European Journal of Pharmaceutics and Biopharmaceutics 45 (1998) 231–238

Review article

Application of infrared spectroscopy to development of stable lyophilized protein formulations

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Accepted 26 May 1997

Keywords: Protein formulation; Lyophilization; Infrared spectroscopy

1. Introduction

The preparation of protein therapeutics as lyophilized (freeze-dried) products is often essential to obtain the requisite stability during shipping and long-term storage. When prepared properly, lyophilized proteins can retain stability for months or even years at ambient temperature [1]. A crucial aspect of proper, rational development of a lyophilized formulation is the recognition that the most sensitive element in the system is the protein itself. Unfortunately, in the early days of preparing lyophilized protein formulations, it appears that frequently the focus was primarily on obtaining an acceptable dried cake structure, with the apparent assumption that a protein would be resistant to freezing and dehydration stresses. For example, when only mannitol is employed as an excipient and crystalline bulking agent, the resulting cake structure is usually excellent, but proteins derive essentially no protection from crystalline mannitol [1]. Such approaches, which can be sufficient for more stable low molecular weight drugs, led to dried products in which the protein was so susceptible to physical and/or chemical degradation that shipping and storage at subzero temperatures was required. Obviously, the potential advantages of a lyophilized product are mostly lost when there are such stringent requirements for control of product temperature.

It is now well known that without adequate stabilization by the appropriate amorphous excipients (e.g. sucrose), the protein can be irreversibly denatured after lyophilization and reconstitution and/or after long-term storage in the dried solid and rehydration [1–8]. Since most protein drugs are delivered parenterally, even if only a small fraction of the total protein molecular population (e.g. a few percent) is irreversibly denatured and aggregated, then the product will not be acceptable. Until recently the changes in protein structure arising during the processing steps of lyophilization, which were usually manifested as protein aggregation after rehydration, were unknown. However, with infrared spectroscopy it is now possible to examine directly the secondary structure of a protein in the initial aqueous solution, and in both the frozen state and the final dried solid. This analysis, combined with exploitation of stabilizers that are specific for either freezing or drying stresses, has documented that both freezing and dehydration can induce protein unfolding [1–8]. Unfolding not only can lead to irreversible protein denaturation, if the sample is rehydrated immediately, but, perhaps more importantly for industrial development of lyophilized protein drugs, can also reduce storage stability in the dried solid [1,6,7].

Moreover, simply obtaining a native protein in samples rehydrated immediately after lyophilization is not necessa-

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0939-6411/98/$19.00
PII S0939-6411(98)00005-8

rily indicative of adequate stabilization during processing steps, nor predictive of storage stability. For example, many proteins unfold during lyophilization, but readily refold if rehydrated immediately [1,2,4,5,8]. Without directly examining the structure in the dried solid, it is not possible to know whether an unfolded protein with poor storage stability is present or not.

The purpose of this review is to give an overview of how infrared spectra needed for such examination of protein structure are processed and analyzed, with our main emphasis on summarizing the practical approaches that we have found to be of use for the pharmaceutical scientist. In addition, we will summarize the results of lyophilization studies in which structural information about the protein in the dried solid was obtained with infrared spectroscopy and used to explain effects of excipients on immediate recovery of native protein after rehydration and/or subsequent storage stability in the dried solid. In general, these results document that a native protein secondary structure in the dried solid was necessary for long-term storage stability. Thus, when used properly, infrared spectroscopy can be a valuable tool for speeding the development of stable lyophilized formulations, in that immediate screening of protein structure in the dried solid should aid in the prediction of long-term stability. Finally, at this point, we wish to stress that other factors, which have been addressed in recent reviews, are also essential for long-term stability of dried proteins [1]. These include: (1) designing the formulation to minimize specific routes of chemical degradation (e.g. avoiding extremes in pH to reduce deamidation); (2) obtaining a relatively low residual moisture (e.g. ca. 1–3 g H_2O/100 g dried solid); (3) and storing the product at a temperature below the formulation glass transition temperature (T_g) [1]. With proper insight into design of formulations and the lyophilization process, all of these criteria can be met and a native protein obtained in a dried product that also has an acceptable cake structure [1].

2. Lyophilization-induced structural changes and analysis of infrared spectra

Until recently, the only way to assess the capacity of an additive to stabilize a protein during lyophilization was to measure activity and/or structural parameters after rehydration. To confound matters further, it was proposed in the protein chemistry literature that dehydration did not alter a protein's conformation [9]. Such a claim was clearly counter to the known contributions of water to the formation of the native, folded protein [10,11]. Also, it was difficult to reconcile the finding that proteins could be irreversibly inactivated and aggregated after rehydration with the contention that protein structure was not perturbed by dehydration.

