulfone) result may, again, reflect the fact that protein in the retentate pool is exposed to the membrane surface for a longer time and at a higher local concentration, due to concentration polarization, on average than that in the permeate pool. Truskey and coworkers[8] observed significant changes in the CD spectra of proteins filtered through hydrophobic PVDF microfiltration membranes as compared to surface modified hydrophilic PVDF membranes. Their observations and those made by Soderquist and Walton[30] suggest that protein may lose activity in both the filtrate and retentate due to protein–membrane and protein–protein interactions leading to conformational perturbations.

CD-based secondary structure estimates for filtration experiments conducted in the bicarbonate buffer are also shown in Table 2. Retentate bGH samples had structure contents that are within experimental error of those the feed solution. However, the permeate samples exhibit an even greater structural perturbation than those from the acetate buffer experiments; the nature of the structural changes in terms of loss of helix and gain in sheet and unordered contents are similar to that in the acetate buffer. Permeate from the PES membranes are somewhat more perturbed than that from the RC membrane, again consistent with the lower wettability of the PES membrane.

To determine the perturbation in the tertiary structure of bGH, we used both second-derivative UV absorbance spectroscopy and fluorescence spectroscopy. A typical second derivative UV absorbance spectrum of bGH is shown in Fig. 4. Table 2 shows the peak to valley intensities between 291 and 295 nm for feed, permeate, and retentate bGH samples concentrated using the acetate and bicarbonate buffer systems. Increases in intensity are associated with an increase in the polarity of the reporter group; intensity

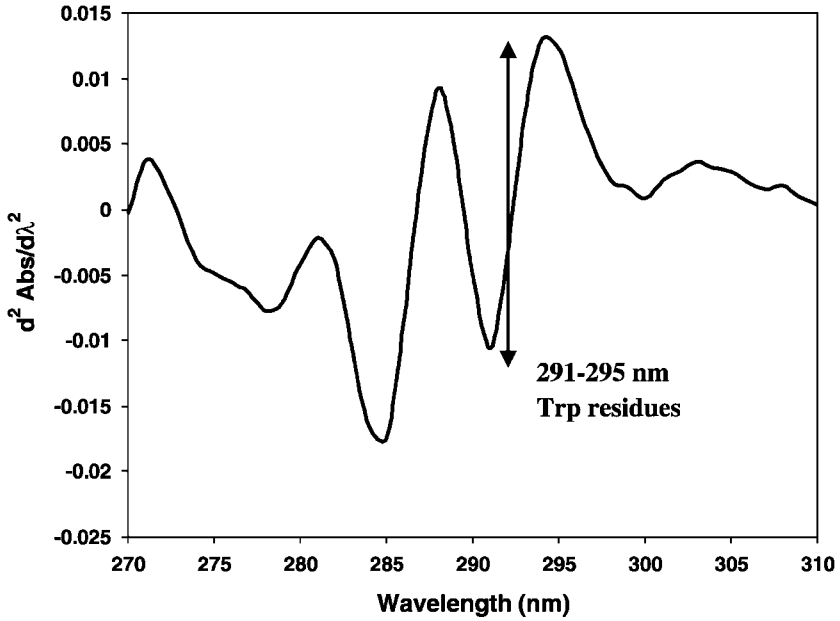

Figure 4. Typical second-derivative UV spectra displayed by bGH. Filtration conditions: poly(ether sulfone) membrane and acetate buffer, pH 4.0.

decreases with a decrease in polarity. For the samples processed in the acetate buffer, the core of bGH becomes more polar for retentate samples and less polar for permeate samples relative to the feed. An increase in core polarity is likely due to increased solvent accessibility and, hence, reflects a somewhat expanded state. A decrease in core polarity likely reflects a compaction of the hydrophobic core of bGH.[28] That protein in the retentate may tend to be somewhat expanded and that in the permeate somewhat more compact than the protein in the feed makes intuitive sense. Both membranes have a nominal molecular weight cutoff (MWCO) of 10 kDa and the protein has a molecular weight of 22 kDa. The more compact a molecule is, the larger its sieving coefficient and the lower its retention. In the bicarbonate buffer, the Trp environment has become less polar in the permeate, consistent with the argument presented previously. However, the core polarities of the protein in the retentates are statistically identical to that of the protein in the feed. This is a rather surprising result given the fact that bGH forms dimers above pH 8.5 due to reversible self-association.[18] The basic buffer leads to extensive

