Isolation and Characterization of a Monomethioninesulfoxide Variant of Interferon α-2b

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Purpose. To isolate and characterize a monomethioninesulfoxide variant of the commercially available therapeutic protein interferon α-2b.
Methods. The methionine (Met)-oxidized variant was isolated by reverse-phase high performance liquid chromatography and characterized by SDS-PAGE, peptide mapping and mass spectrometric analysis of the trypsin/V8-generated peptide fragments. The biological and immunological activities of the isolated variant were also evaluated.
Results. The rHuIFN α-2b variant was found to contain a Met sulfoxide residue at position 111 of the rHuIFN α-2b molecule. The far-UV CD spectra showed a slight loss of α-helical content and an increase in the β-sheet contribution. The CD spectra indicate that both chromatographic conditions and Met oxidation contribute to the observed secondary structure changes. Both interferon α-2b main component and its methionine-oxidized variant showed different reactivity to monoclonal antibodies employed in immunoassays for the protein.
Conclusions. A monomethioninesulfoxide rHuIFN α-2b variant was found to be present in the rHuIFN α-2b bulk drug substance in solution. The Met^{111} residue was identified as Met sulfoxide by comparative tryptic/V8 mapping and mass spectrometric analysis. Nevertheless, the oxidation of the Met^{111} residue did not seem to have a detectable effect on the biological activity of the molecule.

KEY WORDS: protein mapping; mass spectrometry; methionine oxidation.

INTRODUCTION

Interferon (IFN) α proteins are a group of genetically related cytokines with a wide spectrum of biological activities including antiviral, antiproliferative and immunomodulatory properties (1–3). Although genes for several subtypes of the IFN α have been cloned, only IFN α-2a and IFN α-2b proteins have been produced to a high degree of purity in large quantities, and both recombinant-DNA derived IFN α-2a and IFN α-2b are commercialized with a variety of pharmaceutical indications. Whereas IFNs α-2a and α-2b differ by only one amino acid residue, the other subtypes have up to about 25% sequence heterogeneity and have been used as valuable research tools for the investigation of detailed molecular structure and related functional domains. In addition to the easily detectable heterogeneity, subtle microheterogeneity resulting from small but inherent and unavoidable secondary chemical modifications in the structure of proteins could result in altered biological, immunological and even pharmacological properties. Hence, for therapeutic proteins and peptides it is mandatory that detailed microheterogeneity studies be carried out and the quality of the product be maintained for desirable efficacy and carefully evaluated safety profile.

The purity profile of the recombinant DNA-derived human interferon α-2b (rHuIFN α-2b) was investigated employing a variety of chromatographic and electrophoretic methods. Gradient reverse-phase high performance liquid chromatography (RP-HPLC), one of several methods for evaluating the purity of rHuIFN α-2b, revealed a low percent proteinaceous impurity eluting slightly earlier than the main rHuIFN α-2b peak. The chromatographic impurity was isolated employing a RP-HPLC system and was identified and characterized by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), amino acid sequencing, peptide mapping and liquid secondary-ion (LSI) mass spectrometric analysis of peptides isolated from enzymatic hydrolysates.

In an antiviral bioassay [viral cytopathic effect (CPE) assay] employing encephamycarditis virus-infected human diploid foreskin cells, rHuIFN α-2b and its monosulfoxide-rHuIFN α-2b variant were found to be equally bioactive. In addition to structural characterization and biological activity (CPE) evaluation of the monosulfoxide variant of rHuIFN α-2b, this paper includes data alluding to the possibility that the chromatographic isolation procedure itself could affect the structure of proteins and indicate that such issues must be considered, and that appropriate controls must be used for meaningful interpretations of the experimental results.

MATERIALS AND METHODS

Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) of rHuIFN α-2b

The RP-HPLC method for the purity analysis of rHuIFN α-2b employed a Vydac C18 column (5 μm, 300 Å, 4.6 mm × 150 mm), a shallow gradient of 0.1% aqueous trifluoroacetic acid (TFA, mobile phase A) and 0.1% TFA in 90% acetonitrile (mobile phase B), with 0.16% per min increase in the mobile phase B from 48% initial to 55% in the first 45 min, and then to 80% B in 2.5 min. The flow rate was 0.6 ml/min with UV detection at 214 nm.

Isolation of the rHuIFN α-2b-related Impurity

Aliquots of rHuIFN α-2b solution were chromatographed employing the RP-HPLC system described above and the column effluent between the retention time of 5 min to 40 min was collected in separate polypropylene tubes containing 5 μl of 10% sodium dodecyl sulfate (SDS). The protein-containing fractions were lyophilized in a Savant Speed-Vac and then analyzed by SDS-PAGE.

