C-terminal truncation of thymosin β_{10} by an intracellular protease and its influence on the interaction with G-actin studied by ultrafiltration

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Abstract **Two β-thymosins are expressed in most mammalian tissues. We detected small amounts of a third peptide in extracts of rabbit spleen. The portion of this peptide increased when the tissue was first frozen and then thawed at 4°C. Small amounts of the peptide are also present in cells from suspension cultures homogenized immediately in diluted perchloric acid. By means of amino acid analysis and MALDI-mass spectroscopy this peptide was identified to be a C-terminally truncated form of thymosin β_{10}. Having studied the formation in more detail we found that after a 4-h thaw at 4°C all thymosin β_{10} was truncated to thymosin $\beta_{10}{}^{1-41}$, which was further degraded during the next 20 h. On the other hand, thymosin $\beta_4{}^{Ala}$, the second β-thymosin being present in rabbit spleen, was not truncated or degraded even after 22 h. It might be possible that in vivo a truncated form of thymosin β_{10} is formed by a carboxydipeptidase while thymosin $\beta_4{}^{Ala}$ is rather stable against proteolytic modification. By using a newly designed ultrafiltration assay, we determined the dissociation constants of the complexes of G-actin and these three β-thymosins to be 0.28, 0.72, and 0.94 μM for thymosin $\beta_4{}^{Ala}$, β_{10}, and thymosin $\beta_{10}{}^{1-41}$, respectively. The complex with $\beta_4{}^{Ala}$ is unambiguously more stable than the complex with β_{10} or β_4 (0.81 μM). The change in the dissociation constant generated by the truncation of the two C-terminal amino acid residues of β_{10} is small but statistically significant. This demonstrates that even the very last amino acid residues at the C-terminus of β-thymosins are involved in the interaction with G-actin.**

Key words: β-thymosin; Rabbit spleen; Proteolytic modification; Ultrafiltration; Dissociation constant; Actin

1. Introduction

Thymosin β_4 has first been described as a thymic hormone from calf thymus [1,2]. Thymosin β_4 is accompanied by a second highly homologous peptide, thymosin β_8 [3] when isolated from thymosin fraction 5. Extraction of peptides from calf thymus with guanidinium hydrochloride denaturing proteases immediately led to the isolation of thymosin β_9 which is identical to thymosin β_8 with the exception of an additional dipeptide at the C-terminus [3]. The isolation of thymosin β_4 and homologous peptides, which are called thymosin β_4-like peptides or β-thymosins, from various cells [4,5] and tissues of vertebrates [6–8] in high concentrations shaded doubts on their function as thymic hormones. Later on Safer et al. [9] isolated a G-actin sequestering factor, Fx, and showed that this factor is identical to thymosin β_4 [10]. The query that nearly all mammalian tissues investigated up to now express two members of this family has not been clarified [11,12]. Most recently a third β-thymosin (β_{15}) has been detected in Dunning rat prostate carcinoma additionally to thymosin β_4 and β_{10} [13]. In tissues of rabbit [7,14] and trout [15,16] two β-thymosins are found in comparable concentrations. Rabbit spleen contains thymosin $\beta_4{}^{Ala}$ and β_{10} whereas in the spleen of trout thymosin β_{11} and β_{12}-trout are present.

Weber et al. [17] determined the dissociation constant for the interaction of thymosin β_4 with G-actin by measuring the increase in fluorescence of pyrenyl-labeled actin when polymerizing to filamentous F-actin. Jean and coworkers [18] employed the change in tryptophan fluorescence of actin to determine the interactions with thymosin β_4 and $\beta_9{}^{Met}$. However, this method is restricted to those β-thymosins possessing a methionyl residue. In the case of interactions with β-thymosins which do not contain a methionyl residue, or when the methionyl residue is oxidized, only a very weak change in fluorescence intensity could be detected by the authors. Heintz et al. [19] also studied actin-β-thymosin interactions under non-polymerizing conditions by employing G-actin which was modified by monobromotrimethylammoniobimane bromide. In a previous paper we introduced an equilibrium centrifugation assay to study the interactions of β-thymosins and their fragments with non-modified G-actin [20]. This excludes a possible influence of the fluorescent reporter group, as has been demonstrated by Giuliano and Taylor for the interaction of G-actin with profilin [21]. In this paper we report that a C-terminally truncated form of thymosin β_{10} is present in rabbit spleen and a human alveolar cell line. The C-terminal truncation changes the interaction with G-actin as shown by a newly designed, efficient and manageable ultrafiltration assay.

