Development of a Stable Freeze-dried Formulation of Recombinant Human Interleukin-1 Receptor Antagonist

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Purpose. A formulation of recombinant human interleukin-1 receptor antagonist (rhIL-1ra) was developed that provided both acute protection during lyophilization and storage stability in the dried solid.

Methods. The formulation was optimized by monitoring the impact of excipients on protein degradation which was analyzed by turbidimetry and cation-exchange HPLC.

Results. The most appropriate pH was 6.5. Sodium citrate buffer provided better stability than sodium phosphate buffer. Glycine was selected as a bulking agent because the greatest protein stability was noted when this bulking agent was used in combination with an amorphous protein stabilizer. Among the amorphous stabilizers tested, sucrose protected rhIL-1ra best in the presence of glycine. When the protein was freeze-dried in the presence of an inadequate mass ratio of sucrose/protein (< 0.3), the rate of degradation of rhIL-1ra increased. For a formulation containing 100 mg/ml of rhIL-1ra, increasing the sucrose/protein mass ratio to ≥ 0.3 greatly increased storage stability. The moisture content of the dried solid affected the storage stability to a minor degree. Three different stoppers obtained from the WEST Company did not affect the stability of rhIL-1ra.

Conclusions. An optimized formulation could be reconstituted without precipitation after 14 months at 30 or 50°C. At 30°C, there was no loss of native protein due to deamidation, and only a 4% loss at 50°C. These results indicated that the optimized formulation could be stored at ambient temperatures for long periods, without damage to the protein.

KEY WORDS: freeze-dried formulation; rhIL-1ra; protein stability.

INTRODUCTION

One of the main difficulties in the commercial development of protein pharmaceuticals is overcoming their marginal stability (1). Both the physical and chemical stability of proteins in aqueous solution can be greatly perturbed by stresses (including extremes in temperature and agitation) that are routinely encountered during the processing, shipping, storage, and administration (1,2). The physicochemical modifications often result in reduced efficacy and in undesirable byproducts (e.g., protein aggregates) that may increase the risk of adverse side effects. Theoretically, the long-term stability of a protein should be much greater in a freeze-dried solid than in aqueous solution. However, the freezing and dehydration induce protein unfolding (3). Unfolding not only can reduce protein recovery in a formulation rehydrated immediately after lyophilization, but also has been shown to reduce storage stability in the dried solid (4).

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In addition to the efficacy of stabilizing excipients in the preservation of proteins during freeze-drying and subsequent storage, many other factors can affect the proteins' stability (5,6). Individual parameters, such as buffer, pH, bulking agents, moisture content, protein concentration and the effect of moisture transfer from the stopper must be evaluated (5,6). The current study details the steps taken to obtain a stable lyophilized formulation for recombinant human interleukin-1 receptor antagonist (rhIL-1ra), which was potential in treating various cytokine-mediated illnesses. The optimum formulation was fully stable during storage for 14 months at 30°C and had only a 4% loss protein due to deamidation after 14 months at 50°C. This formulation also gave the desired cake morphology and dissolution properties (7,8) and was amenable to being dried rapidly and efficiently (9). The formulation was optimized by understanding the modes of protein degradation and by rationally choosing solution conditions (e.g., pH) and excipients to minimize degradation. Although this work focused on a single protein, the principles followed are general and, thus, should also be applicable to other proteins.

MATERIALS AND METHODS

Materials

RhIL-1ra was produced and purified to pharmaceutical quality (> 98% purity) at Amgen, Inc. (Boulder, CO). Each formulation was made by exchanging the buffer with an excess amount of test formulation using an Amicon stirred cell with YM10 membrane. Vials (3 ml) and stoppers (13 mm) were obtained from the WEST Co. (Phoenixville, PA). Three different lots of stoppers, V50 9310 (Butyls with sulfur resin cure), V50 4416 (Bromobutyl with zinc oxide cure), and V50 4405 (Bromobutyl with sulfur resin cure), were examined in this study. Buffer salts and excipients were obtained from Spectrum Chemical Mfg. Co. (Gardena, CA).

Lyophilization

The protein samples were lyophilized in an EDWARDS Supermodulyo lyophilizer (EDWARDS vacuum products, Tonawanda, NY). Each vial was filled with 1 ml of the formulation to be tested. Vials were frozen on a pre-chilled shelf at −40°C for 1 hour. The shelf temperature was then warmed to −15°C at a heating rate of 2°C/min. This warming was sufficient to promote crystallization of bulking agents such as glycine and mannitol (7). A vacuum was pulled to initiate primary drying, and the chamber pressure was maintained at 50 μmHg. The shelf temperature during the primary drying was −15°C. During primary drying, the sample temperature was maintained at −38°C, which is well below the lowest Tg′ (−32°C) of the formulations tested. Primary drying was continued for 20 hours. Secondary drying was initiated by raising the shelf temperature to 30°C at a heating rate of 2°C/min. Secondary drying was continued for 8 hours. After drying was complete, the head space was filled with dry nitrogen gas before stoppering. In order to generate samples with different moisture contents, secondary drying was completed at 0, 1, 2, 3, 4, and 8 hours after the shelf temperature reached 30°C. Moisture content was analyzed by Karl Fisher assay as reported by May et al. (10). The values reported are the means for three separate vials.