Reconciliation of this apparent dilemma was provided by infrared spectroscopy, which can be used to study protein secondary structure in any state (i.e. aqueous, frozen, dried, or even as an insoluble precipitate). Infrared spectroscopy has long been used for studying stress-induced alterations in protein conformation and for quantitation of protein secondary structure (e.g. [5,12–17]). Structural information is obtained by analysis of the conformationally-sensitive amide I band, which is located between 1600 and 1700 cm^{-1}. This band is due to the in-plane C=O stretching vibration, weakly coupled with C–N stretching and in-plane N–H bending [12,13,15,17]. Each type of secondary structure (i.e. α-helix, β-sheet, β-turn and disordered) gives rise to different C=O stretching frequencies [12–17], and, hence, results in characteristic band positions, which are designated by wavenumber, cm^{-1}. Band positions are used to determine the secondary structural types present in a protein. The relative band areas (determined by curve fitting) can then be used to quantitate the relative amount of each structural component. Therefore, an analysis of the infrared bands in the amide I region can provide quantitative as well as qualitative information about protein secondary structure [5,12–17].

For studies of lyophilization-induced structural changes and assessment of effects of excipients on these alterations, quantitation of secondary structure by curve-fitting is: (1) usually not necessary; (2) can lead to more subjectivity in analyzing data (due to the guess work that is often required to obtained close fits of the curves to the component spectral bands); and (3) actually can be misleading. For example, in one case we found that α-chymotrypsinogen's secondary structure was grossly perturbed in the dried solid, based on a visual comparison of the infrared spectra of the native aqueous and dried protein [5]. In contrast, the total secondary structural contents were no different for the two samples. In this case the lyophilization-induced perturbations led to large rearrangements of structural elements, which coincidentally caused no change in relative proportions of α-helix, β-sheets and turns, and random components. There are cases in which quantitative analysis may prove useful; e.g. for determining the percentage of intermolecular beta sheet in aggregated samples or documenting an alteration in a specific band area such as that for random coil (e.g. [8]). However, even in these cases the other data analysis methods described below should also be followed.

Let us now consider the most useful data analysis methods and what they reveal about lyophilization-induced alterations in protein secondary structure. First, to obtain detailed structural information, it is necessary to enhance the resolution of the protein amide I band, which usually appears as a single broad absorbance contour (Fig. 1). The widths of the overlapping component bands are often greater than the separation between the absorbance maxima of neighboring bands. Because the band overlapping is beyond instrumental resolution, several mathematical band-narrowing methods (i.e. resolution enhancement methods) have been developed to overcome this problem [5,12–17]. For studies of lyophilization-induced structural transitions, calculation of the second derivative spectrum is

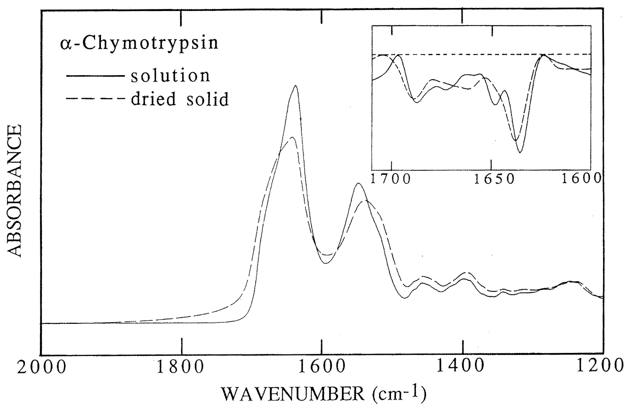

Fig. 1. Comparison of infrared spectra of α-chymotrypsin in aqueous solution and dried solid state. The inset shows the second derivatives in the amide I region for the spectra in the main panel. Reproduced from Dong et al. [5].

recommended [5]. This method is objective, and alterations in component band widths, heights and positions, which are due to protein unfolding, are preserved in the second derivative spectrum.