In order to detect any changes induced by the sample preparation, an aliquot of the rHuIFN α-2b drug substance was spiked with 50% acetonitrile in 0.1% TFA. The unfractionated acetonitrile-treated protein sample was dried under vacuum and served as control.

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Hydrogen Peroxide-induced Oxidation of rHuIFN α-2b

Aliquots of 8–10 mg rHuIFN α-2b in 2.5% acetic acid solutions containing 0.04% hydrogen peroxide were incubated at room temperature for 10 min. The forced oxidation of rHuIFN α-2b was performed under acidic conditions because the primary reaction is the oxidation of methionine (Met) to methionine sulfoxide (4), while other amino acids, e.g., cysteine, tryptophan, histidine, and tyrosine, are modified at neutral or slightly alkaline conditions.

N-terminal Amino Acid Sequencing

N-terminal amino acid sequencing was performed by automated Edman degradation on an ABI 470A or a Porton Protein Sequencer equipped with on-line chromatographic systems for the analysis of phenylthiohydantoin-amino acids.

SDS-PAGE

The non-reducing SDS-PAGE analysis (5) with silver-staining for the detection of proteins (6) was carried out employing a 14% gel with approximately 0.8 μg protein sample/well applied for electrophoresis.

Enzymatic Hydrolysis and Isolation of Peptides from the Enzymatic Hydrolysate

Aliquots of unchromatographed rHuIFN α-2b, RP-HPLC-isolated rHuIFN α-2b, and the RP-HPLC-isolated rHuIFN α-2b impurity were digested at 37°C in 0.1 M sodium phosphate buffer, pH 8.0 with: (1) TPCK-treated trypsin for 16 to 18 hours at a substrate to enzyme ratio of 20:1 (w/w), and (2) TPCK-treated trypsin for 6 hours followed by V8 protease for 16 hours. The second type of enzymatic digests ("dual enzyme" digests) were carried out at a substrate (rHuIFN α-2b) to trypsin ratio of 100:1 (w/w), and a substrate to V8 protease ratio of 15:1 (w/w). The enzymatic hydrolysis was terminated by adding 10 μl of 1% aqueous TFA and 100 μl of 8 M guanidine hydrochloride. In the case of the "dual enzyme" digest of the peroxide-treated rHuIFN α-2b, hydrogen peroxide was removed by lyophilization prior to incubation with the proteolytic enzymes.

After termination of hydrolysis, the enzymatic digests were loaded onto a Vydac C18 column (4.6 mm × 150 mm) for peptide mapping employing a linear gradient of 0–60% mobile phase B in 100 min at a flow rate of 0.7 ml/min and with UV detection at 214 nm. Desired peptide fractions were collected, lyophilized, and stored in polypropylene tubes at −80°C for N-terminal amino acid sequencing and mass spectrometric analysis.

Mass Spectrometry

LSI Mass Spectrometry (MS) was performed on a MAT 90 double-focusing mass spectrometer operating at 5 kV accelerating voltage and equipped with a cesium ion gun generating a primary beam of 10 μA Cs^+ at 20 kV. All LSI mass spectra were acquired at an instrument resolution of m/Δm 2,000 (10% valley definition.) The lyophilized RP-HPLC-isolated peptide fractions were dissolved in 2 μl of 1% aqueous TFA and deposited onto a stainless-steel probe tip, followed by addition of 1–2 μl of 1:1 mixture of glycerol and thioglycerol. All the peptide fractions were also analyzed by plasma desorption MS using a BIOION 20 Californium-252 plasma desorption time-of-flight mass spectrometer (7) operating at a resolution of ca. 300 at 50% of peak height.

Circular Dichroism

Far-UV (190 nm to 270 nm) circular dichroism (CD) spectra were obtained using a Jasco Model J-600 spectropolarimeter on unchromatographed rHuIFN α-2b, RP-HPLC-isolated rHuIFN α-2b and RP-HPLC-isolated methionine-oxidized (from hydrogen peroxide-treated) rHuIFN α-2b. Samples were prepared as 6.1×10^{-5} M solutions in 10 mM phosphate buffer, pH 7.0. The mean residue ellipticity, $[\theta]_{mrw}$, is given in units of degree · centimeter^2/decimole. The mean residue weight (mrw) of rHuIFN α-2b is 116.8 based on the molecular weight of 19265 Da for 165 amino acid residues. Secondary structure estimation was performed using Jasco's SSE program based on the method of Chen, et al (8).

Assessment of Biological Activity

The biological activity of the rHuIFN α-2b and its RP-HPLC-isolated impurity was assessed using an antiviral cytopathic effect assay. The antiviral assay for rHuIFN α-2b is based on protection against viral cytopathic effect. Human diploid foreskin cell are incubated with dilutions of standard and the rHuIFN α-2b test preparations, then infected with mouse encephalomycarditis virus, and later examined for virus CPE. The dilution of the standard rHuIFN α-2b preparation that gives approximately 50% protection against the virus infection compared to similar results obtained with the rHuIFN α-2b test preparation are used to calculate the titer of the test sample.

