Characterization of the Chicken Telokin Heterogeneity by Time-of-Flight Mass Spectrometry†

Filippo Rusconi,*,‡ Marie-Claude Potier,‡ Jean-Pierre Le Caer,‡ Jean-Marie Schmitter,§ and Jean Rossier‡

Laboratoire de Neurobiologie et Diversité Cellulaire, Centre National de la Recherche Scientifique Unité de Recherche Associée 2054, Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris, 10 rue Vauquelin, 75231 Paris Cedex 05, France, and Laboratoire de Physico- et Toxicochimie des Systèmes Naturels, Université de Bordeaux I, 351 cours de la Libération, 33405 Talence Cedex, France

Received April 1, 1997; Revised Manuscript Received June 11, 1997⊗

ABSTRACT: Chicken gizzard telokin was purified to apparent homogeneity by sodium dodecyl sulfate−polyacrylamide gel electrophoresis. This preparation yielded upon mass spectrometry analysis seven mass peaks spanning from 15 858 to 17 100 Da. Anion exchange−high performance liquid chromatography of the purified telokin revealed a high diversity of telokin molecules. By combining protein chemistry to chromatography and mass spectrometry, the telokin heterogeneity was analyzed. Three acetylated N-termini were found, AMI, MIS, and SGR. Cyanogen bromide cleavage of telokin yielded six different C-terminal peptides corresponding to the removal of one to six C-terminal glutamyl residues from the protein sequence deduced from the cDNA. Phosphorylation of telokin was detected, thus increasing the heterogeneity of the telokin preparation. In addition, peptide sequencing has shown that telokin contained either an aspartyl or a glutamyl residue at position 27, probably resulting from chicken polymorphism.

Telokin, also known as KRP (kinase related protein), is a 17.3 kDa protein whose sequence is identical to the C-terminal region of the smooth muscle myosin light chain kinase (MLCK). Telokin concentration in gizzard has been estimated to 90 μM (Shirinsky et al., 1993). The telokin promoter most probably lies within an intron in the 3′ region of the MLCK gene (Guerriero et al., 1986; Collinge et al., 1992). Thus, the primary structure of chicken telokin was first inferred from the sequence of the C-terminal region of MLCK. However, the actual translational start site on the telokin mRNA has not been defined, first because the N-terminus of telokin is blocked and cannot be sequenced, secondly because telokin mRNA contains three possible ATG codons in the same open reading frame. On the basis of cDNA sequence analysis and amino acid composition of telokin, Ito et al. (1989) proposed, for the turkey gizzard telokin N-terminus, the Ile[4] residue contained within the N-terminal sequence ^{1}MAMISGM^{7}, while others proposed the ^{2}AMISGM^{7} N-terminal sequence (Gallagher & Herring, 1991; Collinge et al., 1992). The whole telokin primary structure has not been determined by direct amino acid sequencing yet, and partial sequencing experiments did not encompass the C-terminal tail of the protein (Ito et al., 1989).

In the present report, purified telokin was shown by MALDI-TOF[1] mass spectrometry to contain several molecular species. Mass spectrometric analyses of enzymatically or chemically generated telokin N-terminal and C-terminal peptides have shown that this heterogeneity was related both to different translational start sites and to variable C-termini. In addition, N-terminal peptides were found also as phosphorylated species, as previously indicated by in vitro phosphorylation with cAMP-dependent protein kinase (Ito et al., 1989).

The numbering of the amino acids used in the following sections of this report is according to the scheme ^{1}MAMISGM...$(EG)_4$EEDEEEEEE^{157} as illustrated in the Figure 1C.

EXPERIMENTAL PROCEDURES

Purification of Chicken Gizzard Telokin. Our purification method was based upon protocols described for the purification of MLCK or telokin, as outlined below, and was conducted at 4 °C unless otherwise stated. Fresh chicken gizzards were processed as described previously for the preparation of washed myofibrils with the difference that the first two supernatants were pooled for further purification of telokin (Adelstein & Klee, 1982). Subsequent steps of purification were as described (Ito et al., 1989) with the following modifications: dialysis of the recovered 70% ammonium sulfate saturation pellet was against 30 mM Tris-HCl (pH 7.5, 4 °C), 1 mM DTT, and 1 mM PMSF. No isoelectric precipitation of the dialysate was performed. DEAE Q FastFlow chromatography was carried out with a step-gradient of NaCl starting at 0 mM NaCl and ending at 500 mM NaCl in steps of 50 mM NaCl. Phenyl-Sepharose CL4B chromatography was carried out with a step-gradient of ammonium sulfate starting at 2 M $(NH_4)_2SO_4$ and ending at 0.5 M $(NH_4)_2SO_4$ with 0.1 M $(NH_4)_2SO_4$ steps. The purity

† This work forms a portion of the doctoral thesis of F.R., who received a predoctoral fellowship from the Ministère de la Recherche. It was supported by the Association pour la Recherche contre le Cancer (ARC, France).