For most unprotected proteins (i.e. lyophilized in the presence of only buffer) the second derivative spectra for the dried solid are greatly altered, relative to the respective spectra for the native proteins in aqueous solutions [1–8]. For example, Fig. 1 compares the original and second derivative spectra for α-chymotrypsin in solution and in the dried solid. Second derivative spectra for aqueous and dried lactalbumin and lactate dehydrogenase, which are also greatly altered by lyophilization, and granulocyte colony stimulating factor (GCSF), which is minimally perturbed, are shown in Fig. 2. For dozens of proteins studied to date, lyophilization induces varying degrees of shifts in band positions and intensities, loss of bands, and broadening of bands.

The lyophilization-induced spectral alterations in the conformationally-sensitive amide I region are due to protein unfolding and *not* simply to the loss of water from the protein. The intrinsic effects of water removal on the vibrational properties of the peptide bond, and hence protein infrared spectra, were found to be insignificant by Prestrelski et al. [2]. If the direct vibrational effects of water removal were responsible for drying-induced spectral changes, then the infrared spectra of all proteins should be altered to the same degree in the dried solid, which is not the case.

The exact nature of the non-native protein molecules in the dried solid is unknown. Investigations by other spectroscopic methods (i.e. NMR and Raman) have also shown that dried protein samples contain some fraction of non-native molecules (reviewed in [4]). Since the infrared spectral signal is an average of the contributions of all protein molecules in the sample, it is not possible to discern whether the alterations induced by freeze-drying are due to partial unfolding (or complete) of most (or all) molecules or due to 'complete' unfolding of some fraction of the population. If the latter condition were met, then one might expect to see an increase in the absorbance of the infrared band due to disordered elements in the protein. However, the opposite has been noted. For example, in one study with chymotrypsinogen, the prominent band (at 1649 cm^{-1}) in the spectrum for the aqueous protein, which is assigned to these elements, was not apparent in the spectrum for the dried protein [8].

Actually, it appears that extended, random portions of the peptide backbone are not favored in the dried solid, due to

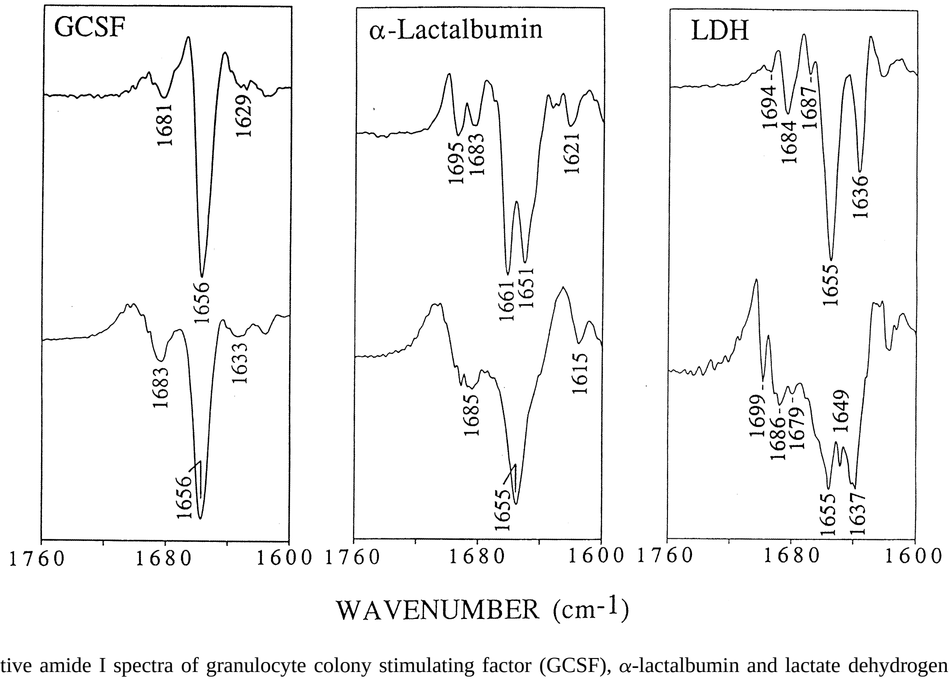

Fig. 2. Second derivative amide I spectra of granulocyte colony stimulating factor (GCSF), α-lactalbumin and lactate dehydrogenase in aqueous solution (upper spectra) and dried solid (lower spectra) states. Figure taken from Dong et al. [5], employing data from Prestrelski et al. [2,3].