Stability Studies

Storage stability was assessed by incubating the lyophilized vials at 8, 30, and 50°C. For screening pH, buffer, bulking agent and amorphous protein stabilizer, appropriate formulations containing 10 mg/ml rhIL-1ra were stored for 12 weeks and analyzed every 3 weeks. To understand the relationship between protein concentration and sucrose concentration (i.e., the mass ratio of sucrose/protein), samples were stored for 14 months and analyzed every 12 weeks. For the study of the effects of residual moisture and stopper lot, formulations containing 50 mg/ml rhIL-1ra, 2% glycine, 1% sucrose, in 10 mM citrate buffer at pH 6.5 were stored for 6 months and analyzed every 4 weeks.

Analyses of Degradation

All the high performance liquid chromatography (HPLC) assays were performed with a Beckman HPLC system, which was interfaced to system GOLD software (Beckman) for programming and data acquisition. Purity was assessed by Cation-exchange HPLC (CEX-HPLC) on a Bio-gel® SP-5-PW (7.5 mm × 7.5 cm, Bio Rad) column. The running buffer was 20 mM MES at pH 5.5. A gradient of 0 M to 1 M sodium chloride was used. The flow rate was 0.5 ml/min, and 500 μg of protein was injected for the analysis.

The turbidity of reconstituted formulations was determined by measuring UV absorption at 500 nm. To establish the validity of using turbidity, which can be rapidly and easily measured, as a quantitative measure of aggregation, we examined the relationship between the amount of precipitated protein and the degree of turbidity. The precipitated protein was collected by centrifugation. Various amounts of the collected material were then added back to fresh buffer. After the suspension was mixed by vortexing, the turbidity of the reconstituted precipitate was determined. A linear relationship was observed between turbidity and protein concentration in the range of 0.1–0.3 mg/ml protein. Hence, in the linear portion of the plot, turbidity, which ranged from 0.05 to 2.0 Abs_{500}, could be used directly as a quantitative measure of protein aggregation. Deionized water was used as a reference.

Reaction rates were determined by calculating the slope of the purity decrease or the turbidity increase versus duration of storage. One standard deviation for the slope obtained by linear regression was used as the error for the reaction rate. For the rate constants presented without standard errors, estimated experimental errors for degradation rate and turbidity increase rate are 20% and 10%, respectively, of the values.

Isoelectric focusing was performed with a precast gel purchased from Intermountain Scientific (Rockland, ME). The pH range was between 3.5 and 9.5. The gel was silver stained after focusing. Standard pI markers were purchased from Pharmacia LKB (Piscataway, NJ).

Tryptic mapping of rhIL-1ra was initiated by alkylating the protein with iodoacetic acid in the presence of 1.6 M guanidine hydrochloride, without a reducing agent. The alkylated protein was digested with trypsin overnight at 37°C. The digested sample was analyzed with a reverse-phase HPLC using Vydac C4 column. Acetonitrile was used to develop a gradient of 0% to 100%.

Mass spectrometry was performed on a Sciex triple quadrapole mass spectrometer (Perkin Elmer Sciex Instruments, Thornhill, Ontario, Canada), which was calibrated with polypropylene mass standards. The samples were infused at 10 μl/minute and ionized by an orifice potential of 70 Volts. Sample ion streams were scanned in the first quadrapole from 400 to 2400 atomic mass units (a.m.u.). Deconvolution of raw mass data was performed using Mac Biospec software provided by Sciex.

RESULTS AND DISCUSSION

Degradation of rhIL-1ra in Lyophilized Formulations

Prior to testing various stabilizing additives, it was important to determine the major degradation products that formed during lyophilization and storage. Once the protein damaged was characterized, then by rationally altering solution conditions and choosing excipients, the formulation could be optimized for protein stability. Protein degradation was first analyzed with CEX-HPLC. Comparison of a native protein sample to a lyophilized sample, which was prepared without stabilizers, stored at 50°C and then rehydrated, indicated that the major degradation product eluted just prior to the native protein (Figure 1b). This fraction was designated the pre-shoulder peak 2 (PS2). To determine the effect of storage temperature and duration of storage, the purity of the rehydrated material was quantified by measuring the relative area of the main native protein peak. Loss of purity was accelerated as the storage temperature was increased.

