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Stability of Ala 125 recombinant human interleukin-2 in solution

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Abstract

Herein, we describe the preformulation study of Ala 125- recombinant human interleukin-2 (rhIL-2A^{125}) in solution. This modified form of the natural human IL-2 is obtained by the replacement of cysteine with alanine at position 125. The compatibility of this rhIL-2A^{125} with type I borosilicate glass vials showed no significant adsorption at liquid–vial interface. The effect of single excipients on the stability of this lymphokine was evaluated through RP-HPLC, SDS-PAGE and biological activity assay. Polysorbate 80 at high concentrations decreased the stability of rhIL-2A^{125} in solution. On the other hand, the use of antioxidants (methionine and EDTA Na_2) diminished the oxidation rate of the active ingredient. Additionally, a group of amino acids (glutamine, alanine, glycine and histidine) stabilized rhIL-2A^{125} in different grades, and glycine at 5 mg mL^{-1} allowed for the best stability behaviour. Taken together, these preformulation results can be used to design an adequate liquid vehicle for rhIL-2A^{125} to be manufactured for human use.

Introduction

Interleukin 2 (IL-2) is a glycoprotein synthesized and secreted by activated T helper lymphocytes (Morgan et al 1976). This lymphokine induces the expansion of antigen-specific T cells and the activation of B and natural killer cells. IL-2 also stimulates the proliferation of tumour-attacking lymphocyte-activated killer cells and tumour-infiltrating lymphocyte cells (Rosemberg et al 1985). In addition, some clinical trials have revealed that IL-2-based therapies can be promising for the treatment of chronic infectious diseases, especially for HIV infection (Chun et al 1999).

Human IL-2 has an apparent molecular weight of 15–18 kDa and its coding gene has been cloned and subsequently expressed (Taniguchi et al 1983). The mature protein contains three cysteine residues, two of which form a disulfide bond that is required for its biological function (Smith 1984). The third cysteine residue is not essential for its activity (Mark et al 1985), although this amino acid is free to form undesirable intermolecular or intramolecular links, which complicate the obtainment of an acceptable active ingredient. This obstacle has been solved by producing recombinant human analogues of IL-2 in transformed *Escherichia coli*. These analogues have been obtained by replacing Cys-125 with other amino acid residues (Bergmann et al 1991; Rodríguez et al 2001; Moya et al 2002).

Proleukin (Chiron Corporation) has been widely used in the treatment of carcinogenic disorders. This IL-2 has Cys at position 125 replaced with Ser and is produced as a lyophilized powder containing 22 million IU (1.3 mg) IL-2-S^{125}, 10.92 mM mannitol and 0.06 mM sodium dodecyl sulfate, buffered with approximately 0.03 mM monobasic sodium phosphate and 0.15 mM dibasic sodium phosphate to pH 7.2–7.8.

Freeze-dried formulations of human IL-2 such as that described for Proleukin are known to be more stable than their equivalent liquid forms (Wang 1999). However, the liquid formulations have the advantages of simple processing, less manipulation and easier application. Therefore, the development of stable liquid formulations of IL-2 to be used in man is highly desirable.

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Herein, we describe the preformulation study of IL-2A^{125} in the liquid state. This recombinant human IL-2 was obtained at the Center for Genetic Engineering and Biotechnology (CIGB, Havana, Cuba) by replacing Cys at position 125 with Ala.

Materials and Methods

Materials

The Center for Genetic Engineering and Biotechnology (CIGB, Havana, Cuba) supplied rhIL-2A^{125} with the characteristics previously described (Moya et al 2002).

All chemicals used were of analytical grade. Polysorbate 80, EDTA, reduced glutathione, methionine, glutamine, alanine, histidine, sodium acetate and acetic acid were purchased from Merck (Darmstadt, Germany). Acetonitrile and trifluoroacetic acid were purchased from Caledon (Georgetown, ON) and PIERCE (Rockford, IL), respectively.