* Corresponding author. Tel: 33-1-40-79-47-67. Fax: 33-1-40-79-47-57. E-mail: rusconi@atlas.bio.espci.fr.

‡ Laboratoire de Neurobiologie et Diversité Cellulaire.

§ Université de Bordeaux I.

⊗ Abstract published in *Advance ACS Abstracts,* August 1, 1997.

[1] Abbreviations: MALDI-TOF, matrix-assisted laser desorption ionization−time-of-flight; PTH-amino acid, phenyl thiohydantoin-amino acid; RP−HPLC, reversed phase−high performance liquid chromatography; DEAE, diethylaminoethyl; TFA, trifluoro acetic acid; CNBr, cyanogen bromide; SDS−PAGE: sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

S0006-2960(97)00752-6 CCC: $14.00

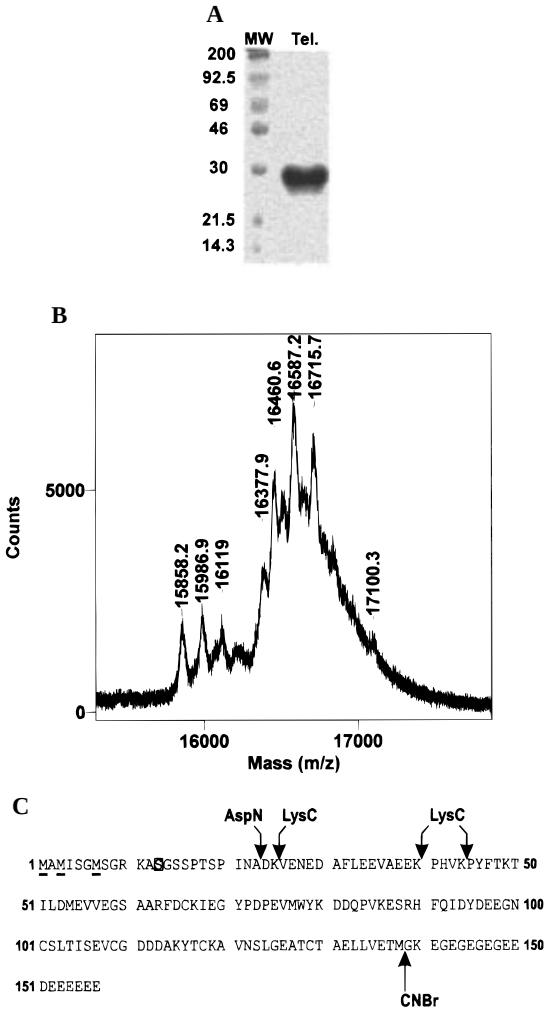

FIGURE 1: Analysis of the purified telokin by sodium docecyl sulfate−polyacrylamide gel electrophoresis and mass spectrometry. (A) Coomassie staining of a 12.5% SDS−PAGE of the purified telokin. Tel., 50 μg of purified protein; MW, molecular weight markers. (B) MALDI-TOF mass spectrometry analysis of the telokin preparation. Numbers indicate the *m*/*z* value of the peaks. For details see Experimental Procedures. (C) Primary structure of the chicken gizzard telokin deduced from the cDNA sequence (Guerriero et al., 1986; Ito et al., 1989). The underlined methionyl residues correspond to the potential initiation sites. Box indicates the serine that has been shown to be phosphorylatable (Ito et al., 1989). Arrows indicate the cleavage sites of interest (AspN, *endo*-proteinase AspN; LysC, *endo*-proteinase LysC; and CNBr, cyanogen bromide cleavage site).

of the resultant telokin preparation was monitored by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE, Figure 1A).