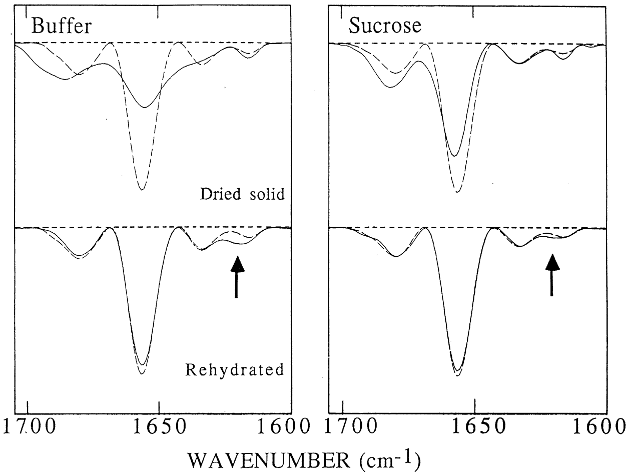

Fig. 3. Comparisons of second derivative spectra of interferon-γ in the dried solid and rehydrated states, with or without 1 M sucrose, with the spectrum of the native aqueous state. The spectrum of the native aqueous state is shown by the dashed line. The arrows indicate the band arising from non-native intermolecular β-sheet. Reproduced from Dong et al. [5].

the propensity of such regions to form intermolecular interactions in the absence of water. For example, with casein, which is essentially completely without secondary structure in solution, the amide I spectrum for the dried protein is dominated by bands arising from intermolecular β-sheet, a major structural component in non-native protein aggregates [3,6]. Similarly, when sodium thiocyanate was used to induce additional unfolding of chymotrypsinogen, the result was intermolecular β-sheet formation during freeze-drying and not an increase in disordered components in the dried protein.

Two different behaviors of proteins unfolded in the dried solid are displayed during rehydration. (1) The protein regains the native conformation upon rehydration (reversible unfolding), as observed for α-lactalbumin, lysozyme, chymotrypsinogen, ribonuclease, β-lactoglobulins A and B, α-chymotrypsin and subtilisin [1–8]. (2) A significant fraction of the protein molecules aggregate upon rehydration (irreversible unfolding), as noted for lactate dehydrogenase, phosphofructokinase, interferon-γ, basic fibroblast growth factor and interleukin-2 [1–6]). It has been documented with several proteins in the latter class that prevention of aggregation and recovery of activity after rehydration correlate directly with retention of the native structure in the dried solid [1–6]. Thus, the mechanism by which stabilizing additives (e.g. sugars) minimize loss of activity and aggregation during lyophilization and rehydration is to prevent unfolding during freezing and drying [1–6]. Also, unfolding of proteins that refold, if immediately rehydrated, can be inhibited by stabilizing additives [1–8]. As will be explained in more detail below, it appears crucial that even these proteins should be stabilized against lyophilization-induced unfolding, in order to maintain stability during long-term storage in the dried solid [1,6,7]. A good example of the effects of sucrose on a protein's structure during lyophilization is provided by results with interferon-γ. The spectrum for the protein lyophilized in the presence of 1 M sucrose is similar to that for the native aqueous protein, whereas that for the protein dried alone is greatly altered (Fig. 3). For analysis of these data, a baseline was fitted to the second derivative spectra and they have been normalized for total area (see [5,18]). This data presentation is useful because it allows visualization of the relative shifts of area from one component band to another, and, hence, the redistribution from native to non-native secondary structural elements. For example, for the sample dried without sugar, there is a loss of α-helix as indicated by the decreased absorbance in the 1656 cm^{-1} band, which is compensated by increased absorbance in bands for β-sheet and turn (ca. 1640–1645 and 1665–1695 cm^{-1}). These changes are atte-