The nature of the major degradation product was examined by purifying and characterizing the PS2 fraction. Isoelectric focusing showed that the pI of PS2 was 5.7, which is significantly lower than that the pI of 6.2 for native rhIL-1ra. Hence, the less positive charge of the PS2 fraction, under the conditions used for CEX-HPLC, would account for the earlier elution time during chromatography. Tryptic mapping indicated that a single peptide (designated T13) was modified in PS2, relative to the native protein. Mass spectrometry showed that modified, T13 peptide had a mass almost identical (within 1–2 mass units) to that for the unmodified peptide from native rhIL-1ra (data not shown). Taken together, these results strongly suggest that deamidation led to the formation of the PS2 fraction (1).

Another major degradation product, due to physical instability (see below), was aggregated protein, which made the reconstituted solution turbid. The precipitated aggregates could be dissolved in 0.1% sodium dodecyl sulfate, indicating that the intermolecular interactions in the aggregate were not covalent. The increase in turbidity, in samples stored at 8, 30, and 50°C prior to reconstitution, was a linear function of storage time.

Finally the interrelationship between deamidation and aggregation was investigated. The precipitated protein was collected by centrifugation, washed several times with buffer to remove "trapped" soluble protein molecules and subjected to peptide mapping. The precipitate contained T13 peptide from PS2 protein molecules, as well as from native protein molecules. The ratio of PS2:Native in the precpitate was equivalent to the ratio in the supernatant. Thus, although some of the studies discussed below show that protein aggregation and deamidation often were positively correlated, it appears that the two degradation pathways are not wholly interdependent.

Protection of the Protein During the Lyophilization Cycle

Prior to attempting to optimize storage stability of a protein, it is essential to design a formulation that protects the protein during the lyophilization cycle itself. Protein unfolding due to the freezing and drying stresses arising during lyophilization can be minimized by stabilizing additives (3,11–14). Samples formulated with only 4% mannitol or 2% glycine in a citrate buffer at pH 6.5 formed a hazy solution after lyophilization and reconstitution. Mannitol and glycine most likely fail to prevent protein aggregation because, as indicated by differential scanning calorimetry (cf., 7), these compounds crystallized during the lyophilization cycle. Several studies have documented that to protect a protein during dehydration, an additive must not crystallize and must interact with the protein in the amorphous phase (3,8,11–13). Inclusion of 1% sucrose, an excipient known to remain amorphous (7), resulted in a clear solution after reconstitution (data not shown). It has been shown with infrared spectroscopy that protein aggregation during rehydration can be inhibited if the appropriate stabilizers (e.g., sugars) are used to prevent unfolding during lyophilization (3,11,13,14). The role of sucrose in preventing precipitation of rhIL-1ra during freeze-drying and reconstitution is consistent with the proposal that the sugar inhibited lyophilization-induced protein unfolding.

Stabilization During Storage

Selection of pH and Buffer

Having established a formulation (e.g., 1% sucrose with 2% glycine) that protected the protein during the lyophilization process, our next goal was to optimize parameters for storage stability. When samples were lyophilized in 2% glycine, 1% sucrose, and 10 mM sodium citrate buffer with an initial pH < 6.0, severe precipitation was observed after storage and reconstitution (Figure 1a). Conversely, there was less chemical degradation at lower pHs (Figure 1b), which is consistent with the general finding that the deamidation rates are slowed as pH is lowered across the range tested in the current study (1,2). The differing pH-dependency of the chemical and physical degradation supported the contention that the two processes were at least partially independent and indicated that a compromise was needed to minimize both types of damage. To obtain minimum chemical degradation, without significant precipitation, pH 6.5 was chosen for further studies.

At pH 6.5, formulations containing phosphate buffer were found to form aggregates more rapidly than the formulations containing citrate buffer, even though other excipients were identical (Figure 2). The effect of phosphate buffer may be due to selective crystallization of the dibasic salt, which would expose the protein to a pH as low as 4–5 during freeze-drying (7,17). However, factors other than the presence of sodium phosphate buffer must be important, because greater damage was noted with mannitol/phosphate formulations than with glycine/phosphate formulations. One possible explanation is that different amount of the dibasic salt crystallized in these formulations (7), with the greatest crystallization in those formulations conferring the most protein damage.