Immunochemical Assays

The rHuIFN α-2b content of the unchromatographed protein solution and its phosphate buffer reconstituted RP-HPLC fractions was assayed by Immunoradiometric assay (IRMA) employing polystyrene beads with immobilized polyclonal anti-rHuIFN α-2b capture antibody and an I^{125} labeled monoclonal anti-rHuIFN α-2b antibody for detection.

The bioassay and immunoassay results are expressed as specific activity, IU per mg rHuIFN α-2b where the IU is an International Unit of biological activity and the protein content was determined by RP-HPLC. The rHuIFN α-2b Reference Standard used for the bioassay and immunoassay was calibrated against the WHO standard.

RESULTS AND DISCUSSION

The analytical RP-HPLC purity assay developed for evaluating the rHuIFN α-2b batch-to-batch consistency showed a minor proteinaceous impurity eluting before the main rHuIFN α-2b peak (Fig. 1). This impurity, referred to as Component A, was isolated for structural analysis and identification following the chromatographic procedure described under Materials and Methods. Automated Edman degradation showed that the N-terminal amino acid sequence of Component A was identical to the N-terminal amino acid sequence of the native rHuIFN α-2b.

Based on the non-reducing SDS-PAGE analysis (Fig. 2), the apparent molecular weights of Component A and rHuIFN α-2b on SDS-PAGE were identical indicating that Component

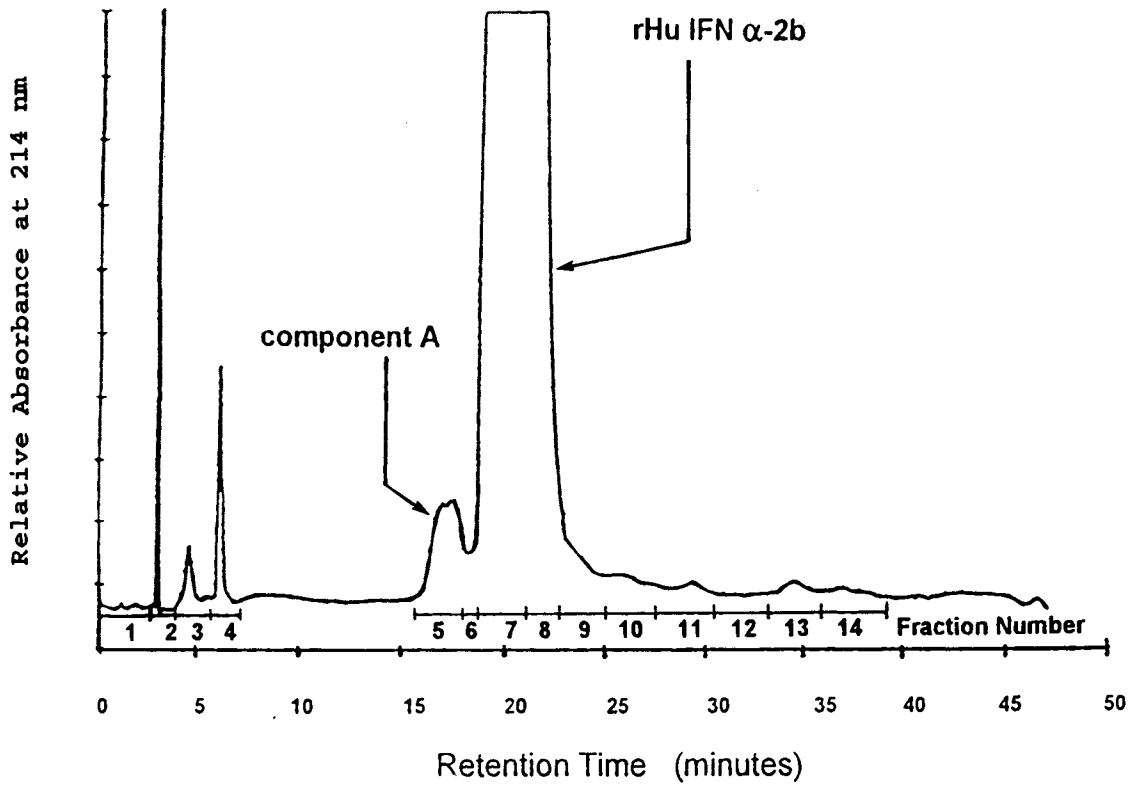

Fig. 1. RP-HPLC isolation of the rHuIFN α-2b Component A using the chromatographic conditions described under Materials and Methods. The column effluent was collected in 14 fractions at the indicated retention times, lyophilized and then analyzed by non-reducing SDS-PAGE.