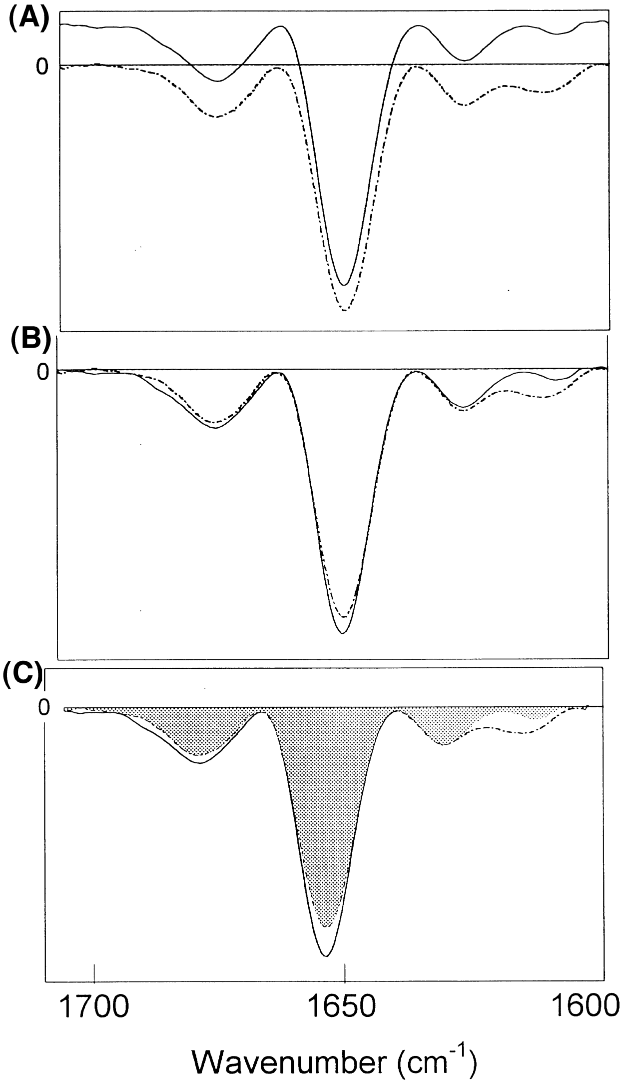

Fig. 4. Second derivative amide I spectra of interferon-γ. (A) Uncorrected spectra, correlation coefficient = 0.80; (B) baseline offset corrected, correlation coefficient = 0.995; (C) area-normalized spectra, area of overlap = 0.92. Solid lines indicate the native aqueous state, dashed lines indicate rehydrated aqueous state, and the gray fill (panel C) indicates the area of overlap. Reproduced from Kendrick et al. [17].

nuated when the protein is lyophilized in the presence of sucrose, documenting an increased retention of native structure in the molecular population.

After rehydration, the spectra of both samples are very native-like, indicating that the majority of non-native molecules have refolded (Fig. 3). However, in spectrum of the sample lyophilized without sucrose, the appearance of a new band near 1625 cm^{-1}, which is assignable to intermolecular β-sheet structure, and the decreased intensities in vibrational bands ascribed to α-helix (1656 cm^{-1}) and turn (1688–1665 cm^{-1}) structures, indicate the formation of protein aggregates upon rehydration (see [5] for a detailed review of the study of protein aggregation with infrared spectroscopy.). In this sample, 18% of the protein formed insoluble aggregates. In contrast, in the sample lyophilized with sucrose, only 9% insoluble aggregate was noted after rehydration. This reduction in aggregation is reflected in a much weaker 1625 cm^{-1} band in the spectrum of the rehydrated sample.

As exemplified by the results discussed above, the best way to begin analysis of protein infrared spectra is to compare visually second derivative spectra that are area-normalized and overlaid. Often lyophilization of a protein without stabilizer leads to large scale changes in all of the component bands. Then, a visual examination of spectra of samples prepared with different amounts of stabilizing excipient (e.g. sucrose) can reveal a ‘titration’ of the spectral features towards those for the native protein. In other cases (e.g. proteins with a very high α-helix content such as interferon-γ), it is sometimes useful to monitor a single band which is most sensitive to lyophilization-induced damage and the effects of stabilizers. In this situation, quantitative data can be obtained by determining the relative height of the band of interest in spectra that have been area-normalized. This approach has recently been applied successfully in studies of recombinant hemoglobin [19].

However, often it is not adequate to focus on a single band for quantitative analysis. Then, a ‘global’ quantitation of similarity between the second derivative infrared spectrum for the native protein, which is used as a reference state, and the spectra for experimental samples is needed. For this analysis, Prestrelski et al. [2,3] originally developed a mathematical procedure to calculate the spectral correlation coefficient (similarity) between two second derivative spectra. More recent analysis indicated that this method can occasionally provide misleading information [18]. If the spectra have offset baselines, then the correlation coefficient is much lower than that expected based on a visual assessment of spectral similarity (Fig. 4A). In contrast, if the spectra are dominated by a large band of high symmetry, the value is too great (Fig. 4B). These shortcomings are avoided by simply normalizing the areas of the reference (e.g. aqueous native protein) second derivative spectrum and that for the experimental sample (e.g. unfolded protein in the dried solid), and then determining the fractional area that the spectra share. The method of determining this ‘area of overlap’ is described in detail in a recent paper by Kendrick et al. [18] and an example is presented in Fig. 4C.