Preparation of Polyclonal Antibodies to Telokin and Identification of the Epitope. Antibodies against telokin were produced in rabbits. The initial injection was 100 μg of purified telokin from chicken gizzard, in complete Freund's adjuvant. Two subsequent boosts were made at 1 month intervals with the same amount of telokin in uncomplete Freund's adjuvant. Western blotting experiments were conducted with Hybond-C Extra Supported Nitrocellulose membranes from Amersham Corp., and the dot blot analysis membrane was Immobilon from Millipore Corporation (Bedford, MA). Typical dilution of the rabbit antiserum to telokin was 1/150000 (incubation 2 h), and dilution of the anti-rabbit IgG antiserum was 1/4000 (incubation overnight). The immunoreactive proteins were visualized on film with the enhanced chemiluminescent detection system (ECL Western Blotting detection system, Amersham Corp.).

The specificity of the antibodies was assessed by Western blot experiments using the samples from different steps of the telokin purification procedure. The antibodies recognized chicken gizzard telokin and MLCK. Reactivity of the antibodies against chicken gizzard MLCK was assessed by comparison of their reactivity with that of an antibody specific for the rabbit smooth muscle MLCK active site (kind gift of Dr. J. Stull). In order to identify the telokin epitope, a telokin band from a SDS−PAGE was digested in the gel with *endo*-proteinase LysC as described below. The resultant peptides were separated by reverse phase−high performance liquid chromatography (RP−HPLC) and fractions collected as single peaks. The fractions were dotted on a membrane and tested for reactivity with the anti-telokin antibody. Three fractions were positive. Mass spectrometry analysis of the recognized fractions yielded four peaks. The two major peaks with observed [M + H$^+$] *m*/*z* values of 1750.86 and 2212.18 were present along with two minor peaks whose *m*/*z* values differred from the first ones by 14 Da. The *m*/*z* values of the major two peaks were close to the calculated masses of the peptides ^{26}VENEDAFLEEVAEEK^{40} (calcd [M + H$^+$] *m*/*z* value, 1750.80) and ^{26}VENEDAFLEEVAEEK-PHVK^{44} (calcd [M + H$^+$] *m*/*z* value, 2212.08), respectively. Sequencing of the peptides eluting in these fractions yielded two similar sequences, namely V**X**NEDAFLEEVAEEK, where the phenyl thiohydantoin-amino acid (PTH-amino acid) for the **X** residue was either a PTH-glutamate or a PTH-aspartate, thus explaining the −14 Da shift observed by mass spectrometry. The PTH-Glu:PTH-Asp signal intensity ratio was 70:30. This observed amino acid variation at position 27 could be due to polymorphism.

Anion Exchange−HPLC of the Purified Telokin. Telokin was injected in a 5PW diethyl amino ethyl−high performance liquid chromatography (DEAE−HPLC) column (Waters Corp. Milford, MA). The elution was performed at room temperature at a flow rate of 700 μL/min. Solvent A was deionized water, 20 mM Tris-HCl, pH 8.0, and 20 mM NaCl, and solvent B was 20 mM Tris-HCl, pH 8.0, and 500 mM NaCl. The gradient used was from 0 to 34% B in 10 min, 34% B for 3 min, and from 34 to 100% B in 59 min. Fractions were collected as single peaks or shoulders.

Proteolytic Digestion and Cyanogen Bromide Cleavage of the Purified Telokin. Proteolysis of purified telokin (approximately 5 μg) by *endo*-proteinase LysC (Boehringer Mannheim) was carried out in the SDS−PAGE as previously described (Rosenfeld et al., 1992) with the following modifications: telokin was migrated in a 12.5% SDS−PAGE. Following Coomassie Brilliant Blue staining (0.2% in methanol 20%, acetic acid 0.5%), destaining (30% methanol), and washing (deionized water), the telokin-containing band was excized and rinsed three times 30 min at 30 °C in 150 μL of rinsing buffer (50% acetonitrile in 100 mM ammonium carbonate). The gel slice was partially dried at room temperature, cut into smaller pieces and rehydrated with 10 μL ammonium carbonate 100 mM. The enzyme was added (diluted in 10 μL of ammonium carbonate 100 mM) to a final enzyme:substrate ratio of 1:40 (w/w). Digestion was carried out in a final volume of 50 μL for 17 h at 37 °C. Peptides were extracted and vacuum-dried and recovered in 0.1% trifluoro acetic acid (TFA) in deionized water for the RP−HPLC separation.

Telokin contained in different DEAE−HPLC fractions was dialyzed against water and vacuum-dried. The dried protein was recovered in formic acid and the amount of CNBr (in 70% formic acid) added was to a molar excess of 1000-fold. Reaction was carried out overnight at room temperature in the dark, and the reagents were then dried by flushing with nitrogen. The sample was recovered in 0.1% TFA and analyzed by either mass spectrometry or RP−HPLC.