A is a monomeric variant of rHuIFN α-2b. The Component A containing RP-HPLC fraction (Fig. 2, lane 8) showed an additional diffused band (ca. 25 kDa.) Similar diffused protein bands also appeared in the SDS-PAGE analysis of the RP-HPLC-isolated rHuIFN α-2b fractions 7 and 8 (Fig. 2, lanes 10 and 11.) Since these bands appear in both Component A and rHuIFN α-2b fractions from RP-HPLC, but not in the reference rHuIFN α-2b sample (Fig. 2, lane 2), it is possible that the chromatographic isolation procedure itself could have induced their formation. Component A appeared to be a monomeric species because it migrated similarly to rHuIFN α-2b monomer on the SDS-PAGE gels. The results of these preliminary experiments indicated that Component A was a chemically modified rHuIFN α-2b monomer species.

Methionine (Met) oxidation and deamidation of asparagine (Asn) and glutamine (Gln) are commonly observed chemical modifications in non-glycosylated proteins (9–11). rHuIFN α-2b contains 12 Gln, 4 Asn, and 5 Met residues, making it susceptible to chemical modifications such as oxidation and deamidation. Protein degradation products containing oxidized Met residues are usually more hydrophilic, and they elute at earlier retention times than the parent protein in RP-HPLC systems. Hence, there was a high probability that Component A contained an oxidized Met residue. To confirm this hypothesis, an aliquot of rHuIFN α-2b was incubated with hydrogen peroxide under acidic conditions specific for Met oxidation. RP-HPLC analysis of the peroxide-treated rHuIFN α-2b showed a significant increase in the Component A content from 2.8% to

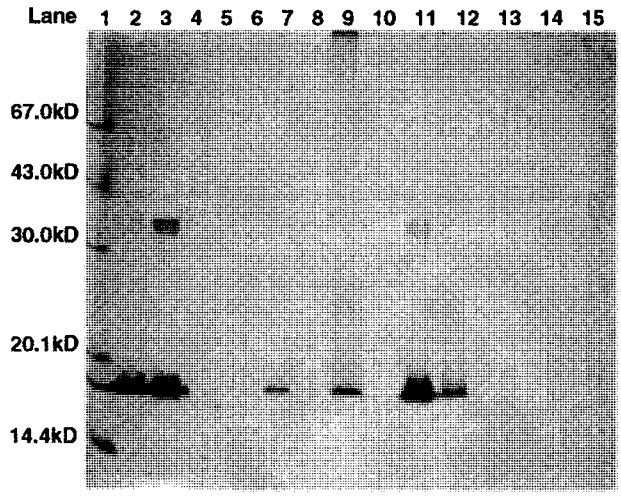

Fig. 2. Analysis of RP-HPLC isolated fractions of rHuIFN α-2b by non-reducing SDS-PAGE. Approximately 0.8 μg of protein was loaded per lane. Two aliquots of rHuIFN α-2b dissolved in aqueous buffer and 50% acetonitrile in 0.1% TFA were included in the analysis as reference (lane 2) and control (lane 3) samples, respectively. Lane 1, protein molecular weight markers in decreasing order of molecular weight from the top of the gel. Lanes 4–11, RP-HPLC fractions 1 to 8. Lane 12, fraction 10; Lane 13, fraction 11; Lane 14, fraction 13; Lane 15, fraction 14.

Table I. Expected Tryptic, V8 Protease, and "Dual Enzyme" (Trypsin Followed by V8 Protease) Peptides of rHuIFN α-2b