2. Materials and methods

2.1. Materials

Reagents were obtained from the following sources: LiChroprep RP-18 (40-63 μm) and trifluoroacetic acid (TFA, Uvasol) from Merck (Darmstadt); ovalbumin from Sigma and fluorescamine from Serva.

2.2. Proteins and peptides

Actin was prepared from bovine heart muscle by the method of Pardee and Spudich [22] and further purified by gel-filtration [23] on a Sephacryl S300 column (Pharmacia) equilibrated with G-buffer (2 mM Tris-HCl, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM mercaptoethanol, 0.05% NaN_3, pH 8.0). Pyrene-labeled actin was prepared according to Kouyama and Mihashi [24]. G-actin was stored in G-buffer at 0°C. The peptides were isolated from rabbit spleen according to the method described for thymosin $\beta_9{}^{Met}$ from pork spleen [25]. The purity of the preparations was demonstrated by reverse-phase high-performance liquid chromatography (RP-HPLC). The concen-

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Abbreviations: K_d, dissociation constant; PCA, perchloric acid; TFA, trifluoroacetic acid; RP-HPLC, reversed-phase high-performance liquid chromatography; MALDI-MS, matrix-assisted laser desorption mass spectrometry

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trations of β-thymosins and actin were determined by amino acid analysis after acid hydrolysis (6 M HCl, 155°C, 1 h) and precolumn derivatization with *o*-phthaldialdehyde/3-mercaptopropionic acid [26].

2.3. Time course of proteolytic degradation

Six freshly prepared rabbit spleens were frozen in separate tubes for 24 h and then thawed for 0, 0.5, 1, 2, 4 or 22 h at 4°C. Thereafter, the spleens were homogenized in four volumes of ice-cold 0.4 M PCA and a defined amount of the dipeptide Phe–Phe (5 μg/g tissue) was added as an internal standard. After 30 min on ice the extracts were centrifuged for 5 min at 20 000×*g*. The supernatant solution was carefully removed and adjusted to pH 4 with 10 M KOH. After another 30 min on ice the precipitated $KClO_4$ was removed by centrifugation and an aliquot of the supernatant was analyzed by RP-HPLC.

2.4. HPLC

Chromatographic conditions were controlled by a Merck-Hitachi L-6200 system supplemented with an reaction pump for post-column derivatization (655A-13, Merck-Hitachi) and with a fluorometer (F-1050, Merck-Hitachi). The fluorescence signal was recorded on an integrator (D-2500, Merck-Hitachi).

2.4.1. Analytical separations. Flow rate was 0.75 ml/min. The buffer was 0.1% TFA; gradients are as described in figure legends. The column was a Beckman ODS Ultrasphere (5 μm, 4.6×250 mm). For detection post-column derivatization with fluorescamine was used.

2.4.2. Preparative separation of the peptides. Flow rate was 0.75 ml/min. The buffer was 0.1% TFA. The gradient was linear from 0 to 40% acetonitrile in 120 min. The column was a Pharmacia SuperPac Pep-S (5 μm, 4×250 mm); detection was at 215 nm (UV-detector L-4000, Merck-Hitachi).

2.5. Matrix-assisted laser desorption mass spectrometry (MALDI-MS)

Mass determinations were performed with a LaserTec Research time-of-flight mass spectrometer (Perseptive Biosystems, USA). The instrument is equipped with a nitrogen laser (λ = 337 nm, pulse duration 3 ns, 20 pulses/s). Laser-desorbed positive ions were analyzed after being accelerated by 20 kV resulting in a mass range for single charged ions up to 5.6 kDa in the reflection mode. External calibration was performed by using $[M+H^+]^+$ of melittin (M_r = 2847.5). Usually 40–80 laser shots were averaged to produce a mass spectrum.

2.6. Sample preparation for MALDI-MS

Peptide samples were diluted with H_2O to a concentration of about 5–20 pmol/μl. As matrix solution, 10 mg of α-cyano-4-hydroxy-cinnamic acid were dissolved in 1 ml containing 33.3% acetonitrile and 0.1% TFA. Equal volumes of analyte solution and matrix solution were mixed and 2 μl of the mixture were pipetted onto the roughened 2.5-mm diameter sample positions on a stainless-steel plate.