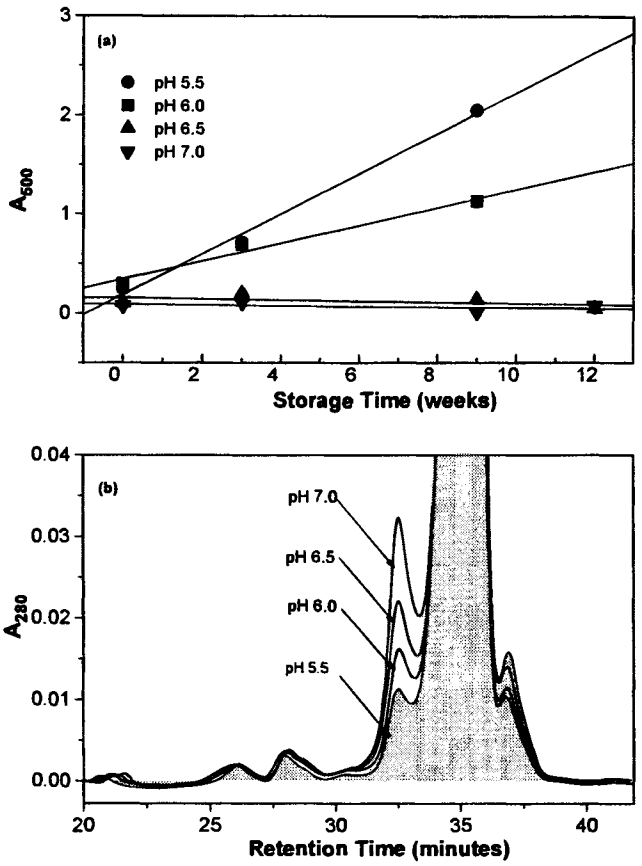

Fig. 1. Effect of pH on the storage stability of lyophilized rhIL-1ra. (a) Effect of pH on turbidity. Samples were stored at 50°C, prior to reconstitution and analysis. (b) Effect of pH on chemical degradation as determined by CEX-HPLC. Samples were stored for 4 weeks at 50°C. See text for formulation components.

Selection of Bulking Agent

Often, crystalline bulking agents are preferred for lyophilization because they form stronger dried cakes, with better dissolution properties, than amorphous agents (7). All of the tested rhIL-1ra formulations contained mannitol, glycine, or alanine as crystalline bulking agents and formed desirable cakes after freeze-drying (data not shown). In the presence of 1% sucrose, glycine provided the greatest stability among tested bulking agents (Table I). Speculatively there are several, nonexclusive mechanisms for this observed difference in stability. 1) There may be a difference in the capacity of the bulking agents to aid in protecting the protein during freezing and drying (3,11,13,15). 2) The contribution of the bulking agents to the amorphous fraction may vary, as may the glass transition temperature of this fraction (7). Reducing the glass transition temperature would be expected to reduce stability at the higher temperatures studied (16). 3) A fraction of the bulking agent remaining amorphous during lyophilization could crystallize during storage and release moisture to the remaining amorphous components, including the protein (7).

Selection of an Amorphous Stabilizer

As discussed earlier, for rhIL-1ra formulated with glycine and citrate buffer, both the protection of the protein during

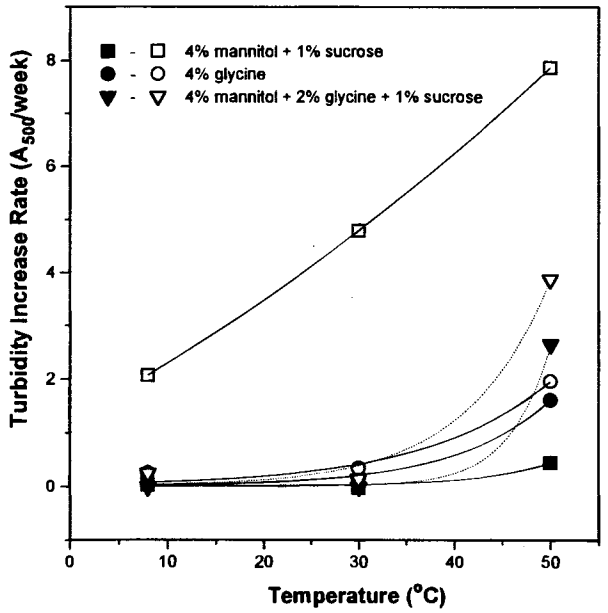

Fig. 2. Effect of buffer salts on storage stability of lyophilized rhIL-1ra. Data for 10 mM sodium phosphate buffer are represented by open symbols and those for 10 mM sodium citrate buffer are represented by closed symbols. The pH of each solution was 6.5. Each formulation contained 10 mg/ml rhIL-1ra.