Tryptic Digest	V8 protease Digest	"Dual Enzyme" Digest
T1: CDLPQTHSLGSR[12]	V1: CD[2]	TV1: CD[2]
T2: R[13]	V2: LPQTHSLGSRRTLMLLAQ-MRRISLFSCLKD[32]	TV2: LPQTHSLGSR[12]
T3: TLMLLAQMR[22]	V3: RHD[35]	TV3: R[13]
T4: R[23]	V4: FGFPQE[41]	TV4: TLMLLAQMR[22]
T5: ISLFSCLK[31]	V5: E[42]	TV5: R[23]
T6: DR[33]	V6: FGNQFQKAE[51]	TV6: ISLFSCLK[31]
T7: HDFGFPQEEFGNQFQK[49]	V7: TIPVLHE[58]	TV7: D[32]
T8: AETIPVLHEMIQQIFN-LFSTK[70]	V8: MIQQIFNLFSTKD[71]	TV8: R[33]
T9: DSSAAWDETLLDK[83]	V9: SSAAWD[77]	TV9: HD[35]
T10: FYTELYQQLNDLEACV-IQGVGVTETPLMK[112]	V10: E[78]	TV10: FGFPQE[41]
T11: EDSILAVR[120]	V11: TLLD[82]	TV11: E[42]
T12: K[121]	V12: KFYTE[87]	TV12: FGNQFQK[49]
T13: YFQR[125]	V13: LYQQLND[94]	TV13: AE[51]
T14: ITLYLK[131]	V14: LE[96]	TV14: TIPVLHE[58]
T15: EK[133]	V15: ACVIQGVGVTE[107]	TV15: MIQQIFNLFSTK[70]
T16: K[134]	V16: TPLMKE[113]	TV16: D[71]
T17: YSPCAWEVVR[144]	V17: D[114]	TV17: SSAAWD[77]
T18: AEIMR[149]	V18: SILAVRKYFQRITLYLKE[132]	TV18: E[78]
T19: SFSLSTNLQESLR[162]	V19: KKYSPCAWE[141]	TV19: TLLD[82]
T20: SK[164]	V20: VVRAE[146]	TV20: K[83]
T21: E[165]	V21: IMRSFSLSTNLQE[159]	TV21: FYTE[87]
	V22: SLRSKE[165]	TV22: LYQQLND[94]
		TV23: LE[96]
		TV24: ACVIQGVGVTE[107]
		TV25: TPLMK[112]
		TV26: E[113]
		TV27: D[114]
		TV28: SILAVR[120]
		TV29: K[121]
		TV30: YFQR[125]
		TV31: ITLYLK[131]
		TV32: E[132]
		TV33: K[133]
		TV34: K[134]
		TV35: YSPCAWE[141]
		TV37: AE[146]
		TV38: IMR[149]
		TV39: SFSLSTNLQE[159]
		TV40: SLR[162]
		TV41: SK[164]
		TV42: E[165]

Note: Numbers in superscripts indicate positions of these amino acids in the rHuIFN α-2b sequence.

14.2%, supporting the hypothesis that Component A contained an oxidized Met residue.

Identification of the Monosulfoxide-rHuIFN α-2b Variant

Enzymatic and chemical fragmentation of proteins followed by mass spectrometric analysis yield their structure-related specific peptide maps which are routinely used for monitoring the quality of therapeutic proteins (7, 12–16). These peptide maps are also useful for the identification of minor chemical modifications or substitutions in the primary structure of proteins. There are 20 tryptic (10 Lys and 10 Arg) and 21 V8 protease (8 Asp and 13 Glu) cleavage sites in rHuIFN α-2b; therefore 21 tryptic peptides and 22 V8 protease peptides are expected from the enzymatic hydrolysis of the rHuIFN α-2b molecule as shown in Table I. The two disulfide-linked tryptic peptide segments of rHuIFN α-2b containing Cys^{1}-Cys^{98} and Cys^{29}-Cys^{138} are expected to be relatively large with expected average MH^{+} values of 4616.3 and 2118.6 (13), respectively.

An overlay of the HPLC tryptic peptide maps of unchromatographed rHuIFN α-2b, RP-HPLC-isolated rHuIFN α-2b, and Component A is presented in Figure 3. When compared with the tryptic peptide map of unchromatographed rHuIFN α-2b, significant changes in the peptide maps of RP-HPLC-iso-