2.7. Determination of K_d values by ultrafiltration

A defined amount of the internal standard Phe–Phe was added to thymosin β_4^{Ala}, thymosin β_{10}, or thymosin β_{10}^{1-41} and diluted to a concentration of 60 μM. This solution was diluted 4-fold with either G-buffer or a solution of G-actin (0.86 mg/ml) or ovalbumin (0.9 mg/ml). The first pair of ultrafiltration units (Nanosep 30 kDa, Pall Filtron Corporation, North Borough, MA 01532, USA) was loaded with 100 μl of the peptide–buffer mixture, the second pair with 100 μl of peptide–actin solution and the third pair with 100 μl of peptide–ovalbumin mixture. All tubes were incubated for 15 min at room temperature and then centrifuged for 15 min at 2700×*g* in a table-top centrifuge (Eppendorf centrifuge 5417, Eppendorf-Netheler-Hinz, Germany). Ultrafiltrates (40 μl) were analyzed by HPLC.

2.8. Determination of K_d values using pyrenyl-actin

To determine the concentration of unpolymerized actin being the sum of the critical concentration of G-actin plus the G-actin sequestered by β-thymosin, the polymerization of actin in the absence or presence of β-thymosins was determined using native actin supplemented with 5% of pyrenyl-actin according to Weber et al. [17]. Excitation and emission wavelengths were set at 365 and 386 nm, respectively, using a Kontron fluorometer (SFM 25). In presence of β-thymosins the amount of unpolymerized actin in equilibrium with F-actin was derived from fluorescence measurements of serially diluted solutions of F-actin containing a constant amount of the β-thymosin. These experiments were done in a buffer consisting of 2 mM $MgCl_2$, 2 mM Tris-HCl, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM mercaptoethanol and 0.05% NaN_3, pH 8.0.

2.9. Calculation of dissociation constants

For an interacting system like G-actin (G) and β-thymosin (βT):

$$G + \beta T \rightleftharpoons G{-}\beta T$$

The dissociation constant can be calculated by applying the following equation:

$$K_{d,app} = \frac{[\beta T]_{free} \cdot [G]_{free}}{[G{-}\beta T]} \quad (1)$$

where $K_{d,app}$ = apparent equilibrium constant; [G-βT]=concentration of formed complex; $[G]_{free}$ = concentration of unbound G-actin and $[\beta T]_{free}$ = concentration of unbound β-thymosin, which can be determined from the following equation:

$$[\beta T]_{free} = [\beta T] \cdot \frac{F_{\beta T}}{F'_{\beta T}} \quad (2)$$

where [βT]=concentration of β-thymosin before centrifugation;

$$F = \frac{\text{concentration of peptide in ultrafiltrate}}{\text{concentration of peptide before ultrafiltration}} \quad (3)$$

and $F_{\beta T}$ = in the presence of G-actin and $F'_{\beta T}$ = in absence or in presence of ovalbumin after being proven that there is no interaction between thymosin β_4 and ovalbumin (data not shown). The concentration of the complex [G-βT] can be calculated from the following equation:

$$[G{-}\beta T] = [\beta T] - [\beta T]_{free} \quad (4)$$

and the value for the concentration of unbound actin $[G]_{free}$ can be calculated from

$$[G]_{free} = [G] - [G{\cdot}\beta T] \quad (5)$$

where [G] is the total concentration of actin before centrifugation.

3. Results

3.1. Isolation and characterization of thymosin β_{10}^{1-41}

When we analyzed perchloric acid extracts of freshly isolated rabbit spleens by RP-HPLC to determine their content of β-thymosins, we detected a third peptide eluting between the expected two β-thymosins (Fig. 1). We also observed that the portion of this peptide increased when spleens where first

Table 1
Amino acid composition of the three β-thymosins isolated from rabbit spleen