Type I borosilicate glass vials were acquired from Nuova OMPI (Piombino Dese, Italy) and rubber stoppers plus flip-off seals were from Helvoet Pharma (Alken, Belgium).

Preformulation of rhIL-2A^{125}

Compatibility of rhIL-2A^{125} with type I borosilicate glass vials in aqueous solution

rhIL-2A^{125} was diluted to 0.1, 0.4, 0.7, 1 and 1.2 mg mL^{-1} in 10 mM sodium acetate, pH 4, and dispensed into 1-mL borosilicate glass vials. Each vial was sealed with a chlorobutyl stopper and a 13-mm flip-off aluminium seal, and stored at 4°C for 120 h. The compatibility of rhIL-2A^{125} with glass vials was estimated by determining the concentration of the Lowry-quantified rhIL-2A^{125} present at 24, 48 and 120 h of storage, compared with the initial concentration.

Influence of polysorbate 80 on the stability of rhIL-2A^{125} in aqueous solution

rhIL-2A^{125} was diluted to 1 mg mL^{-1} in 10 mM sodium acetate, pH 4, in the presence of polysorbate 80 at 0.1, 1 or 10 mg mL^{-1} and dispensed in borosilicate vials. Sealed vials were stored at 37°C and periodically analysed by reverse-phase high-performance liquid chromatography (RP-HPLC) and sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reduced and non-reduced conditions, and biological activity was determined. rhIL-2A^{125} without polysorbate 80 was used as the control.

Thus, the kinetics (k_{obs}) constants presented here were calculated from the linear relationships between the logarithms of residual relative purity determined by RP-HPLC or SDS-PAGE under reduced and non-reduced conditions, or biological activity and time.

Influence of selected antioxidants on the stability of rhIL-2A^{125} in solution

rhIL-2A^{125} diluted to 1 mg mL^{-1} in 10 mM sodium acetate, pH 4, was stored at 37°C in the presence of 0.1 mg mL^{-1} EDTA, 0.1 mg mL^{-1} reduced glutathione or 5 mg mL^{-1} methionine. These solutions were then analysed by RP-HPLC at the initial time and after 7, 14, 21 and 30 days of storage and compared with the rhIL-2A^{125} without additives.

Influence of selected amino acids on the liquid stability of rhIL-2A^{125}

rhIL-2 diluted to 0.4 and 1 mg mL^{-1} in 10 mM sodium acetate, pH 4, was stored at 37°C in the presence of 0.25 or 10 mg mL^{-1} glutamine, 5 or 20 mg mL^{-1} glycine, 5 or 20 mg mL^{-1} alanine and 2.5 mg mL^{-1} histidine. rhIL-2A^{125} solutions were then periodically analysed by RP-HPLC and SDS-PAGE under reduced and non-reduced conditions, and the biological activity was determined at time 0, 21, 35, 72 and 90 days of storage. rhIL-2A^{125} without additives was used as control.

Analyses of the formulated rhIL-2A^{125}

Determination of biological activity

The bioactivity of rhIL-2A^{125} was estimated with an IL-2-dependent cell proliferation assay using the murine cytotoxic T-cell line CTLL-2 (ATCC PIB 214) as previously described (Gillis et al 1978). Cell viability was then assessed by the modified MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method (Tada et al 1986).

Reverse-phase high-performance liquid chromatography (RP-HPLC)

RP-HPLC analysis was performed on a Vydac (Hesperia, CA) wide-pore octyl (C8) column (5 μm; 125 × 4.6 mm). Solvents and gradient were: A, 0.1% aqueous trifluoroacetic acid (TFA) and B, 0.1% TFA in acetonitrile (45% to 80% B in 30 min). The flow rate was 0.8 mL min^{-1}. Detection was performed at 226 nm with automatic data processing using the Unicorn version 4.10 software (Amersham Biosciences AB, Uppsala, Sweden) for data acquisition and analysis. Purity was calculated as the relation between the area of the main peak and the area of contaminant peaks.