Proteolysis of purified telokin (25 μg) by *endo*-proteinase Asp-N (Boehringer Mannheim) was carried out in a 30 mM Tris-HCl, pH 8.5, buffer with a 1:300 enzyme:substrate ratio (w/w) for 6.5 h at 36 °C. The reaction mixture was analyzed by RP−HPLC.

RP−HPLC Separation of endo-Proteinase Cleavage Products. The *endo*-proteinase Asp-N cleavage products were subjected to HPLC on a RP column (Nucleosil C18, 1 × 250 mm, SGE Scientific Pty Ltd, Australia). The elution was performed at room temperature at a flow rate of 50 μL/min. Solvent A was deionized water and 0.1% TFA, and solvent B was 80% acetonitrile in deionized water and 0.09% TFA (v/v). The acidified (0.1% TFA) sample was injected at time 0, and buffer B was 5%. The gradient used was from 5 to 18% B in 18 min, then from 18 to 36% B in 92 min, and finally from 36 to 70% B in 40 min.

The *endo*-proteinase LysC cleavage products were processed as for the *endo*-proteinase Asp-N cleavage products, except that the gradient was from 0 to 52% B in 60 min and then from 52 to 100% B in 10 min.

For both separations, fractions were collected as single peaks and further analyzed by mass spectrometry and Edman degradation sequencing.

Mass Spectrometry. MALDI-TOF spectra of the protein and proteolytic peptides were obtained with a Voyager-Elite Biospectrometry Workstation mass spectrometer (PerSeptive Biosystems, Inc., Framingham, MA).

Mass Spectrometry of the Protein. The purified telokin was desalted either by dialysis against deionized water or by desalting on a C18 reversed-phase resin (Sep-Pak, Waters Corp., Milford, MA). The matrix was 3,5-dimethoxy-4-hydroxycinnamic acid (Sinapinic Acid, Aldrich, St. Louis, MO), and the sample was loaded on the target by the dried droplet method. Spectra obtained for the whole protein were calibrated externally using the [M + H$^+$] ion from one protein standard (horse apomyoglobin: *m*/*z* 16952.5). The analysis was performed in the positive and linear modes, with an accelerating voltage of 25 000 V, an extraction delay of 200 ns, and around 70 scans were averaged. Typically, the amount of protein analyzed was of 5−10 pmol.

Mass Spectrometry on the Peptides. The matrix used was α-cyano-4-hydroxycinnamic acid (Sigma, St. Louis, MO), and the sample was loaded on the target by the dried droplet method. Spectra were calibrated either internally, using the monoisotopic [M + H$^+$] ion from two peptide standards (angiotensin I, *m*/*z* 1296.68; and neurotensin, *m*/*z* 1672.92), for peptides obtained with *endo*-proteinase AspN, or externally with the same standards for peptides obtained with cyanogen bromide and *endo*-proteinase LysC. The analysis was performed in the positive and reflector modes, with an accelerating voltage of 20 000 V, an extraction delay of 150 ns, and around 120 scans were averaged. Typically the amount of peptide analyzed was of 1−5 pmol.

Amino Acid Sequencing. Peptides were sequenced by automated Edman degradation using a model 794 Protein Sequencer (Perkin Elmer-Applied Biosystems Division, Foster City, CA). The amount of peptide loaded was typically 150 pmol.

RESULTS

Purification and Identification of Telokin. The most important difference between our purification protocol and the ones described in the literature was that we did not perform any isoelectric precipitation step. Indeed, we observed in a previous experiment that telokin is poorly enriched during this step, indicating that telokin molecules differed by their total electric charge. Figure 1A shows the SDS−PAGE-migrated purified protein. A single broad band was observed at an apparent molecular mass of ∼26 kDa.

The purified protein was analyzed by MALDI-TOF mass spectrometry. The spectrum shown in Figure 1B contains several [M + H$^+$] peaks, spanning from a *m*/*z* value of 15 858 to a *m*/*z* value of 17 100. These values should be compared to the calculated molecular mass of telokin (17 336 Da, if the N-terminal sequence is MAMISGM, Figure1C).

Sequencing of an estimated amount of 100 pmol of the purified protein gave no signal at all, indicating that the telokin molecules were N-terminally blocked.