In addition to quatitating effect of lyophilization on protein structure, we have also used area of overlap analysis to quantitate protein unfolding by guanidine HCl (Fig. 5). As can be seen in Fig. 5, the changes in a protein’s second derivative spectrum induced by chemical denaturation are very similar to those noted after lyophilization. There are alterations in relative band absorbances, widths and positions. Furthermore, the unfolding curve generated with infrared spectroscopic data is essentially identical to that based on circular dichroism spectroscopy (Fig. 6). These results further support the contention that infrared spectroscopy coupled with area of overlap analysis can be used to assess quantitatively protein unfolding.

It is important to note here that with some samples a visual impression may be the only useful means for comparing spectra, because band shifts, which are significant in terms of structure, may result in only a relatively small decrease in the area of overlap parameter. In such cases, the resolution between fully native and unfolded samples becomes so small that an incremental improvement in structure noted with a stabilizer is not resolved with the area of overlap analysis. Then, one must carefully make a qualitative assessment of the spectra to discern what stabilizer

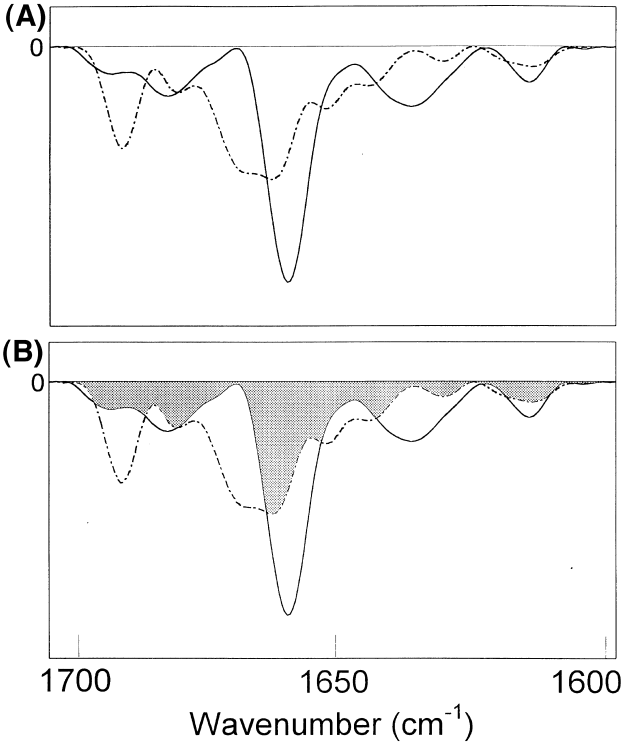

Fig. 5. Second derivative amide I spectra of wild type iso-1-cytochrome *c* in aqueous solution in the presence and absence of guanidine HCl. (A) Baseline offset corrected and area normalized, correlation coefficient = 0.72. (B) Baseline offset corrected and area normalized, area of overlap = 0.63. Solid lines indicate no guanidine HCl, dashed lines indicate 2.5 M guanidine HCl, and the gray fill (panel B) indicates the area of overlap. Reproduced from Kendrick et al. [17].

concentration and types afford the greatest retention of native-like features in the spectrum of the dried protein; for example, by monitoring relative intensities and positions of component bands that are most different between spectra for native and unfolded states.

Finally, preliminary infrared spectroscopic investigations have provided direct evidence for freezing-induced perturbation of protein secondary structure (B. Kendrick, A. Dong, L. Krielgard, T. Anchordoquy and J. Carpenter, unpublished observations). For example, it was found that structure of lactate dehydrogenase was slightly perturbed in the frozen state and that the protein refolded upon thawing. In contrast, recombinant Factor XIII dimer was irreversibly unfolded by freezing. Usually it appears that the degree of structural perturbation noted in the frozen state is intermediate to that noted in dried solid, indicating that freezing-induced unfolding contributes partly to the total protein damage noted during lyophilization. This infrared method will be valuable for assessing the relative contributions of excipients to stabilization during the freezing and drying steps. A caveat is that at the concentrations necessary (i.e. >2 mg/ml) to obtain high-quality protein infrared spectra in the frozen state, many proteins, which are known to be denatured at lower concentrations (e.g. catalase), are not unfolded during freezing.