Table I. Effect of Bulking Agents on the Storage Stability of Lyophilized rhIL-1ra[a]

Bulking agents	Stabilizers	Degradation Rate (%/week) 8°C	30°C	50°C	Turbidity Increase Rate (A_{500}/week) 8°C	30°C	50°C
4% mannitol		0.095	0.213	0.727	0.005	0.003	0.009
4% mannitol	1% sucrose	0.030	0.027	0.450	0.003	0.002	0.006
2% glycine		0.088	0.315	1.77	0.016	0.044	0.075
2% glycine	1% sucrose	0.033	0.007	0.153	0.007	0.002	0.008
4% alanine		0.233	0.223	1.013	0.006	0.05	0.055
4% alanine	1% sucrose	0.017	0.057	0.297	0.007	0.004	0.021

[a] Each formulation contained 10 mg/ml rhIL-1ra and sodium citrate as a buffer at pH 6.5. Bulking agents examined here were selected to form crystalline cake to facilitate drying process and to provide a good appearance.

lyophilization and the storage stability were improved if 1% sucrose was included in the formulation. As shown in Table II, 1% maltose or trehalose, as well as sucrose, essentially completely inhibited aggregation in samples that were reconstituted after storage for more than 3 weeks at 50°C. Sorbitol was less effective at inhibiting aggregation. However, sucrose stabilized rhIL-1ra against chemical degradation better than other stabilizers (Table II). Accelerated chemical degradation with maltose may be because this is a reducing sugar, which could react with proteins' amino groups (18). Both the browning of powder and the appearance of new peaks in CEX-HPLC chromatograms supported this speculation. Both trehalose and sucrose are nonreducing disaccharides, yet chemical degradation was more rapid in the presence of trehalose than sucrose.

Table II. Effect of Different Stabilizers on the Storage Stability of rhIL-1ra[a]

Stabilizers	Degradation Rate (%/week) 8°C	30°C	50°C	Turbidity Increase Rate (A_{500}/week) 8°C	30°C	50°C
None	0.088	0.315	1.77	0.016	0.044	0.075
Sucrose	0.033	0.007	0.153	0.007	0.002	0.008
Maltose	0.087	0.383	6.433	0.000	0.001	0.003
Sorbitol	0.044	0.030	2.297	0.002	0.001	0.037
Trehalose	0.070	0.150	0.430	0.001	0.002	0.005

[a] Each formulation contains 10 mg/ml rhIL-1ra, 2% glycine, and 10 mM sodium citrate buffer at pH 6.5. The sugar concentration was 1% (w/v).

The stability afforded by sucrose can be ascribed, at least in part, to its inhibition of protein unfolding during lyophilization (3,4,13). Protein unfolding could expose amino acids to an environment conducive to degradative chemical reactions during long-term storage (13). This effect has been documented in aqueous solutions (1,2), but has not yet been confirmed for freeze-dried formulations. Since trehalose also inhibits lyophilization-induced unfolding,(3,4,12) it is not known why this sugar is not as effective as sucrose in protecting against chemical degradation during subsequent storage.

Effect of Moisture Content

To examine the effect of moisture content on storage stability, a formulation containing 50 mg/ml rhIL-1ra, 2% glycine, 1% sucrose, and 10 mM citrate buffer at pH 6.5 was lyophilized to 6 different moisture contents, ranging from 0.5% to 3.2% (w/w), and stored at 30°C. Relative stability was significantly affected by moisture content (Figure 3). The formulation was least stable at moisture content around 0.8% (w/w). The greatest stability was noted at moisture content of 3.2% or ≤ 0.5%.

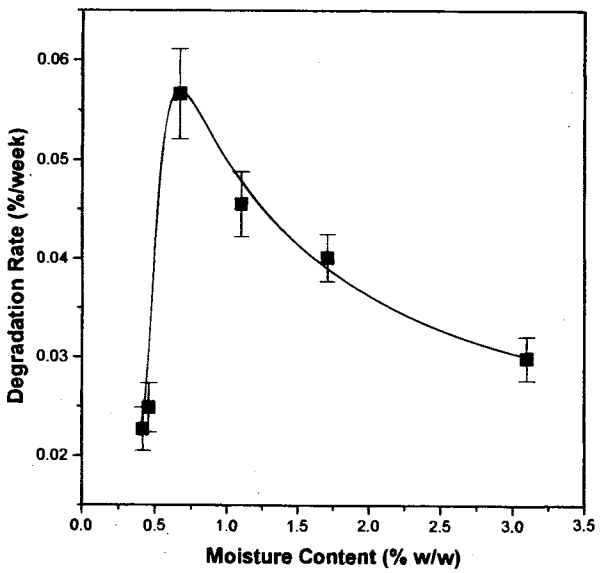

Fig. 3. Effect of moisture content on storage stability of lyophilized rhIL-1ra. See text for formulation components.

However, even at a moisture content of 0.8%, the purity of the formulation after 6 months at 30°C was still higher than 97%. Thus, even though there were relative differences in stability across the residual moisture range tested, in terms of absolute stability, any of the level tested were acceptable for a room temperature product. Thus, the specification for the final moisture content can fairly broad, which will allow the lyophilization process to be more flexible.