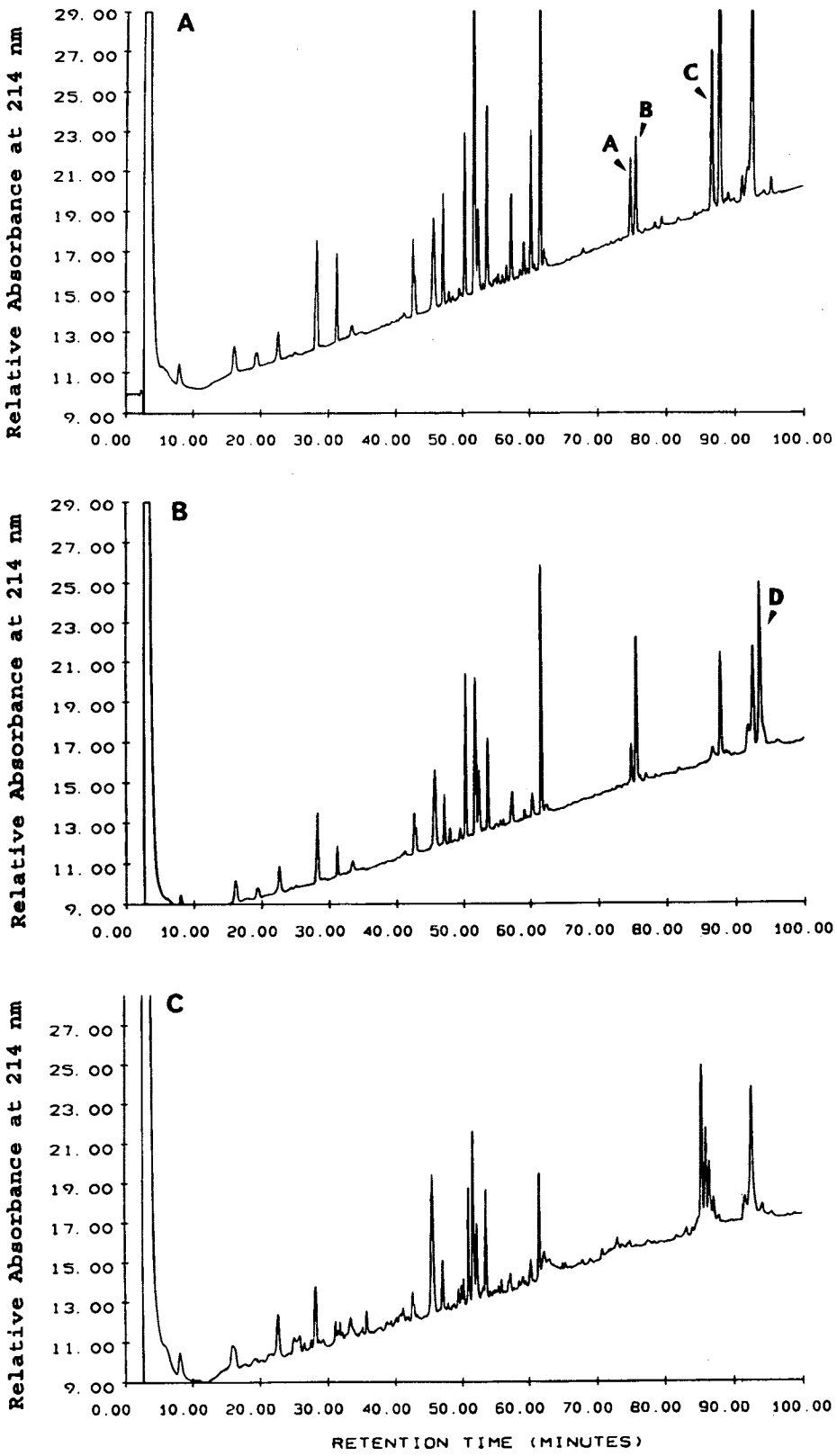

Fig. 3. RP-HPLC tryptic peptide maps of (A) unchromatographed rHuIFN α-2b, (B) RP-HPLC-isolated rHuIFN α-2b, and (C) Component A.

lated rHuIFN α-2b and Component A were detected. These changes were noticed mainly in major peaks eluting after 70 min where usually high-mass fragments and disulfide-linked peptides elute (Fig. 3, peaks A–D). Both peaks A and B were missing and peak C was reduced in the peptide map of Component A (Fig. 3C), with two new peaks appearing adjacent to peak C. Furthermore, peak C was significantly reduced in the tryptic digest of the RP-HPLC-isolated rHuIFN α-2b with a new peak eluting at a longer retention time (Fig. 3B, peak D). The observed differences in the tryptic maps, and the lower hydrophobicity of Component A compared to that of rHuIFN α-2b, indicated the possibility that the Component A is a chemically modified variant of rHuIFN α-2b. Moreover, the differences in the peptide maps of the unchromatographed rHuIFN α-2b and the RP-HPLC-isolated rHuIFN α-2b indicate that there had been isolation-induced changes in the main IFN species. Generally, RP-HPLC separation systems employing acidic mobile phases, organic modifiers, and hydrophobic stationary phases are known to denature proteins subjected to the chromatographic separation and isolation. Since our primary goal was to identify potential chemical modifications in Component A and the preliminary studies indicated that the rHuIFN α-2b itself could change upon RP-HPLC isolation, we used the RP-HPLC-isolated rHuIFN α-2b as the control in the characterization experiments of Component A.

To improve our ability to detect and locate potential chemical modifications in the primary structure of Component A, we carried out fragmentation of rHuIFN α-2b into smaller peptides via a trypsin/V8 protease ("dual enzyme") hydrolysis (Table I.) The "dual enzyme" digestion of the peroxide-treated rHuIFN α-2b and characterization of the generated peptides was included in the study as a positive control for Met oxidation.