3. Practical aspects of infrared spectroscopic analysis of protein

It is obvious from the infrared studies to date that the effect of a given additive may vary depending on the protein (e.g. [2]), the presence of other additives and other specific solution conditions (e.g. pH; [6]). Therefore, the structure of each dried protein in each formulation should be studied with infrared spectroscopy. Unfortunately, this will not be possible with certain formulations. If albumin is used, then, as is the case with any physical measurement, it will not be possible to separate the albumin contribution to the data from that of the protein drug. If other compounds (e.g. polyvinylpyrrolidone, arginine, histidine, glycine, lysine) that absorb strongly in the amide I region are used in the formulation, then they may interfere with the protein spectrum in the dried solid. Sometimes, if relatively low concentrations of such additives are used, it may be possible to gain useful information by focusing on regions on the protein spectrum in which the bands from the interfering substance do not appear. Although contributions from interfering substances can readily be subtracted from the spectra of aqueous protein solutions, this is not the case in the dried solid. The bands for interfering substance dried without protein are shifted in position from those for the substance dried with protein. Thus, it is usually not possible to obtained a quantitative subtraction of the interference.

There should be few barriers to implementing infrared spectroscopic analysis, at least in industrial laboratories. The instrumentation is available commercially at relatively modest costs. Also, high quality spectra can be acquired in less than 5 min and with minimal sample preparation. Moreover, with instruction in a properly equipped lab and from a experienced spectroscopist, a researcher can learn the fundamentals of spectral acquisition in a few days. Also, the skills needed to make rudimentary interpretation of spectra (e.g. at the level of relative comparison between the spectra for an aqueous protein and lyophilized protein) can be acquired fairly rapidly. However, detailed quantitative analysis of spectra and assignment of spectral bands can be difficult for even a spectroscopist with years of experience (cf. [17]).

The main disadvantage of the technique in the past was that a minimum protein concentration of 15 mg/ml is needed to obtain quality spectra of proteins in H_2O solutions. This is because water absorbs strongly in the amide I region. However, with a new instrument introduced in 1998, it is now possible to obtain high quality spectra in H_2O with as little as 3–5 mg/ml protein. The approaches needed to subtract quantitatively the contributions of liquid and gaseous water from the spectrum of a protein solution have been described in detail in earlier reviews (e.g. [16]) and will not be considered here.

The absolute mass of protein needed is not great, since as little as 5 μl of solution is required to load certain sample cells. If solubility is limited, then the protein can be studied at much lower concentrations (ca. <1 mg/ml) in D_2O. However, the researcher must then be aware of the potential difficulties of data interpretation due to the direct effects of H–D exchange on the vibrational frequencies of amide I component bands (see [5,17,20]). In some cases, deuteration of the protein makes assignment of bands to different sec-

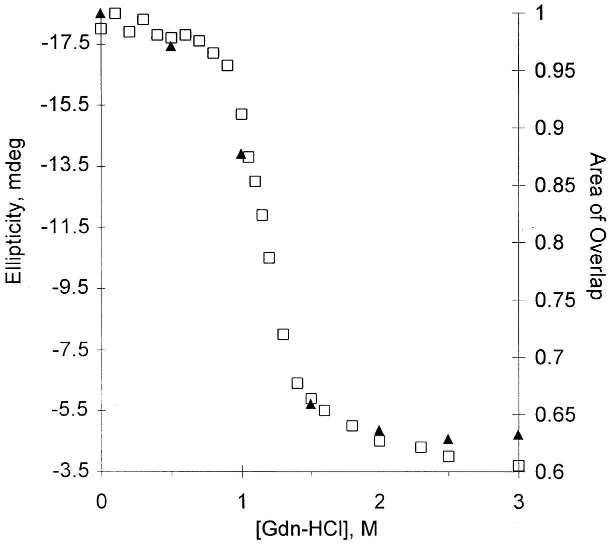

Fig. 6. Guanidine HCl-induced unfolding of wild type iso-1-cytochrome *c*. The unfolding transition is presented as a plot of ellipticity at 220 nm in millidegrees (open squares) and infrared spectral area of overlap (closed triangles) as a function of guanidine HCl concentration. Reproduced from Kendrick et al. [17].

ondary structural types uncertain. This can be a problem if quantitation of secondary structural content is needed. However, if all that is required is a global comparison between a spectrum for an aqueous control sample and that for a freeze-dried protein, then a protein can be studied reliably in D_2O. The only caveat is that sufficient time for H–D exchange must be allowed prior to lyophilization (cf. [20]), so that additional exchange does not arise during freezing and drying.