Effect of Stopper

A common problem in the storage of lyophilized products is the migration of water from the stopped to the dried cake (e.g., 19). Three different lots of siliconized stoppers (9310, 4416 and 9310) were obtained from WEST Co. and tested for their effect on the storage stability of the optimized rhIL-1ra formulation. As shown in Table III, no significant difference in stability was observed between stoppers. This is despite the finding that migration of moisture from stopper to product was significant with 4405 and 4416 stoppers, but not with the 9310 stopper (Table III). It is most likely that the migration of moisture from stopper to the dried product did not decrease stability because the product was reasonably stable over a wide range of moisture contents (Figure 3).

Effect of rhIL-1ra Concentration

The formulation work to this point employed relatively low protein concentrations, compared to concentrations that a commercial product might contain. Therefore, the effect of protein concentration on storage stability was investigated. In the presence of 1% sucrose (w/v), increasing the protein concentration increased degradation at storage temperatures of 30 and 50°C (Figure 4). In samples stored at 8°C, there was minimal degradation at all protein concentrations tested.

Increasing protein concentration most likely enhanced degradation because 1% (w/v) sucrose did not provide a high enough ratio of sugar/protein for stabilization during the dehydration step of the lyophilization cycle (3,11–15) and/or storage in the dried solid (5,8,16). It does not seem probable that the failure of 1% sucrose to protect higher protein concentrations was due to the inability of this sugar concentration to stabilize during the freezing step of the lyophilization cycle. Protection during freezing is due to the preferential exclusion of the excipient from the protein, which increases the chemical potential of the protein (11,15). The degree of exclusion and increase in chemical potential is greater for the denatured than for the native

Table III. Effect of Different Stoppers on Storage Stability of rhIL-1ra[a]

Stoppers	Degradation Rate (%/week)	Turbidity Increase Rate (A_{500}/week)	Moisture content (%) 0 week	Moisture content (%) 24 week
9310	0.022 ± 0.006	0.0000	0.65 ± 0.13	0.83 ± 0.03
4416	0.030 ± 0.007	0.0002	0.58 ± 0.17	1.26 ± 0.19
4405	0.029 ± 0.009	0.0001	0.36 ± 0.13	1.89 ± 0.08

[a] Rate constants were calculated for samples stored at 30°C. Three vials were analyzed for each value.

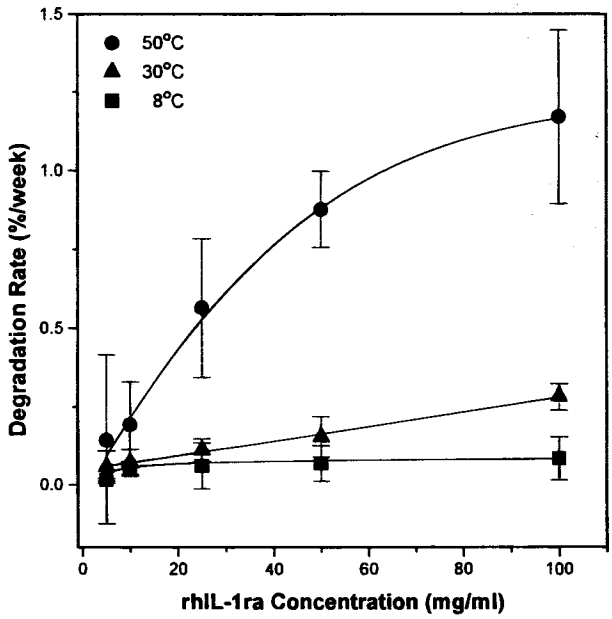

Fig. 4. Effect of rhIL-1ra concentration on storage stability of lyophilized rhIL-1ra. Each formulation contained 2% (w/v)glycine, 1% (w/v) sucrose and 10 mM sodium citrate buffer at pH 6.5.

state. Therefore, a preferentially excluded solute increases the thermodynamic barrier to unfolding (see Reference 11 and 15 for a detailed explanation of this mechanism). With this thermodynamic mechanism, the degree of stabilization is based on the bulk concentration of the excipient and not the mass ratio of excipient to protein. Since 1% (w/v) sucrose is adequate to protect 10 mg/ml rhIL-1ra, this bulk concentration should also protect higher concentrations of protein during freezing. Also, many multimeric proteins have increased intrinsic resistance to freezing damage, and are more amenable to protection by excipients, as protein concentration is increased (11,15). It is not known if the same phenomena exist for monomeric proteins such as rhIL-1ra, but at a minimum, increasing protein concentration would not be expected to decrease protein stabilization by sucrose during freezing.