The "dual-enzyme" digest peptide maps of the RP-HPLC-isolated rHuIFN α-2b, Component A, and peroxide-treated rHuIFN α-2b are shown in Figure 4. A comparison of the three peptide maps showed that a peak appearing at 32.5 min (TV-25) in the peptide map of RP-HPLC-isolated rHuIFN α-2b digest (Fig. 4A) was found to be significantly reduced in the enzymatic digest of Component A (Fig. 4B), while it was absent in the peptide map of the peroxide-treated rHuIFN α-2b digest (Fig. 4C). Two new peaks (TV-25a and TV-25b) eluting at 25.3 min and 27.2 min, appeared in the enzymatic digests of both Component A and the peroxide-treated rHuIFN α-2b (Fig. 4B-C). Edman sequencing of the three peptides TV-25, TV-25a, and TV-25b yielded the amino acid sequences TPLMK, TPLMK, and TPLMKE, respectively. These peptides correspond to rHuIFN α-2b segments containing amino acid residues from position 108 to 112 (TPLMK), and 108 to 113 (TPLMKE).

The LSI mass spectra of the three peptides, TV-25, TV-25a, and TV-25b, showed signals at *m/z* of 589.4, 605.3, and 734.4, respectively (Table II). The Edman-derived amino acid sequence TPLMK for the peptide TV-25 from the RP-HPLC-isolated rHuIFN α-2b, and the MH^+ value observed by LSIMS are in agreement with the theoretically expected sequence and mass of the "dual enzyme" peptide TPLMK. The MH^+ value of peptide TV-25a (amino acid sequence TPLMK) from Component A and peroxide-treated rHuIFN α-2b (Table II) indicated an increase of 16 Da over the expected value. This is due to oxidation of the Met residue at position 111 of the protein, because Met^{111} is the amino acid in the TPLMK peptide which is most susceptible to oxidation. The peptide TPLMKE (TV-25b), appearing in the digests of both Component A and peroxide-treated rHuIFN α-2b (Fig. 4), could have resulted from an incomplete tryptic cleavage at Lys^{112} possibly due to the presence of the neighboring modified Met residue. The observed MH^+ value for this peptide was 734.4, which is 16 Da higher than the theoretical mass value, accounting for the oxidation of Met^{111}. Thus, Component A is identified as a variant of rHuIFN α-2b with the oxidized Met at position 111.

The CD spectra indicate a lower α helix content in the Met-oxidized (40%) and RP-HPLC-isolated rHuIFN α-2b (46%) compared to the unchromatographed rHuIFN α-2b (55%). At the same time, β sheet contribution is 30%, 20% and 6% for the Met-oxidized, RP-HPLC-isolated and unchromatographed rHuIFN α-2b, respectively. These estimates indicate that both the chromatographic conditions and Met oxidation contribute to the observed major changes in the secondary structure.

Evaluation of Biological and Immunological Activities

Methionine oxidation has been reported for other protein therapeutics, e.g. rh-growth hormone (rhGH), insulin, OKT3 antibody, parathyroid hormone (PTH), with varying effect on the biological activity of the protein. For example, the Met oxidized rhGH retains complete biological activity (17), whereas the PTH loses biological activity depending upon the number of oxidized Mets in the molecule (18). In the case of rHuIFN α-2b, both Component A and the RP-HPLC isolated protein had comparable biological activity as evidenced from the CPE assays (Table III).

However, Component A showed low reactivity in the IRMA assay while the RP-HPLC-isolated rHuIFN α-2b had the expected reactivity in that assay. It is possible that the conformation, and the monoclonal antibody specific epitope of Component A were altered due to Met oxidation. Since a monoclonal antibody was employed in the IRMA assay, the potential conformational changes could interfere with antigen-antibody reactions necessary for the detection of Component A. The RP-HPLC-isolated rHuIFN α-2b was fully reactive in IRMA, thus indicating that the RP-HPLC isolation-related changes in this rHuIFN α-2b species did not

Table II.

Protein	Trypsin/V8 Peptide	Observed mass Value[a]
RP-HPLC isolated rHuIFN α-2b	TV-25	589.4
Component A	TV-25a	605.3
	TV-25b	734.4
Peroxide-treated rHuIFN α-2b	TV-25a	605.3
	TV-25b	734.5

[a] Monoisotopic MH^+ mass value.