Protein determination

The concentration of rhIL-2A^{125} was determined as previously described by Lowry et al (1951). Bovine serum albumin at 2 mg mL^{-1} was used as the standard protein.

Electrophoresis profile of the formulated rhIL-2A^{125}

rhIL-2A^{125} was analysed by SDS-PAGE as described by Laemmli (1970). The gel-separated bands were detected by Coomassie blue staining. A reference standard of rhIL-2A^{125} provided by the Quality Control Division of CIGB was used as the positive control.

Statistical analysis

The results were analysed statistically by the Kruskal–Wallis test (Sigarroa 1985).

Results and Discussion

Compatibility of rhIL-2A^{125} with type I borosilicate glass vials

We did not find any evidence of rhIL-2A^{125} adsorption to the borosilicate vials. Statistical differences between the recovery of rhIL-2A^{125} at the initial time and after 24, 48 or 120 h of storage at 4°C were not significant (Table 1). This clearly indicated that there is no risk of losing significant amounts of rhIL-2A^{125} by irreversible adsorption to glass vials, at least under our conditions.

Proteins can be adsorbed at many surfaces (Wang 1999). This process can significantly change the secondary structure of these macromolecules, resulting in the loss of biological activity or destabilization. The rate of protein adsorption depends on the surface material and the protein itself, but in any case, it is usually a very rapid process (Johnston 1996).

Tzannis et al (1997) determined that IL-2 at 0.1 mg mL^{-1} in 10 mM sodium phosphate (pH 7.4), 150 mM NaCl, loses about 90% of its activity after being pumped through silicone rubber catheter tubing for 24 h, principally due to adsorption-induced protein denaturation. Our results were largely different. The acidic pH (pH 4) of our conditions would increase the charge density of rhIL-2A^{125}, which has an isoelectric point of 7.68 (Rodríguez et al 2001). Therefore, this pH can inhibit any hydrophobic protein–protein or protein–surface interaction. In fact, it is known that pH can affect the degree of adsorption, probably by increasing the charge density of proteins, and thus reducing the hydrophobic effect that drives these molecules to aggregation or adsorption (Wang 1999).

These results indicated that the use of additives (e.g. detergents) to inhibit any unwanted rhIL-2A^{125} adsorption at glass vials is not necessary, at least under our conditions.

Table 1 Compatibility of borosilicate glass vials with rhIL-2A^{125} in solution

Concn (μg mL^{-1})	Time (h)	Lowry concn (μg mL^{-1})	Recovery (%)	α (0.05)
100	0	96.22 ± 0.79	—	0.99
	24	97.54 ± 0.52	101.37	
	48	97.18 ± 0.27	100.99	
	120	98.32 ± 0.23	102.18	
400	0	399.28 ± 0.72	—	0.92
	24	388.42 ± 0.37	97.28	
	48	388.73 ± 0.53	97.36	
	120	395.55 ± 0.23	99.07	
700	0	699.26 ± 0.68	—	0.97
	24	710.12 ± 0.64	101.55	
	48	701.56 ± 0.32	100.33	
	120	699.13 ± 0.27	99.98	
1000	0	990.65 ± 0.32	—	0.99
	24	1002.31 ± 0.55	101.18	
	48	991.73 ± 0.92	100.11	
	120	998.61 ± 0.97	100.80	
1200	0	1201.62 ± 0.72	—	0.99
	24	1201.38 ± 1.23	99.98	
	48	1200.72 ± 0.22	99.93	
	120	1202.38 ± 0.45	100.0	

The results are expressed as mean ± s.d., n = 3.

Influence of polysorbate 80 on the stability of the rhIL-2A^{125} in solution

In the RP-HPLC analysis, polysorbate 80 accelerated the formation of pre- and post-main rhIL-2A^{125} native peak by-products (Figure 1A–F). As shown in Figure 1, the hydrophilic peak was mostly induced in the presence of polysorbate 80 at the higher concentration and it affected the purity of rhIL-2A^{125}, at a larger extension than the hydrophobic one.