DEAE−HPLC Chromatography of the Telokin Preparation. In an attempt to separate the molecular species detected by mass spectrometry, the telokin preparation was subjected to a DEAE−HPLC. We obtained 13 peaks that were arranged in six doublets plus a last single peak (Figure 2A). All these fractions were shown to contain telokin by Western blot analyses performed with the antibody described in Experimental Procedures (Figure 2B).

In order to characterize the molecular differences responsible for the elution pattern, each DEAE−HPLC fraction was treated with cyanogen bromide, expected to generate the C-terminal peptide ^{139}GKEGEGEGEGEEDEEEEEE^{157} (Figure 1C). Reaction products were analyzed by MALDI-TOF mass spectrometry.

Table 1 shows the sequence of all the C-terminal peptides identified. The C-terminus of telokin appeared to be variable, with one to six C-terminal glutamyl residues being removed.

As an example, cyanogen bromide-treated fraction DEAE-2 generated a single C-terminal peptide with a [M + H$^+$] *m*/*z* value of 1321.50, corresponding to the C-terminus of telokin to which the last six Glu (Glu^{152}−Glu^{157}) had been removed (calcd [M + H$^+$] *m*/*z* value, 1321.50). Amongst all the DEAE fractions, the longest C-terminal peptide that we detected had a [M + H$^+$] *m*/*z* value of 1966.49, corresponding to the C-terminus of telokin to which the last glutamyl residue had been excized (calcd [M + H$^+$] *m*/*z* value, 1966.72). The complete C-terminal peptide was not detected in any of the fractions analyzed (Table 1, $n = 6$). Several DEAE−HPLC fractions contained the same C-terminal peptides, showing that the six variants ($n = 1-6$) could not account for all the 13 peaks observed in the DEAE−HPLC separation.

Each DEAE−HPLC fraction was also directly analyzed by mass spectrometry. Figure 3 shows the spectrum obtained for DEAE fraction 2 (Table 1, $n = 0$). The major peak (peak 5) had a [M + H$^+$] *m*/*z* value of 16 481, close to the calculated [M + H$^+$] *m*/*z* value of 16 473 Da corresponding to telokin molecules with the following acetylated N-terminus: ^{2}AMI. Peak 4 had a [M + H$^+$] *m*/*z* value of