4. Effect of protein structure on storage stability in the dried solid

Despite our rather confident claims that native protein structure is a requirement for long-term stability in the dried solid, there have been only two published studies to date that address this issue. Our confidence comes from the data in these studies, but also from the results of several unpublished studies that have been conducted by us and others on proprietary industrial formulations. Hopefully, these latter studies will also soon make it into the peer reviewed literature.

In published reports Prestrelski and colleagues first suggested that in addition to storage below T_g, long-term stability is dependent on retaining the native protein structure in the dried solid [4]. The first published data supporting this proposition came from their study with interleukin-2, in which they found that when lyophilized from a solution of pH 7 the protein was unfolded in the dried solid and unstable during storage at 45°C [6]. In contrast, lyophilization from a solution with pH 4 led to a native protein and storage stability. This storage temperature was most likely below the sample T_g, because characteristically dried proteins have T_gs greater than 100°C [21]. Using protein in a pH 7 solution, they also compared protection afforded during lyophilization and storage stability conferred by carbohydrates of increasing molecular weight [6]. As molecular weight increased, the inhibition of unfolding during processing decreased and the formulation glass transition temperature increased. The optimum stability during storage at 45°C was noted in samples stabilized with the tetrasaccharide, stachyose, in which the dried protein was native and T_g was greater than 45°C. The protein degraded in samples with lower molecular weight carbohydrates, which had native protein, but a T_g less than 45°C. Thus, even with a native protein, stability is dependent on maintaining the temperature below T_g. With the highest molecular weight dextrans, for which $T_g > 45°C$, the protein was initially unfolded. However no further damage was noted during storage, which supports the stabilizing role of the glassy state.

More recently, Chang et al. [7] have studied the role of protein structure in the storage stability of lyophilized interleukin-1 receptor antagonist. A series of formulations were prepared (100 mg/ml protein) with initial sucrose concentrations ranging from 0 to 10% (w/v), all of which had a T_g of 66 ± 2°C. Those with sucrose concentrations less than 5% degraded rapidly (via deamidation and aggregation, which were measured after rehydration) during storage below T_g at 50°C. In contrast, formulations with higher sucrose concentrations had less than 2% deamidation and no detectable aggregation after 14 months at 50°C. Infrared spectroscopy indicated that these stable formulations contained native protein in the dried solid, whereas the protein was unfolded in the unstable formulations. Thus, as was the case with interleukin-2, in addition to storage of a dried formulation below T_g, with interleukin-1 receptor antagonist it was also necessary to obtain a native protein during the lyophilization cycle.

Finally, the importance of inhibiting protein unfolding during the lyophilization process in long-term storage stability also can be inferred from earlier studies that did not determine protein structure in the dried solid. For example, Isutzu et al. [22] found that storage stability of β-galactosidase at 70°C for 7 days was greatest in the presence of stabilizing excipients (e.g. trehalose and sucrose), which also led to full enzyme activity recovery in samples rehydrated immediately after lyophilization. Acute, irreversible loss of activity and further instability during storage were noted in samples lyophilized without stabilizers or with agents that did not protect well during lyophilization (e.g. mannitol). Since minimizing acute loss of enzyme activity correlates with inhibition of unfolding during lyophilization [2,3], Isutzu's data support the contention that increased storage stability conferred by certain excipients is due the maintenance the native protein structure during lyophilization. The T_gs of the final dried solids were not measured. It is most likely that the T_gs of the stable formulations were higher than the storage temperature of 70°C and/or the duration of storage was not sufficient for degradative processes to be manifested in samples with T_gs lower than 70°C.

5. Conclusions

Infrared spectroscopy provides a powerful means to assess the effects of lyophilization and excipients on protein structure in the dried solid. Although only limited published studies have documented the importance of protein structure in storage stability in the dried solid, it seems clear that infrared spectroscopic analysis of protein structure should be considered as an essential tool for the pharmaceutical scientist working on protein products.

Acknowledgements

We gratefully acknowledge support by grants to J.F.C. from the National Science Foundation (BES9505301 and BES9520288), the Whitaker Foundation, Genentech Inc., Boehringer Mannheim Therapeutics, Amgen, Genetics Institute and Genencor International. We also thank the National Science Foundation, the American Foundation of Pharmaceutical Education, the Pharmaceutical Manufacturers and Research Association and the Colorado Institute for Research in Biotechnology for providing pre-doctoral fellowships to students in J.F.C.'s laboratory.

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