Residue[a]	Thymosin β_4^{Ala}	Thymosin β_{10}	Thymosin β_{10}^{1-41}
Asx	4.1 (4)[b]	4.3 (4)[b]	4.2 (4)[c]
Glx	10.3 (11)	8.4 (9)	8.7 (9)
Ser	2.0 (3)	2.1 (3)	1.2 (2)
Gly	1.1 (1)	1.3 (1)	1.3 (1)
Thr	2.7 (3)	4.1 (5)	3.9 (5)
Arg	0.0 (0)	0.8 (1)	0.6 (1)
Ala	3.3 (3)	3.3 (3)	3.0 (3)
Met	0.3 (1)	0.3 (1)	0.3 (1)
Ile	2.3 (2)	3.1 (3)	2.1 (2)
Phe	0.9 (1)	0.8 (1)	0.8 (1)
Leu	2.3 (2)	2.2 (2)	2.0 (2)
Lys	8.1 (9)	7.6 (8)	7.1 (8)

[a]Proline was not determined, all other amino acids not listed were < 0.1 residue per peptide.
[b]Values in parentheses are from the known sequences of thymosin β_4^{Ala} [7] and thymosin β_{10} [14,30–32].
[c]Values in parentheses are from the sequence of thymosin β_{10}^{1-41} reported here.

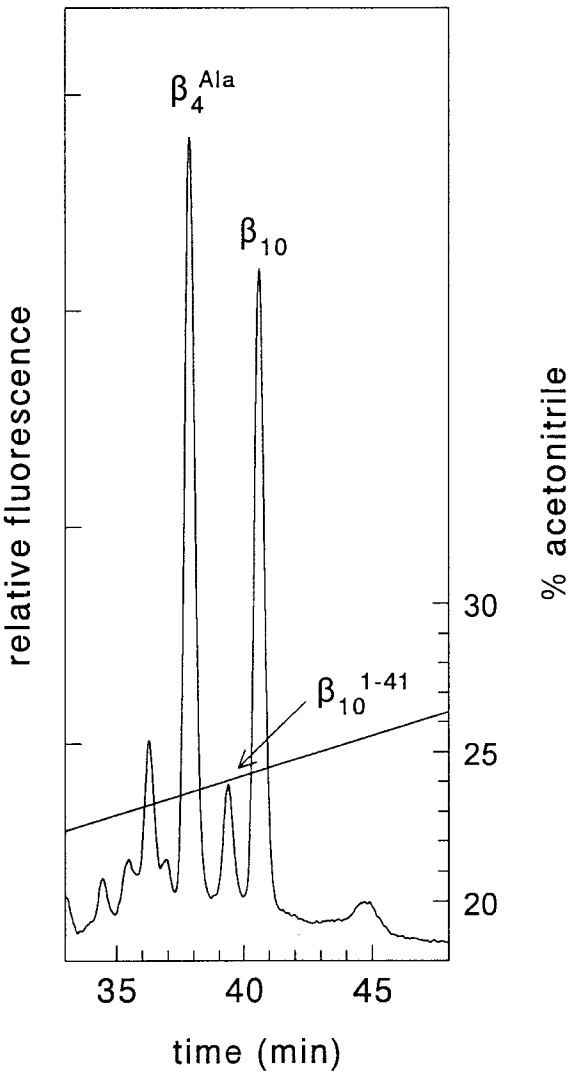

Fig. 1. HPLC analysis of the PCA-extract of rabbit spleen. An aliquot (100 μl) of the PCA extract after neutralization was analyzed by RP-HPLC on a Beckman ODS Ultrasphere column. Peptides were eluted with the following gradient: 0–16% acetonitrile in 0.1% TFA in 10 min and from 16 to 40% during 90 min. Detection was carried out by post-column derivatization with fluorescamine [29]. Retention time (min): thymosin β_4^{Ala} (38.0); thymosin β_4^{1-41} (39.5); thymosin β_{10} (40.7).