The stability of a formulation containing 100 mg/mL rhIL-1ra was dramatically improved by increasing the concentration of sucrose (Figure 5). At sucrose concentrations ≥ 3% (w/v), protein aggregation was negligible even after storage at 50°C (Figure 5a). Increasing the sucrose concentration to ≥ 3% (w/v) also greatly decreased chemical degradation rhIL-1ra (Figure 5b). These data indicate that an optimized lyophilized formulation rhIL-1ra must contain a minimum sucrose/protein mass ratio of 0.3 to provide stability during storage. These results are most likely due to two factors. 1) There should be improved protection from unfolding conferred by the higher sucrose concentration of the protein from unfolding during freeze-drying (3,11–15), which has been shown to correlate with improved storage stability in the dried solid (4), 2) Increasing the sucrose:protein ratio in the dried solid could lead to increased spatial separation between protein molecules, which in an amorphous system has been proposed to minimize protein aggregation during storage (16). Finally, it is also most likely that the stable formulations had a glass transition temperature above the storage temperature, because in several instances this physical

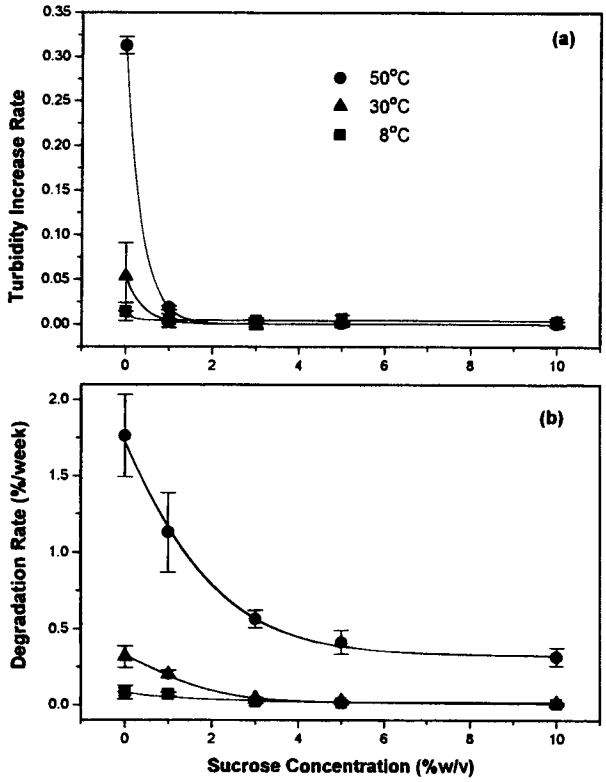

Fig. 5. Effect of sucrose concentration on storage stability of lyophilized rhIL-1ra. Each formulation contained 100 mg/ml rhIL-1ra, 2% (w/v) glycine and 10 mM sodium citrate buffer at pH 6.5. (a) Effect on rate of turbidity increase. (b) Effect on chemical degradation rate.

factor has been shown to be necessary for long-term stability of dried protein formulations (4,6,16,20).

Long Term Stability of Optimized Formulation

This study was designed to examine the long-term stability of the lyophilized rhIL-1ra in the optimized formulation with 100 mg/ml rhIL-1ra, 2% (w/v) glycine, 10% (w/v) sucrose, and 10 mM sodium citrate buffer at pH 6.5. Samples were stored at 8°C, 30°C, and 50°C. Even after 14 months there was essentially no change in the product stored at 8 and 30°C. Although there was a slight increase in turbidity of the samples stored at 50°C, there was no visual precipitation observed. Also, the purity based on CEX-HPLC was still greater than 94% in these samples. Since no damage has arisen after this duration of storage at 30°C, it is probable that the storage could be extended, without significant damage to the protein.

Stability during Exposure to Extreme Temperature

To simulate the temperature extremes to which a product might be exposed during shipping and storage, the optimized lyophilized formulation was heated to 100°C (at a heating rate of 10°C/minute) and kept at this temperature for 5 minutes before reconstitution and analysis. The rhIL-1ra was not damaged by this treatment. Loss of purity was not detected by CEX-HPLC analysis and the reconstituted sample was visually clear. Thus, the optimized formulation could safely shipped and stored in regions of the world where temperature control cannot be assured.

SUMMARY AND CONCLUSIONS

A stable lyophilized formulation of rhIL-1ra was developed by identifying the degradation products that formed during storage and then preventing these routes of degradation by optimizing various formulation factors. In the absence of stabilizers, the protein degraded chemically and physically by deamidation and precipitation, respectively. The formulation factors that were most important for minimizing degradation, and meeting our other goal of obtaining a morphologically-intact dried cake, were: 1) the combination of crystallizing bulking agent and amorphous protein stabilizer; 2) buffer and pH; and 3) mass ratio of the amorphous stabilizer sucrose to protein. We were also able to obtain the desired stability and cake integrity, under the constraint that the formulation be amenable to rapid and efficient lyophilization (9). For 100 mg/ml rhIL-1ra, the optimum lyophilized formulation contained 2% (wt/vol) glycine, 10% (w/v) sucrose and 10 mM citrate buffer at pH 6.5. This same combination of excipients may prove useful for the lyophilization of other proteins. However, it is clear that for each protein several combinations of excipients may have to be screened before the optimum formulation is developed. We suggest that following the approach that we have taken with rhIL-1ra should help make such screening a rapid and rational process.