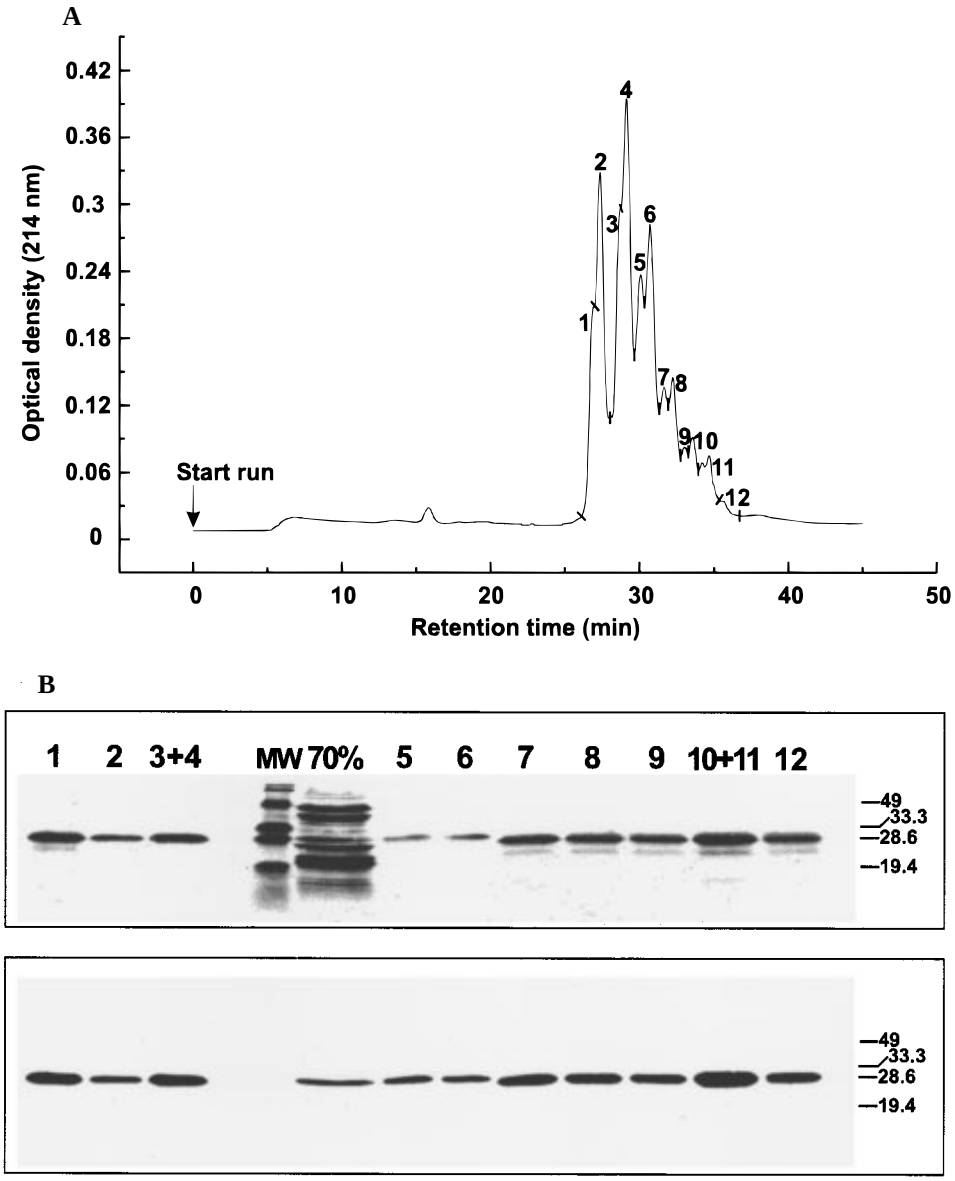

FIGURE 2: DEAE−HPLC of the purified telokin and Western blot analysis of the fractions with an anti-telokin antibody. (A) Elution profile after injection of 300 μg of telokin on a DEAE−HPLC column. Salt gradient was from 20 to 500 mM NaCl in three steps as described in Experimental Procedures. Numbers identify peaks or shoulders that were collected. (B) DEAE fractions from the chromatogram in panel A were TCA precipitated, migrated on a 15% SDS−PAGE and blotted on to a nitrocellulose membrane. Top shows a Ponceau red staining of the membrane and the number of the fractions. 70%, 70% ammonium sulfate precipitation pellet from the purification process (see Experimental Procedures). MW, prestained molecular weight markers (Bio-Rad Laboratories, CA), values are indicated on the right of the panel. Blot was rinsed and incubated with the anti-telokin antibody. Bottom shows the chemiluminescent revelation of the immunoreactivity. Each DEAE−HPLC fraction contained telokin.

16 410, corresponding to telokin starting at ^{3}MIS (calcd [M + H$^+$] *m*/*z* value, 16 402 Da if the N-terminal residue is acetylated). Peak 1 (observed [M + H$^+$] *m*/*z* value of 15 887 Da) could correspond to telokin with a shorter N-terminus, ^{8}SGR (calcd [M + H$^+$] *m*/*z* value, 15 882 if the N-terminus is acetylated ^{8}SGR). Peaks 1 and 5 were followed by two minor peaks (2 and 6) with a mass increment of about 80 Da (15 969 and 16 563 Da, respectively), suggesting that phosphorylation could account for part of the observed telokin heterogeneity. Finally, peaks 3 and 7 corresponded to phosphorylated telokin found predominantly in DEAE fraction 3 (Table 1, $n = 1$), with the same N-terminus as in peaks 1 and 5, respectively.

N-Terminal Sequence and Phosphorylation of Telokin. In order to identify precisely the N-terminus of telokin and its phosphorylation, we analyzed by mass spectrometry N-terminal peptides generated by enzymatic proteolysis of purified telokin using the *endo*-proteinase AspN. Digestion products were separated by RP−HPLC and fractions collected as single peaks or shoulders. Each fraction was analyzed both by MALDI-TOF mass spectrometry and by Edman degradation sequencing.

Table 2 shows the sequence of all the N-terminal peptides detected with their observed and calculated [M + H$^+$] *m*/*z* values. Three acetylated N-terminal peptides were identified, starting at positions 2, 3, and 8 of telokin sequence (Figure 1C). All of these peptides were found as phosphorylated and unphosphorylated species, the latter ones being the most abundant species.

None of these six N-terminal peptides could be sequenced by Edman degradation, confirming that they were N-terminally blocked.