frozen and then thawed at 4°C, whereas the portion of thymosin β_{10} decreases. This was a first hint that the unknown peptide may derive from thymosin β_{10}. We used a freeze–thaw cycle to generate higher amounts of this peptide for the isolation and characterization of the unknown peptide. All three peptides were isolated using the protocol described under Section 2. Afterwards we determined the amino acid composition of the unknown peptide by total acid hydrolysis. We found that the amino acid composition of the unknown peptide is nearly identical to that of thymosin β_{10} except that it contained one serine and one isoleucine residue less than thymosin β_{10} (Table 1). The comparison of the amino acid composition with the known amino acid sequence of thymosin β_{10} suggested that the unknown peptide might be a C-terminally truncated form of thymosin β_{10}. To verify this, we employed MALDI-MS to determine the molecular mass of all three isolated peptides. As shown in Fig. 2 the molpeak $[M+H^+]^+$ for protonated thymosin β_4^{Ala} appeared at a molecular mass of 4947.8 (m/z) (Fig. 2A), the molpeak for thymosin β_{10} at 4937.6 (Fig. 2B) and $[M+H^+]^+$ for the unknown peptide at 4734.5 (Fig. 2C). The small peak at 4933.9 in the spectra of the unknown peptide represents a small amount of contamination with thymosin β_{10}. Table 2 summarizes observed and calculated molecular masses of the three peptides. These data substantiated that the unknown peptide represented thymosin β_{10} truncated for two amino acid residues at the C-terminus. This peptide is named thymosin β_{10}^{1-41}.

Further studies of the time course of proteolytic degradation showed that after a 4-h incubation period thymosin β_{10} was completely converted to thymosin β_{10}^{1-41}. Thymosin β_{10}^{1-41} disappeared almost completely after a thaw of 22 h, whereas the amount of thymosin β_4^{Ala} remained constant (data not shown).

3.2. Detection of thymosin β_{10}^{1-41} in cells from suspension culture

To verify that this peptide is also present in cells from suspension culture, we analyzed several cell lines after immediate homogenization in diluted perchloric acid. In all cases a small peak between thymosin β_4^{Ala} and thymosin β_{10} was present. As an example Fig. 3 shows the HPLC chromatogram of a perchloric acid extract from the human alveolar cell line A549. To confirm that this peak represents thymosin β_{10}^{1-41}, we collected the HPLC eluate between 38 and 41 min, containing thymosin β_4 and the unknown peptide, but excluding thymosin β_{10}. This eluate was then analyzed by MALDI-MS. As expected, the mass spectra shows a large peak at 4946.3, representing thymosin β_4 and a small peak at 4737.1 representing thymosin β_{10}^{1-41} (Fig. 4).

3.3. Determination of dissociation constants

To study the influence of C-terminal truncation on the interaction with G-actin in the absence of a fluorescent reporter group in G-actin and under non-polymerizing conditions, we developed an ultrafiltration assay. Depending on the difference in molecular mass between the β-thymosins (5 kDa) and their complexes with actin (48 kDa), one can separate by ultrafiltration free β-thymosin from β-thymosin complexed with actin. The amount of free β-thymosin can be determined

Table 2
Characterization of isolated peptides

	Composition	m/e (observed)	(calculated)	Deviation in ‰
Thymosin β_4^{Ala}	$[M+H^+]^+$	4947.8	4948.9	0.22
Thymosin β_{10}	$[M+H^+]^+$	4937.6	4938.0	0.08
Thymosin β_{10}^{1-41}	$[M+H^+]^+$	4734.5	4738.7	0.89

Molecular mass of the purified peptides $[M+H^+]^+$ were determined by MALDI-MS.