dimerization which presumably protects the tertiary structure of bGH molecules from perturbation as in indicated by the signal from the Trp^{86} environment of retentate samples. This is in contrast to the results with the acidic buffer where no such protection is afforded to the monomeric molecules and changes to a more polar Trp^{86} environment are observed.

We obtained corroborative results from fluorescence spectroscopy experiments. Figure 5 depicts representative fluorescence spectra of bGH in solution. Table 2 indicates the wavelength of maximum emission for the feed, permeate and retentate bGH samples concentrated under acidic and basic conditions. Again, we observed that in the acetate buffer, the Trp environment of the retained molecules became more polar, as indicated by the red shift in the emission maximum, and that of the permeate became less polar environment, as indicated by a blue shift in the emission maximum as compared to the feed sample.[26] In the bicarbonate buffer there was no appreciable change in the retentate samples, again, suggesting that dimerization provides some degree of structural stabilization, whereas a blue shift was observed in the permeate samples.

The size exclusion chromatography results of protein molecules in feed, retentate, and permeate are shown in Table 2 with a characteristic chromatogram shown in Fig. 6. Feed sample results corroborate those of Bewley[18]: bGH dimerizes to a significant degree above pH 8.5.

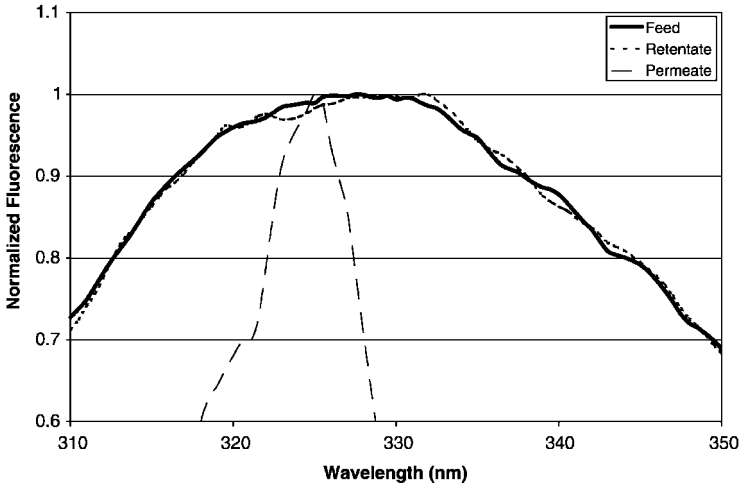

Figure 5. Typical fluorescence spectra displayed by bGH in the feed, retentate, and permeate stream pools; bicarbonate buffer, pH 9.0.

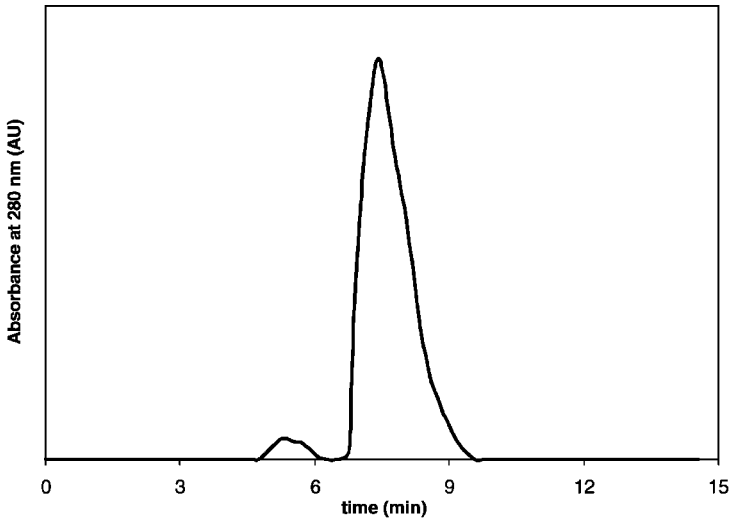

Figure 6. Typical size exclusion chromatogram displayed by bGH; bicarbonate buffer, pH 9.0 feed solution.