References

1. M. C. Manning, K. Patel, and R. T. Borchardt. Stability of protein pharmaceuticals. *Pharm. Res.* **6**:903–918 (1989).
2. J. L. Cleland, M. F. Powell, and S. J. Shire. The development of stable protein formulations: a close look at protein aggregation, deamidation, and oxidation, *Critical Reviews in Therapeutic Drug Carrier Systems*, **10**(4): 307–377 (1993).
3. S. J. Prestrelski, N. Tedeschi, T. Arakawa, J. F. Carpenter. Dehydration induced conformational transitions in proteins and their inhibitions by stabilizers. *Biophys. J.*, **65**(2): 661–671 (1993).
4. S. J. Prestrelski, K. A. Pikal, and T. Arakawa. Optimization of lyophilization conditions for recombinant human interleukin-2 by fried-state conformational analysis using FT-IR spectroscopy. *Pharm. Res.* (In press).
5. M. W. Townsend, P. R. Byron, and P. P. DeLuca. The effects of formulation additives on the degradation of freeze-dried Ribonuclease A. *Pharm. Res.* **7**(10): 1086–1091 (1990).
6. M. J. Pikal, K. M. Dellerman, M. L. Roy, and R. M. Riggin. The effects of formulation variables on the stability of freeze-dried human growth hormone. *Pharm. Res.* **8**(4): 427–436 (1991).
7. B. S. Chang and C. S. Randall. Use of thermal analysis to optimize protein freeze-drying. *Cryobiology* **29**:632–656 (1992).
8. M. J. Pikal. Freeze-drying of proteins. *BioPharm.* September: 18–27 (1990).
9. B. S. Chang and N. L. Fischer. Development of an efficient single-step freeze-drying cycle for protein formulations. *Pharm. Res.* **12**:831–837 (1995).
10. J. C. May, E. Grim, R. M. Wheeler, and J. West. Determination of residual moisture in freeze-dried viral vaccines: Karl Fischer, gravimetric and thermogravimetric methodologies. *Journal of Biological Standardization*, **10**:249–259 (1982).
11. T. Arakawa, S. J. Prestrelski, W. C. Kenney, and J. F. Carpenter. Factors affecting short-term and long-term stabilities of proteins. *Advanced Drug Delivery Reviews*, **10**:1–28 (1993).
12. J. F. Carpenter and J. H. Crowe. An infrared spectroscopic study of the interactions of carbohydrates with dried proteins, *Biochemistry*, **28**:3916–3922 (1989).
13. S. J. Prestrelski, T. Arakawa, and J. F. Carpenter. Structure of proteins in lyophilized formulations using FTIR spectroscopy. in

"Formulation and delivery of proteins and peptides" American Chemical Society Symposium Series No. **567**:148–169 (1994).
14. A. Dong, S. J. Prestrelski, S. D. Allison, and J. F. Carpenter. Infrared spectroscopic studies of lyophilization- and temperature-induced protein aggregation. *J. Pharm. Sci.*, **84**:415–424 (1995).
15. J. F. Carpenter, S. J. Prestrelski, T. J. Anchordoguy, and T. Arakawa. Interaction of stabilizers with proteins during freezing and drying. in "Formulation and delivery of proteins and peptides" American Chemical Society Symposium Series No. **567**:134–147 (1994).
16. F. Franks, R. H. M. Hatley, and S. F. Mathias. Material science and the production of shelf stable biologicals. *BioPharm* **4**(9): 38–55 (1994).
17. L. van den Berg, and D. Rose. The effect of freezing on the pH and composition of sodium and potassium solutions: The reciprocal system KH_2-PO_4-Na_2HPO_4-H_2O. *Arch. Biochem. Biophys.* **81**:319–329 (1959).
18. G. P. Ellis. The maillard reaction. *Advances in Carbohydrate Chemistry* **14**:63–134 (1959).
19. M. J. Pikal and S. Shah. Moisture transfer from stopper to product and resulting stability implications. *Develop. Biol. Standard.* **74**:165–179 (1991).
20. M. L. Roy, M. J. Pikal, E. C. Rickard, and A. M. Maloney. The effects of formulation and moisture on the stability of freeze-dried monoclonal antibody-vinca conjugate: A test of the WLF glass transition theory. *Dev. Biol. Standard.* **74**:323–340 (1990)