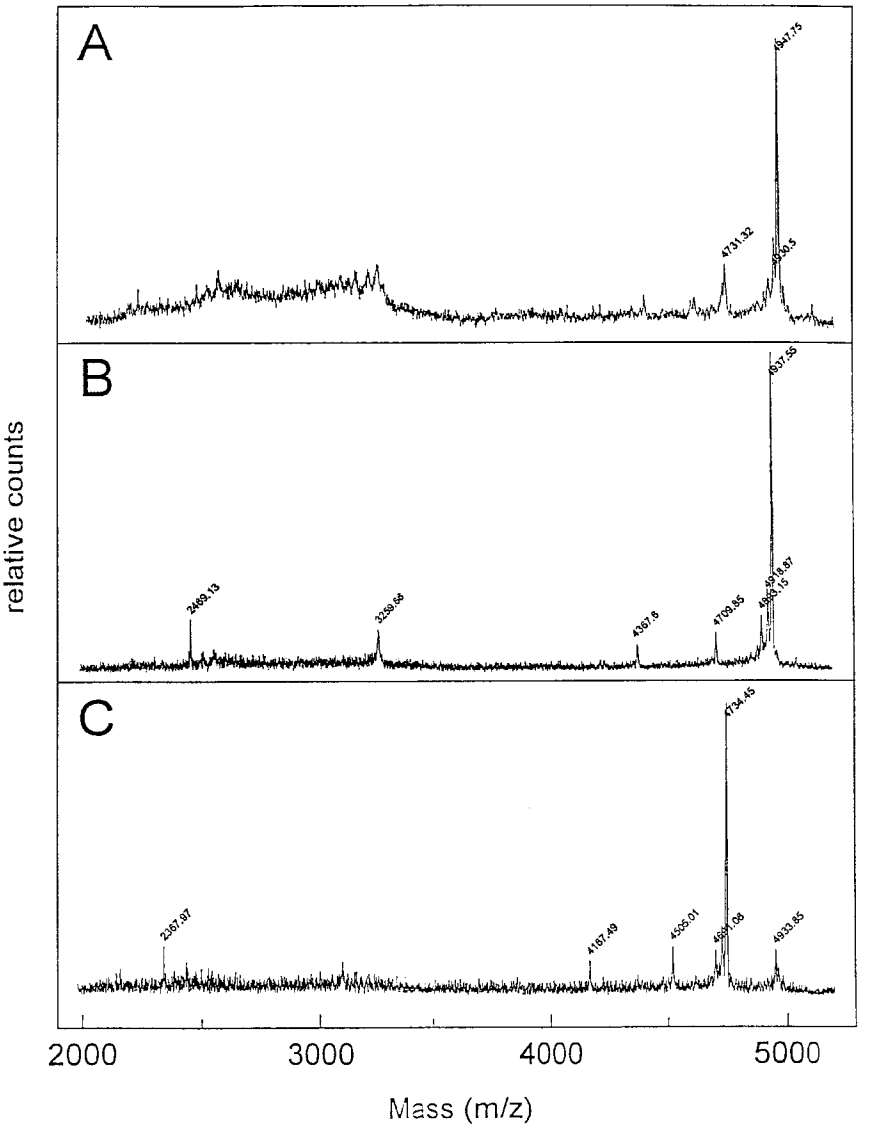

Fig. 2. MALDI mass spectra of the isolated peptides: (A) thymosin β_4^{Ala}, $[M+H^+]^+$ identification: m/z 4947.8; (B) thymosin β_{10}, $[M+H^+]^+$ identification: m/z 4937.6; (C) thymosin β_{10}^{1-41}, $[M+H^+]^+$ identification: m/z 4734.5.

by analyzing the ultrafiltrate by RP-HPLC. We were interested to investigate whether the truncation of thymosin β_{10} to thymosin β_{10}^{1-41} influences its interaction with G-actin. Therefore, we determined the dissociation constants for these two peptides from rabbit spleen and also for thymosin β_4 and β_4^{Ala} with the described ultrafiltration assay. The difference in the K_d value of the complex of G-actin with thymosin β_{10} and thymosin β_{10}^{1-41} is small, but statistically significant (Student t-test, $2P < 0.001$) and comparable to the dissociation constant of the G-actin–thymosin β_4 complex. In contrast the K_d value of the complex with thymosin β_4^{Ala} was about 3-fold lower (0.28 μM). For validation of the ultrafiltration method, we compared the obtained data with the data obtained by using pyrenyl-labeled G-actin (Table 3).

Table 3
Comparison of the apparent dissociation constants for the interaction between G-actin and β-thymosins

Thymosin	$K_{d,app}$ (μM)			
	Determined by ultrafiltration		Determined by pyrenyl-labeled actin	
	($\bar{x} \pm$ SD)	(n)	($\bar{x} \pm$ SD)	(n)
β_4^{Ala}	0.28 ± 0.03	7	0.28 ± 0.04	3
β_4	0.81 ± 0.11	9	0.75 ± 0.06	3
β_{10}	0.72 ± 0.09	5	0.66	
β_{10}^{1-41}	0.94 ± 0.06	4	0.80	

The apparent dissociation constants for the interaction between β-thymosins and G-actin from bovine heart were determined by ultrafiltration assay or with pyrenyl-labeled actin. The exact concentrations of β-thymosin and actin were estimated by amino acid analyses before use. All assays were carried out with a single actin preparation.