Table 1: Heterogeneity at the C-Terminus of Telokin[a]

DEAE fraction number	GKEGEGEGEGEED(E)$_n$						
	$n = 0$	$n = 1$	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$
DEAE-1	+						
DEAE-2	+						
DEAE-3	+	+					
DEAE-4	+						
DEAE-5		+	+				
DEAE-6			+				
DEAE-7			+	+			
DEAE-8		+	+	+			
DEAE-9+10			+	+	+		
DEAE-11+12		+	+	+	+	+	

[a] DEAE−HPLC fractions from the chromatogram described in Figure 2A were treated with cyanogen bromide and analyzed by MALDI-TOF mass spectrometry. Sequence of the C-terminal peptides is shown on the top (*n* is the number of C-terminal glutamyl residues). Crosses indicate the peptides contained in the DEAE−HPLC fractions.

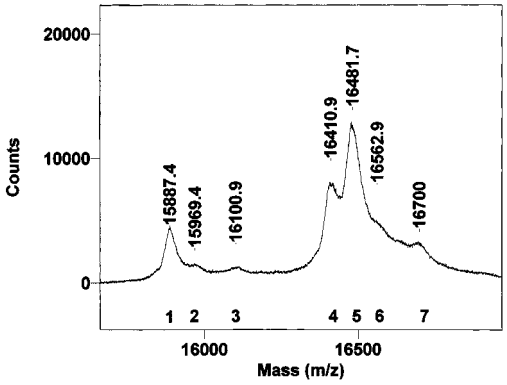

FIGURE 3: Mass spectrometry analysis of DEAE fraction 2 from the chromatogram described in Figure 2A. DEAE fraction 2 was desalted and submitted to MALDI-TOF spectrometry. Numbers indicate the *m*/*z* value of the peaks.

Table 2: Heterogeneity at the N-Terminus of Telokin[a]

sequence of the N-terminal peptides	calculated $[M + H^+]$ *m*/*z* value	measured $[M + H^+]$ *m*/*z* value
Ac-^{2}AMISGMSGRKASGSSPTSPINA	2149.04	2149.07
Ac-^{2}AMISGMSGRKASGSSPTSPINA + P	2229.01	2228.99
Ac-^{3}MISGMSGRKASGSSPTSPINA	2078.00	2078.02
Ac-^{3}MISGMSGRKASGSSPTSPINA + P	2157.92	2157.92
Ac-^{8}SGRKASGSSPTSPINA	1558.78	1558.75
Ac-^{8}SGRKASGSSPTSPINA + P	1638.74	1638.70

[a] Purified telokin was treated with *endo*-proteinase AspN. Products were separated by RP−HPLC and analyzed by mass spectrometry. Table shows the sequences of the N-terminal peptides deduced from the measured $[M + H^+]$ *m*/*z* values. Ac- indicates that the peptides are acetylated. + P indicates that the peptides are phosphorylated.

DISCUSSION

In this report we show that telokin is rather heterogeneous, even if it appears homogeneous upon SDS−PAGE electrophoresis followed by Coomassie staining of the gel. This heterogeneity was clearly visible upon analysis of the telokin preparation by high-resolution MALDI-TOF mass spectrometry. The telokin mass spectra consisted of up to seven peaks (from 15 858 to 17 100 Da; Figure 1B), and the *m*/*z* values observed for the $[M + H^+]$ ions appeared to be lower than the calculated molecular mass of telokin (17 336 Da).

DEAE−HPLC of the purified telokin yielded as much as 13 peaks. This elution pattern was shown to be due in part to variable C-termini. MALDI-TOF mass spectrometry analysis of CNBr-generated C-terminal peptides revealed six variants differing in their number of C-terminal glutamyl residues.

The six C-terminal variants described could not, however, account for the thirteen DEAE−HPLC peaks observed (Figure 2A). Analysis of the mass data obtained from the different DEAE−HPLC fractions has shown that each fraction contained more than one molecular species with different N-termini and phosphate content (Figure 3, Table 2). Upon analysis of *endo*-proteinase AspN digest of telokin by mass spectrometry, we demonstrated the existence of three N-terminal peptides, the shortest starting at Ser^8, the longest at Ala^2, and the intermediate at Met^3. Semiquantitative analysis of our RP−HPLC data indicated that the longest and the intermediate peptides were present in almost the same amount, corresponding to around 3-fold the amount of the shortest peptide. All the described N-terminal peptides were acetylated explaining why, by Edman degradation sequencing, no signal for the N-terminus could be obtained. Anderson et al. (1991) and Holden et al. (1992) have found that the 32 N-terminal and the 19 C-terminal residues of turkey gizzard telokin were not visible in the electron density map, indicating that these regions are not firmly structured, probably because of the heterogeneity described here.