The rhIL-2A^{125} SDS-PAGE profile was also affected due to the presence of the detergent (data not shown). This profile was characterized by the formation of rhIL-2A^{125} aggregates, probably due to the same factors affecting the RP-HPLC profile.

Note that polysorbate 80 at 0.1 mg mL^{-1} increased the RP-HPLC-determined purity of rhIL-2A^{125} about 1.19 fold (Table 2) compared with the control samples. However, differences in the biological activity and SDS-PAGE results obtained from the control and 0.1 mg mL^{-1} polysorbate 80 samples (Table 2) were not statistically significant.

In contrast, polysorbate 80 at 1 mg mL^{-1} reduced the biological activity and the RP-HPLC- and SDS-PAGE-determined purity in the range of 1.42–4.69 fold compared with the control samples. Additionally, polysorbate 80 at 10 mg mL^{-1} induced the worst behaviour on the basis of the stability of the control samples. Thus, the detergent at this concentration increased the degradation rate of rhIL-2A^{125} by 2.6 to 14.16 fold as determined by the biological assay, SDS-PAGE and RP-HPLC (Table 2).

Correlation coefficients (r^2) in this experiment varied from 98.24 to 99.99.

Polysorbate 80 can stabilize proteins at low concentrations (Bam et al 1995). This nonionic detergent competes with proteins for adsorption to various interfaces where physical instability can be induced. This detergent can also bind weakly to proteins, covering hydrophobic sites and thus inhibiting their aggregation (Wang 1999). Therefore, the use of this surfactant has been widely described in the literature as a stabilizer in pharmaceutical formulations (Wang 1999). Nevertheless, it has been well-documented that surfactants with alkyl polyoxyethylene chains, such as polysorbates, undergo autoxidation with subsequent chain-shortening degradation (Ha et al 2002). Autoxidation leads to the formation of peroxides and changes in the physico-chemical properties of the detergent (Ha et al 2002).

These results definitively indicated that the use of polysorbate 80 can be problematic for the stability of formulated rhIL-2A^{125}, probably due to the presence of remnant or induced peroxides in the detergent solution.

In agreement with these findings, several studies have focused on the oxidative effect of peroxides from surfactants