4. Discussion

In 1981 Low et al. reported the isolation and sequence analysis of a peptide from thymosin fraction 5 which they named thymosin β_4 [1]. A year later we characterized a second highly homologous peptide in thymosin fraction 5, thymosin β_8 [3]. However, when we treated calf thymus with guanidinium hydrochloride, which minimizes proteolytic activity during preparation, we isolated another peptide that was identical to thymosin β_8 with the exception of an additional dipeptide at the C-terminus [3]. Therefore, we concluded that thymosin β_8 was proteolytically generated probably during the preparation of thymosin fraction 5 [3]. In the following years several reports showed the ubiquitous distribution of high intracellular concentrations of thymosin β_4 and several homologous peptides [4–8]. Nevertheless, no information on proteolytic modifications of β-thymosins has been reported. Safer et al.

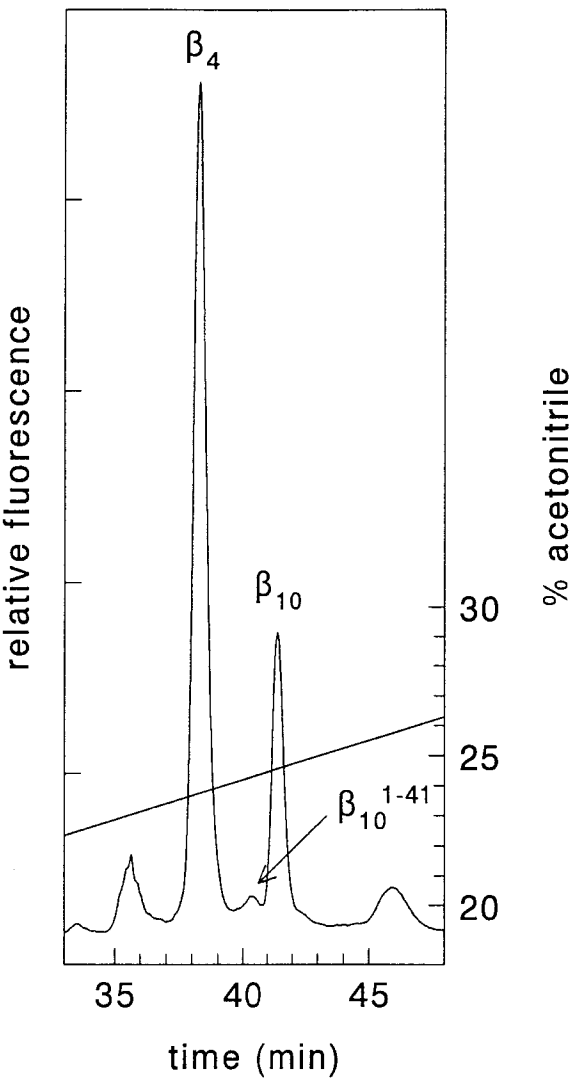

Fig. 3. HPLC analysis of the PCA extract of A549 cells. About 5×10^6 cells were homogenized in 400 μl of 0.4 M perchloric acid. After 30 min at 4°C and centrifugation for 5 min at $14000\times g$ the supernatant was adjusted to pH 4 using 10 M KOH. The precipitated $KClO_4$ was removed by centrifugation and the supernatant was analyzed by RP-HPLC with conditions as described in the legend to Fig. 1. Retention time (min): thymosin β_4 (38.3); thymosin $\beta_{10}{}^{1-41}$ (40.5); thymosin β_{10} (41.7).

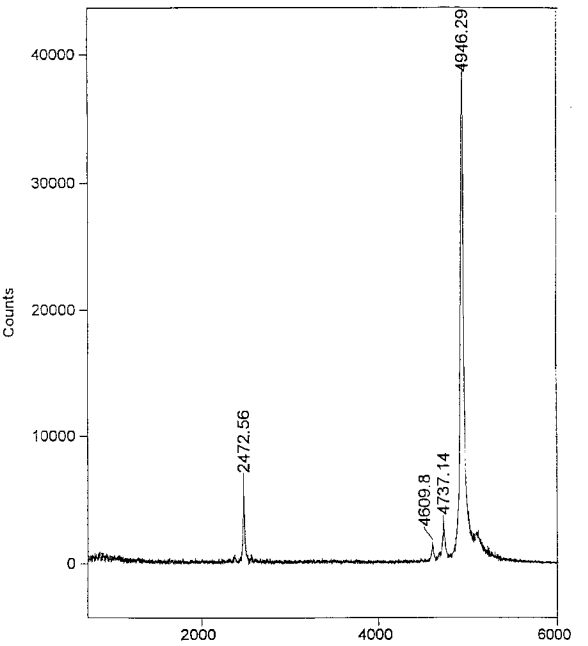

Fig. 4. MALDI mass spectra of the HPLC eluate from A549 cells. Eluate of HPLC run between 38 and 41 min was collected, dried in a speed-vac and further treated as described in Section 2.