This work shows that the combination of protein chemistry, chromatography and MALDI-TOF mass spectrometry, allowed us to delineate the heterogeneity of the N-terminus and the C-terminus of telokin. Recent advances in mass spectrometry resolution and sensitivity provide a means to access structural data pertaining to blocked N-termini and C-termini of proteins. This is of valuable interest, since N-terminal sequencing is unfeasible if the N-terminal residue is blocked and C-terminal sequencing is still not a routinely used technique.

The lack of an accurate and readily applicable carboxyl-terminal sequencing method [for a review, see Inglis (1991)] that would complement the N-terminal Edman degradation procedure has led to a little knowledge of the post-translational modifications of the C-terminal primary structure of proteins, such as truncation or carboxyl-terminal proteolytic excisions. Studies of the C-terminal structure of proteins have first been done by using carboxypeptidases and specific antibodies. One such example of C-terminal post-translational processing by glutamyl residue removal is the *des*-glutamylation of tubulins: the detyrosinated α tubulin C-terminal tail undergoes excision of the C-terminal glutamyl residue. This observation was first made by use of specific antibodies to nontyrosinatable tubulin. Mass spectrometry later brought confirmation of these results by analysis of biological peptides (Paturle-Lafanechère et al., 1991; Redeker et al., 1996).

In the case of chicken gizzard telokin, described in this report, time-of-flight mass spectrometry proved to be the key technical method to gain direct access to the structure of blocked N-terminal peptides and of C-terminal peptides of the protein, thus explaining the observed high heterogeneity of the purified protein.

In vitro, Shirinsky et al. (1993) showed that micromolar concentrations of telokin bind unphosphorylated myosin and promote the assembly into myosin filaments in the presence of ATP. In gizzard tissue, telokin concentration is estimated at 90 μM, comparable to myosin concentration (Shirinsky

et al., 1993), making telokin a good candidate for stabilizing the myosin filaments in vivo. Interaction of variably charged telokin molecules with myosin would lead to differently charged complexes varying in their ability to assemble into filaments. Our work shows that telokin is heterogeneous both in its N- and C-termini, providing a large number of different structures varying both in the length and in the total charge of the protein, indicating that its interaction with other proteins might be finely regulated at the structural level.

REFERENCES

Adelstein, R. S., & Klee, C. B. (1982) *Methods Enzymol. 85*, 298−308.

Anderson, T. A., Ito, M., Hartshorne, D. J., & Rayment, I. (1991) *J. Mol. Biol. 217*, 621−623.

Collinge, M., Matrisian, P. E., Zimmer, W. E., Shattuck, R. L., Lukas, T. J., Van Eldik, L. J., & Watterson, D. M. (1992) *Mol. Cell. Biol. 12*, 2359−2371.

Gallagher, P. J., & Herring, B. P. (1991) *J. Biol. Chem. 266*, 23945−23952.

Guerriero, V., Jr., Russo, M. A., Olson, J. J., Putkey, J. A., & Means, A. R. (1986) *Biochemistry 25*, 8372−8381.

Holden, H. M., Ito, M., Hartshorne, D. J., & Rayment, I. (1992) *J. Mol. Biol. 227*, 840−851.

Inglis, A. S. (1991) *Anal. Biochem. 195*, 183−196.

Ito, M., Dabrowska, R., Guerriero, V., Jr., & Harshorne, D. J. (1989) *J. Biol. Chem. 254*, 13971−13974.

Paturle-Lafanechère, L., Eddé, B., Denoulet, P., Van Dorsselaer, A., Mazarguil, H., Le Caer, J.-P., Wehland, J., & Job, D. (1991) *Biochemistry 30*, 10523−10528.

Redeker, V., Rusconi, F., Mary, J., Promé, D., & Rossier, J. (1996) *J. Neurochem. 67*, 2104−2114.

Rosenfeld, J., Capdevielle, J., Guillemot, J.-C., & Ferrara, P. (1992) *Anal. Biochem. 203*, 173−179.

Shirinsky, V. P., Vorotnikov, A. V., Birukov, A. K. N., Collinge, M., Lukas, T. J., Sellers, J. R., & Watterson, D. M. (1993) *J. Biol. Chem. 268*, 16578−16583.

BI970752E