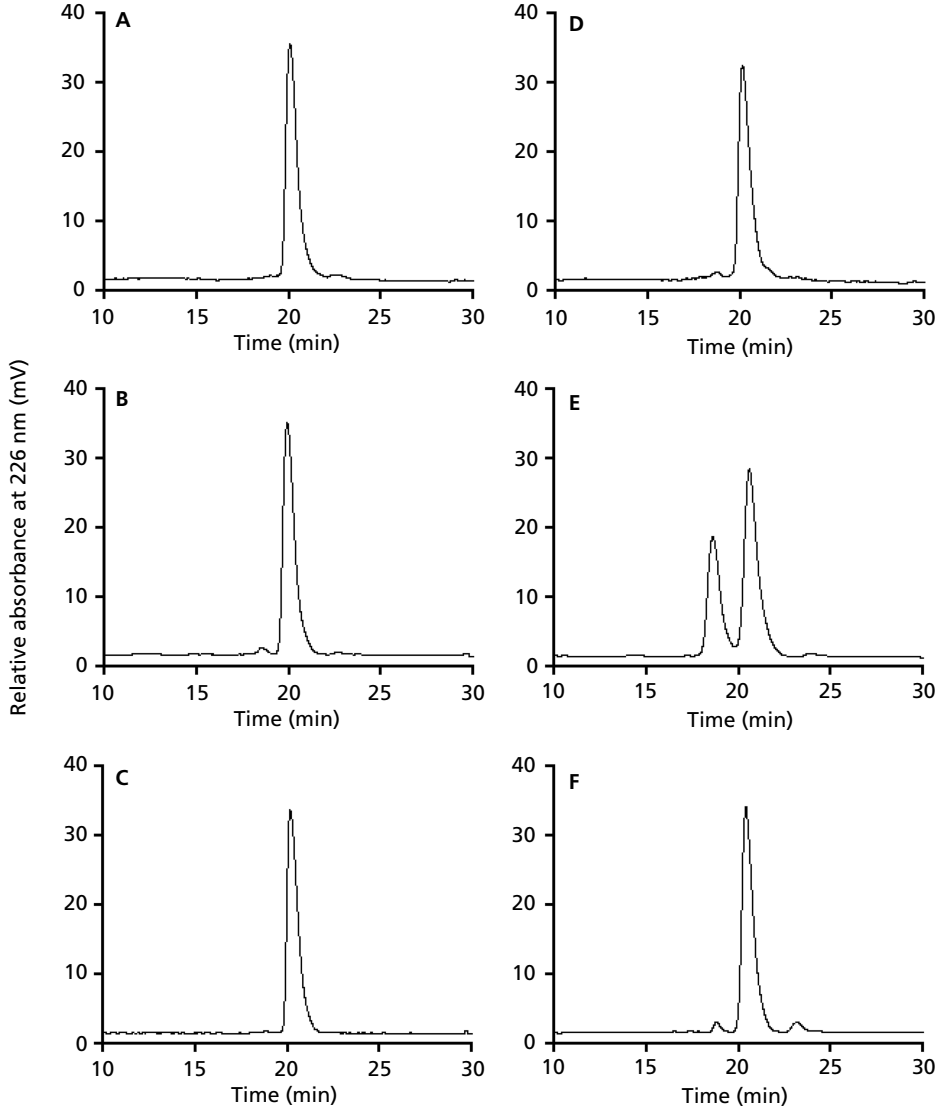

Figure 1 Effect of polysorbate 80 on the RP-HPLC profile of rhIL-2A^{125}. Samples were stored at 37°C and systematically analysed. A. Polysorbate 80 at 0.1 mg mL^{-1} at time 0. B. Polysorbate 80 at 10 mg mL^{-1} at time 0. C. Control at the time 0. D. Polysorbate 80 at 0.1 mg mL^{-1} after 18 days of storage. E. Polysorbate 80 at 10 mg mL^{-1} after 18 days of storage. F. Control after 18 days of storage.

on proteins. Knepp et al (1996) demonstrated that alkyl hydroperoxides in polysorbate 80 induced oxidation, dimerization and aggregation of recombinant human ciliary neurotrophic factor in solution. In other studies, Herman et al (1996) described the oxidation of human granulocyte colony stimulating factor in the presence of polysorbates 20 and 80.

Influence of selected antioxidants on the liquid stability of rhIL-2A^{125}

The main chemical degradation product of rhIL-2A^{125} has been shown to be Met-104 sulfoxide rhIL-2A^{125} (Moya et al 2002). In an attempt to avoid or minimize the increment of this oxidized product or any other potential oxidized form of rhIL-2A^{125}, we evaluated three antioxidants (EDTA, methionine and glutathione). These compounds were chosen on an empirical basis, since the protective effect of these agents is variable, depending on the protein to be stabilized (Andersson et al 2000).

EDTA

In our study, EDTA protected rhIL-2A^{125} against the formation of Met-104 sulfoxide rhIL-2A^{125} (Table 3). Kinetic analyses (Table 3) showed that 0.1 mg mL^{-1} EDTA increased the RP-HPLC-determined purity of the active ingredient 3.71 fold as compared with the control samples. Correlation coefficients (r^2) in this experiment varied from 97.09 to 99.44.