[9] and Weber et al. [17] found that the main physiological role of thymosin β_4 seems to be the sequestering of actin monomers. In most mammalian tissues investigated, two β-thymosins are expressed, whereby in most cases thymosin β_4 is the main peptide which represents about 70–80% of the total β-thymosin content. One of the two known exceptions is rabbit spleen in which thymosin $\beta_4{}^{Ala}$ and thymosin β_{10} were found in comparable amounts. Our finding that in rabbit spleen and several cell lines a small portion of thymosin β_{10} is truncated at the C-terminus for two amino acids, is the first report of a proteolytic modification of β-thymosins. We also showed that when the tissue is first frozen and then thawed at 4°C for 2 h almost all thymosin β_{10} was truncated to thymosin $\beta_{10}{}^{1-41}$, which was then further degraded during the next 20 h. Thymosin $\beta_4{}^{Ala}$, the second β-thymosin, expressed in rabbit spleen was not degraded under these conditions at all. The demonstration, that small amounts of thymosin $\beta_{10}{}^{1-41}$ are present in cells aside from β_{10} is important when studying the concentration or distribution of thymosin β_{10} by antibodies. The antibodies may recognize thymosin β_{10} as well as the truncated thymosin $\beta_{10}{}^{1-41}$.

Several groups studied the interaction of actin and β-thymosin by labeling actin at Cys^{374} with various fluorescent reporter groups [17–19,27,28]. These methods are bound of the following restrictions and disadvantages. Some of them can only be used at salt conditions that initiate actin polymerization and can, therefore, only be used to study interactions with peptides that inhibit actin polymerization. Labeling of actin can interfere with the interaction of actin with β-thymosins and some methods are restricted to those β-thymosins containing a methionine residue. In a previous paper [20], we introduced an equilibrium centrifugation assay, which did not require any derivatization of actin and can be used at low salt concentrations. This enabled us to determine dissociation constants of N-terminally truncated β-thymosins, which do not inhibit actin polymerization. This method, how-

ever, has the disadvantage that a calibration curve with molecular mass standards in the used molecular weight range had to be generated and that one equilibrium centrifugation run consumed at least 16 h. Therefore, we established an ultrafiltration assay, which can be efficiently and easily handled and is carried out with non-labeled G-actin. The method is based on the work of Sophianopoulos and coworkers [33,34], who showed that during ultrafiltration of a system at equilibrium, although the concentration of the macromolecule-ligand complex increases, the total amount of it in the ultrafiltration cell remains constant. Since no net uptake or removal of ligand takes place, the rate of the filtrate does not disturb the equilibrium. Free β-thymosin can be separated from actin-bound β-thymosin by employing ultrafiltration tubes equipped with a 30-kDa cut-off membrane and an adjustable tabletop centrifuge. The concentration of free β-thymosin can then be determined by HPLC analysis of the ultrafiltrate. The dissociation constant of the actin–β-thymosin complex can be calculated knowing the initial concentrations of β-thymosin and actin. The values obtained by this simple, fast and economic ultrafiltration procedure are in good agreement with our values observed after overnight incubation of β-thymosins with pyrenyl-labeled G-actin and we therefore conclude, that the equilibrium is reached during ultrafiltration.

The complex of G-actin with thymosin β_4 is stabilized when the N-terminally acetylated seryl residue is replaced by an alanyl residue (β_4^{Ala}) as it is the case in rabbit tissues. While the N-terminus is strongly involved in the interaction with G-actin, the two C-terminal amino acid residues seem to be less important for the interaction. However, even the removal of only these two amino acid residues generate a small but statistically significant increase in the dissociation constant of the complex with G-actin. This suggests that the small β-thymosins interact in their entire length with G-actin.

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