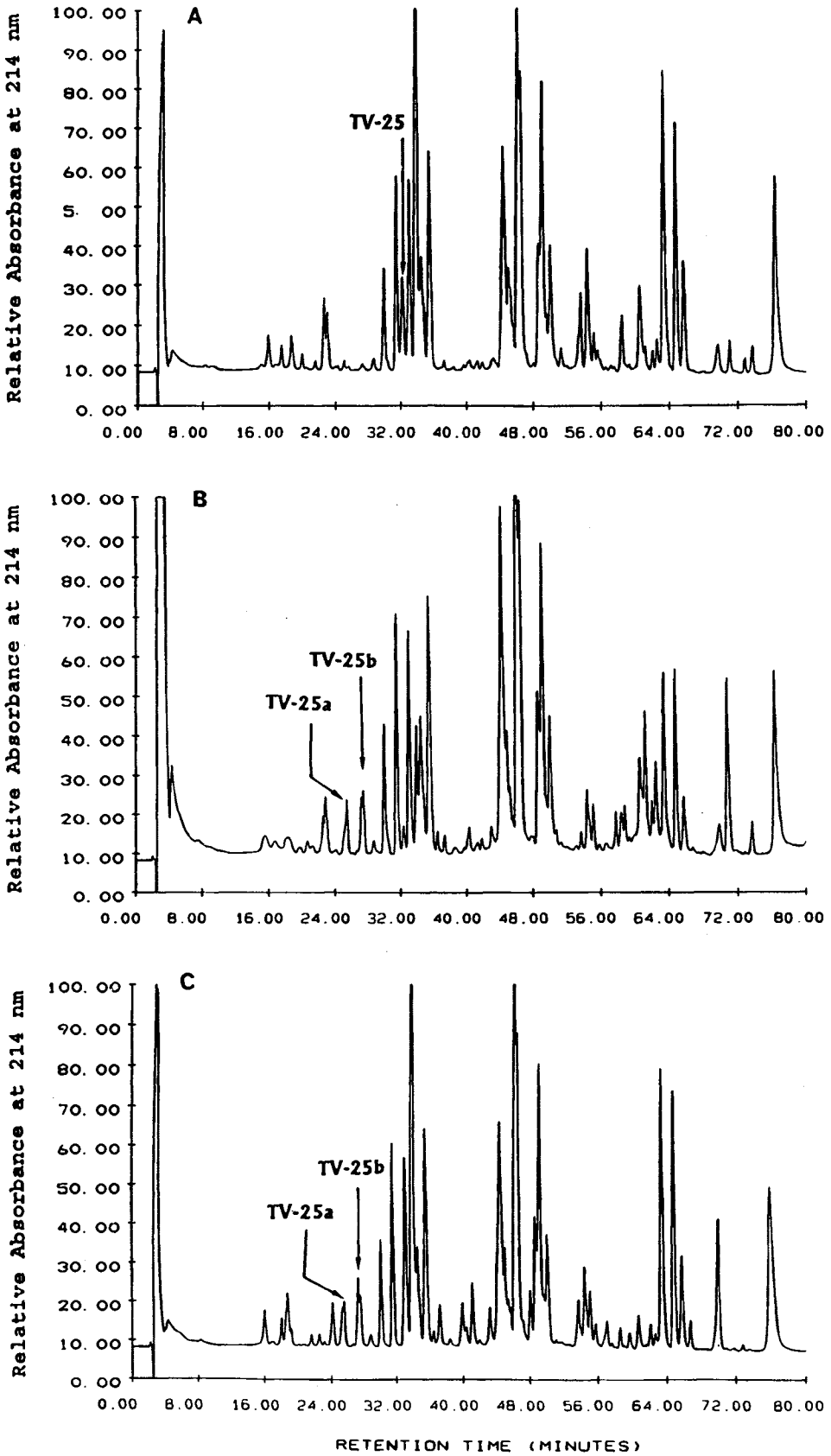

Fig. 4. RP-HPLC peptide maps from the trypsin/V8 protease digests of (A) RP-HPLC-isolated rHuIFN α-2b, (B) Component A, and (C) peroxide-treated rHuIFN α-2b.

Table III. Specific Activity of rHuIFN α-2b Component A and RP-HPLC-isolated Control Based on the Antiviral CPE Bioassay and IRMA Immunoassay

	CPE (IU/mg)	IRMA (IU/mg)[a]
Component A:	2.88×10^8	0.68×10^8
RP-HPLC-isolated rHuIFN α-2b:	2.88×10^8	3.19×10^8

[a] The immunoassay results were converted to IU/mg using a conversion factor for equivalence between the unit (mg) rHuIFN α-2b measured by the immunoasssay and its biological activity measured by the antiviral CPE assay.

seem to have significant effects on its monoclonal antibody binding sites.

CONCLUSIONS

A monosulfoxide-rHuIFN α-2b variant was found to be present in the rHuIFN α-2b bulk drug substance in solution. The Met residue at amino acid position 111 of the rHuIFN α-2b molecule was identified as Met sulfoxide by comparative tryptic/V8 mapping and LSI mass spectrometric analysis of relevant fractions. LSIMS was essential to the identification of the Met sulfoxide-containing peptides, since Edman sequencing could not differentiate between Met and Met sulfoxide residues. The specific acivity of rHuIFN α-2b and its monomethioninesulfoxide variant were comparable as evidenced from the antiviral CPE bioassay.

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