This chelating agent has been used as a stabilizer in protein formulations due to the inhibition of the oxidation reactions by the removal of metal ions (Wang 1999). Under our experimental conditions, the role of EDTA

Table 2 Kinetic parameters of rhIL-$2A^{125}$ thermal degradation in the presence of polysorbate 80

Concn of polysorbate 80	$k \times 10^3$ per day
Biological activity	
0.01%	13.45 ± 0.82
0.1%	17.92 ± 1.37
1%	45.17 ± 2.55
Control	11.89 ± 0.97
SDS-PAGE purity[a] (under reduced conditions)	
0.01%	1.73 ± 0.13
0.1%	2.25 ± 0.52
1%	4.56 ± 0.82
Control	1.75 ± 0.07
SDS-PAGE purity[a] (under non-reduced conditions)	
0.01%	3.51 ± 0.09
0.1%	4.79 ± 0.13
1%	13.91 ± 2.03
Control	3.38 ± 0.11
RP-HPLC purity[b]	
0.01%	3.96 ± 0.15
0.1%	22.17 ± 1.27
1%	66.98 ± 2.64
Control	4.73 ± 0.21

[a]Determination of the purity of rhIL-$2A^{125}$ main band. [b]Determination of the purity of native rhIL-$2A^{125}$, as determined by RP-HPLC. The results are expressed as mean ± s.d., n = 3.

Table 3 Kinetic parameters of rhIL-$2A^{125}$ thermal degradation in the presence of antioxidants

Antioxidant	RP-HPLC purity[a] ($k \times 10^3$ per day)	% of the oxidized peak[b] ($k \times 10^3$ per day)
EDTA	1.24 ± 0.02	59.04 ± 1.27
Glutathione	35.59 ± 1.24	161.67 ± 1.66
Methionine	1.96 ± 0.03	59.61 ± 0.97
Control	4.61 ± 0.04	73.06 ± 0.74

[a]Determination of the purity of native rhIL-$2A^{125}$, as determined by RP-HPLC. [b]Determination of the percent of the oxidized peak affecting the purity of rhIL-$2A^{125}$, as determined by RP-HPLC. The results are expressed as mean ± s.d., n = 3.

may have been the capture of trace amounts of metal ions in solution, which otherwise would accelerate several protein degradation reactions (e.g. oxidation) (Wang 1999).

Results from the evaluation of this chelating agent proved that oxidation reactions can be efficiently inhibited in formulations of this cytokine.

Methionine

The results provided by methionine were similar to those obtained with EDTA (Table 3). Kinetic analyses (Table 3) showed that 5 mg mL^{-1} of this antioxidant increased the RP-HPLC-determined purity of rhIL-$2A^{125}$ 2.35 fold.

Correlation coefficients (r^2) in this experiment varied from 98.14 to 99.85.

This is a sulfur-containing amino acid normally found in proteins. This amino acid plays a central role as an efficient oxidant scavenger, since a wide variety of oxidants can react with this residue to form methionine sulfoxide (Levine et al 1996). As seen with EDTA Na_2, this amino acid was very effective at maintaining the stability of IL-2. This indicated that oxidation in this protein can be inhibited by antioxidants like methionine.

Glutathione

This additive had the worst stability behaviour among the evaluated antioxidants (Table 3). Taking into account the degradation rate in the control samples, we found that gluthathione destabilized rhIL-$2A^{125}$ by promoting its degradation about 7.72 fold. Correlation coefficients (r^2) in this experiment varied from 97.59 to 99.19.

Such effect was more evident in the analysis of the Met-104 sulfoxide rhIL-$2A^{125}$ (Table 3); gluthathione increased the rate of formation of the oxidized rhIL-$2A^{125}$ 2.21 fold while EDTA and methionine reduced this parameter 1.24 and 1.22 fold, respectively. We also might speculate that glutathione induced not only the oxidation of methionine but also disulfide exchange (Figure 2).