The aggregation state of bGH in the retentate streams is unchanged from that in the feed streams. Retention of bGH was uniformly high, regardless of the feed aggregation state. This was expected given the 22,000 Da molecular weight of bGH and the nominal 10,000 MWCO of the membranes used. The bGH from the permeate streams for the RC membrane experiments had increased dimer contents relative to the corresponding feed pools. Structure perturbations occurring on passing through the membrane may have facilitated the formation of dimers. The high retention of the PES membrane precluded an analysis of the corresponding permeate streams.

DISCUSSION

Maa and Hsu[32] used a concentric cylinder shear device and a homogenizer to study the effect of shear on proteins and the results indicated that shear alone may not cause any significant perturbation in protein conformation or formation of protein aggregates. Their observations, and those made by Truskey et al.,[8] suggest that shear stress probably does not initiate conformational changes; whereas, interaction of the proteins with a

solid surface such as a membrane surface and with other proteins appears to induce conformational changes and hence protein activity loss. Additionally, the effect of the number of passes through the membrane on protein conformation as a result of shear, protein–protein and protein–surface interactions in and around the pore has been shown to be important,[10,33] indicating the role of the membrane in protein denaturation. Recent intermolecular force measurements by Koehler and coworkers[34,35] suggest that hydrophobic and hydrophilic surfaces exhibit large and small adhesive forces respectively. However, protein–protein interactions can play a significant role in flux decline for hydrophilic membranes due to concentration polarization. Huisman and coworkers[36] have used streaming potential measurements to conclude that in the initial stages of filtration, hydrophobic protein–membrane interactions dominate the fouling mechanism; whereas, in the later stages of filtration, protein–protein interaction dictates the overall performance. Solution conditions, such as pH, also exacerbate these interactions. The results obtained here support the assertion that the ideal mode in which to ultrafilter protein solutions is to use a hydrophilic membrane and to control the wall concentration of the protein.[37] Further, the solution pH should be maintained slightly above the pI of the protein since most polymeric membranes carry a negative charge.[36] For bGH, the apparent structural stabilization afforded by dimerization at higher pHs is an added benefit of alkaline operation. For any process, the wall concentration should be optimized so as to maximize flux and minimize activity losses due to protein–membrane and protein–protein interactions at the surface of the membrane.[37] Our results show that characterizing the stability of pharmaceutical proteins can lead to process improvements: solution conditions can play a crucial role in recovering active protein. To concentrate bGH, a basic buffer above pH 8.5 should be used even though, or perhaps because, it is present as a dimer, thereafter adjusting back to acidic pH to obtain active monomers. In this case the structural perturbation would be less than that if the concentration are carried out under acidic conditions. There are of course other caveats when imposing dramatic pH changes on protein-containing process streams. bGH is minimally soluble at neutral pHs and adjusting the pH of a bGH solution from basic to acidic may lead to precipitation.

CONCLUSION

This investigation was conducted to provide a basis for a greater understanding of membrane fouling by proteins, the screening of appropriate membranes, and the selection of suitable filtration conditions that preserve the

structural integrity of proteins. The spectroscopic techniques employed here can find its use in screening potential surface or bulk modification chemistries that reduce adsorptive fouling and preserve protein conformation. Solution conditions (pH 4 vs. 9), membrane chemistry (wettable vs. less wettable), and membrane exposure (permeate vs. retentate) effect the structure (secondary and tertiary) and aggregation state (monomer and dimer) of bGH. Desirable conditions for maximum filtration performance and minimum fouling with bGH solutions included processing at high pH with a wettable membrane. This study indicates a good correlation between membrane wettability and adsorptive fouling of bovine growth hormone. A series of membrane adsorption experiments with proteins of different properties including, molecular weight, isoelectric point, surface hydrophobicity, and adiabatic compressibility is clearly needed.

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