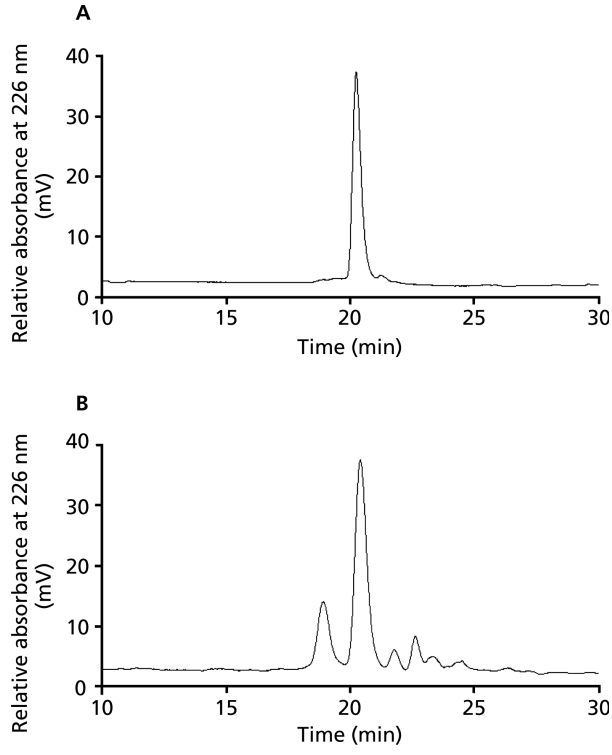

Figure 2 Influence of glutathione on the RP-HPLC profile of rhIL-$2A^{125}$. Samples were dispensed into borosilicate glass vials, stored at 37°C and analysed at time 0 (A) and after 14 days (B) of storage.

This is a sulfur-containing compound whose sulfhydryl groups can be oxidized by peroxides or metal ions during incubation, and thus it decreases the probability of the oxidation of amino acid residues (Ha et al 2002). This agent has therefore been used for the prevention of potential oxidative reactions in proteins (Knepp et al 1996; Ha et al 2002).

In experiments with IL-2, Ha et al (2002) determined that glutathione did exert a significant inhibitory effect on the prevention of the oxidation of this protein after freeze-drying in the presence of polysorbate 80. However, this protection was not completely prevented.

In our experiments, glutathione increased the degradation of IL-2. Although rhIL-2A^{125} does not have any free Cys residue, this observation might be explained on the basis of disulfide scrambling formation, which may still occur in the presence of oxidants such as peroxides, causing the aggregation of the protein (Wang 1999). In fact, we cannot explain the behaviour of IL-2 due to the presence of peroxides. Therefore, due to its ability to be oxidized, we might suggest that gluthathione could reduce the disulfide bond on IL-2 causing disulfide scrambling and consequently the formation of aggregates of the protein.

Influence of selected amino acids on the liquid stability of rhIL-2A^{125}

Here, we evaluated the ability of four amino acids (glutamine, alanine, glycine and histidine) to guarantee the liquid stability of rhIL-2A^{125}. Kinetic analyses showed that reduction of the destabilization rate varied from 1.01 to 3.36 fold for biological activity as well as for SDS-PAGE- and RP-HPLC-determined purity of rhIL-2A^{125} (Table 4). Correlation coefficients (r^2) in this experiment varied from 97.91 to 99.39.

Specifically, Gly was the most effective excipient on maintaining the stability of rhIL-2A^{125} among the evaluated amino acids. This additive decreased the inactivation rate of rhIL-2A^{125} as follows: 2.79–3.36 fold for biological activity; 1.44–1.75 fold and 1.48–2.68 fold for SDS-PAGE-determined purity under reduced and non-reduced conditions, respectively; and 1.48–1.91 fold for RP-HPLC-determined purity (Table 4).

Certain amino acids are known to stabilize proteins via an exclusion mechanism whereby the protein is preferentially hydrated in solution (Wang 1999). Given this, many investigators have studied the use of amino acids for the stabilization of proteins. For example, Sebeka et al (2001) determined that aromatic (Phe, Trp), basic (Lys) or acidic amino acids (Glu) and Met were effective on the stabilization of recombinant human interferon α-2b. In addition, Chen et al (1994) increased the half-life of recombinant human keratinocyte growth factor in the presence of Glu and Asp. However, the stabilizing effect of amino acids largely depends on the properties of the protein that will be stabilized.

We did not find a rule to stabilize rhIL-2A^{125} in the presence of these amino acid solutions, but our studies indicated that some of these compounds such as Gly could be useful in additional studies.

Table 4 Kinetic parameters of the thermal stability of rhIL-2A^{125} in the presence of amino acids

Amino acid	IL-2 at 0.4 mg mL^{-1} ($k \times 10^3$ per day)	IL-2 at 1 mg mL^{-1} ($k \times 10^3$ per day)
Biological activity		
Gln 0.25%	15.45 ± 1.85	8.76 ± 1.59
Gln 1%	15.81 ± 1.37	10.94 ± 1.27
Gly 0.5%	4.85 ± 1.16	3.17 ± 0.85
Gly 2%	12.56 ± 1.31	3.48 ± 0.88
Ala 0.5%	9.24 ± 2.67	7.05 ± 0.4
Ala 2%	12.52 ± 1.16	7.96 ± 0.87
His 0.25%	19.81 ± 1.39	9.04 ± 1.29
Control	16.29 ± 2.84	8.87 ± 1.67
SDS/PAGE purity[a] (under reduced conditions)		
Gln 0.25%	0.38 ± 0.03	2.56 ± 0.04
Gln 1%	0.64 ± 0.04	2.19 ± 0.09
Gly 0.5%	0.34 ± 0.02	1.07 ± 0.08
Gly 2%	0.29 ± 0.01	0.94 ± 0.04
Ala 0.5%	0.33 ± 0.05	1.67 ± 0.02
Ala 2%	0.41 ± 0.02	1.56 ± 0.01
His 0.25%	0.37 ± 0.03	1.38 ± 0.02
Control	0.49 ± 0.02	1.87 ± 0.08
SDS/PAGE purity[a] (under non-reduced conditions)		
Gln 0.25%	1.87 ± 0.05	2.45 ± 0.05
Gln 1%	2.14 ± 0.04	3.03 ± 0.06
Gly 0.5%	0.99 ± 0.02	1.34 ± 0.02
Gly 2%	0.82 ± 0.03	1.97 ± 0.01
Ala 0.5%	1.18 ± 0.08	2.54 ± 0.03
Ala 2%	1.27 ± 0.06	2.55 ± 0.08
His 0.25%	1.45 ± 0.04	2.34 ± 0.06
Control	1.47 ± 0.07	3.59 ± 0.08
RP-HPLC purity[b]		
Gln 0.25%	2.94 ± 0.08	4.22 ± 0.09
Gln 1%	3.85 ± 0.08	5.65 ± 0.16
Gly 0.5%	2.12 ± 0.06	2.54 ± 0.09
Gly 2%	2.01 ± 0.17	2.63 ± 0.08
Ala 0.5%	4.27 ± 0.09	5.11 ± 0.18
Ala 2%	3.66 ± 0.04	4.37 ± 0.14
His 0.25%	4.03 ± 0.02	4.99 ± 0.07
Control	3.14 ± 0.04	4.84 ± 0.09

[a]Determination of the purity of rhIL-2A^{125} main band. [b]Determination of the purity of native rhIL-2A^{125}, as determined by RP-HPLC. The results are expressed as mean ± s.d., n = 3.

Conclusions

Polysorbate 80 at high concentration seems unfavourable for the stability of the lymphokine rhIL-2A^{125}, since the remnant or induced peroxides on the surfactant solution can accelerate the rate of formation of the main degradation product, Met-104 sulfoxide rhIL-2A^{125}. On the other hand, the use of antioxidants (methionine and EDTA) can be convenient for decreasing the oxidation rate of the active ingredient. This was not the case for gluthathione, which significantly affected the stability of the lymphokine, probably due to an adverse interaction with the protein. A group of amino acids (glutamine, alanine, glycine and histidine) stabilized rhIL-2A^{125} to different extents. However, glycine

at 5 mg mL^{-1} allowed for the best stability behaviour. Taken together, this preformulation study could permit us to design an adequate liquid vehicle for rhIL-2A^{125} to be manufactured for human use.

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