Degradation of α-actinin during Ca^{2+}-sensitive proteolysis of myofibrils

Dorota Kulesza-Lipka and Anna Jakubiec-Puka[+]

Laboratory of Protein Metabolism, Nencki Institute of Experimental Biology, Pasteur Str. 3, 02-093 Warsaw, Poland

Received 3 June 1985

The noted loss of α-actinin from the Z-line of myofibrils during post-mortem autolysis, probably following the action of calcium-activated protease, has previously been attributed to its release without degradation. This report shows that in isolated myofibrils α-actinin is proteolysed in a Ca^{2+}-sensitive manner presumably via the action of calcium-activated protease.

α-Actinin degradation *Myofibrillar protein degradation* *Ca^{2+}-dependent proteolysis*

1. INTRODUCTION

During post-mortem autolysis of muscle, the Z-line disappears owing to the action of CAF [1–4] situated in striated muscle also within the Z-line [5]. The opinion prevails that CAF removes α-actinin from the Z-line without splitting it [6,7]. Thus, in spite of a similar turnover rate for α-actinin and myosin [8], α-actinin, in contrast to myosin, is resistant to cleavage by skeletal muscle proteases [1,9–12].

Contrary to earlier reports, this work shows that the autolysis of myofibrils, under close to physiological conditions, is accompanied by Ca^{2+}-sensitive degradation of α-actinin.

2. MATERIALS AND METHODS

Crude and purified myofibrils were obtained from soleus muscles of female Wistar rats aged 2½–3 months as described [13]. Myofibrils were incubated 24–48 h at 4°C with 1 mM EDTA or 2 mM $CaCl_2$ in a medium of 40 mM Tris-HCl (pH 7.4), 0.1 M NaCl and 1 mM NaN_3. Myofibrils without incubation served as controls. Inhibitors of proteolytic enzymes were used: DFP, PMSF, chicken ovomucoid, soybean trypsin inhibitor, TPCK, TLCK, iodoacetamide, NEM and pepstatin. After incubation samples that had been dissolved by 10 min boiling with SDS were subjected to SDS gel electrophoresis [14,15]. After separation, proteins from individual discs were eluted from their gels and their amount was measured [16]. In some experiments 2-dimensional polyacrylamide gel electrophoresis was performed with isoelectric focusing in the first dimension [17]. Purified α-actinin from rat skeletal muscle [18] was used as exogenous substrate during incubation of myofibrils. The total amount of protein in myofibrils was estimated by the biuret method [19] and the amount of material soluble in trichloroacetic acid by the method of Lowry et al. [20]. All procedures were exactly quantitative [13].

DFP, PMSF, chicken ovomucoid, pepstatin and soybean trypsin inhibitor were purchased from Sigma (St Louis), TPCK and TLCK from Serva, iodoacetamide from Koch Light, NEM from Merck. All other reagents were of analytical grade.

[+] To whom correspondence should be addressed

Abbreviations: CAF, Ca^{2+}-activated protease (EC 3.4.22); MRC, myosin heavy chains; DFP, diisopropyl fluorophosphate; PMSF, phenylmethyl sulfonylfluoride; TPCK, L-1-chloro-3-[4-tosylamido]-4-phenyl-2-butanone; TLCK, L-1-chloro-3-[4-tosylamido]-7-amino-2-heptanone; NEM, *N*-ethylmaleimide

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

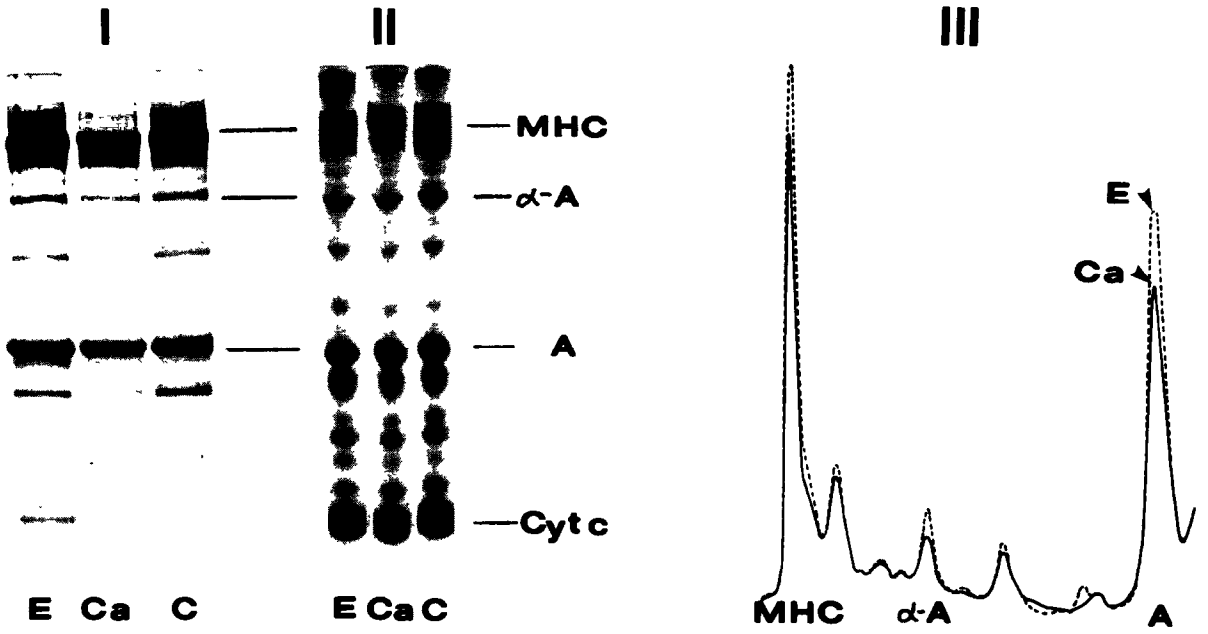

Fig.1. Myofibrils after autolysis in the presence of $CaCl_2$ (Ca) or EDTA (E), control myofibrils without incubation (C). SDS-gel electrophoresis according to (I) Laemmli [15] and (II) Weber and Osborn [14], (III) gel profile of II. α-Actinin (α-A), myosin heavy chain (MHC), actin (A), quantitative standard; cytochrome *c* (Cyt c).

3. RESULTS

Myofibrillar proteins separated by SDS-polyacrylamide gel electrophoresis [14,15] are shown in fig.1. The protein band of about 92 kDa corresponding to α-actinin represents only this protein as shown in 2-dimensional gel electrophoresis (fig.2). During incubation of both crude and purified myofibrils autolytic degradation of proteins was more pronounced in the presence than in the absence of Ca^{2+} (table 1). Simultaneous splitting of α-actinin, MHC, actin and other myofibrillar proteins was observed (fig.1). The rates of degradation of α-actinin, MHC and actin were very similar (table 2). In the absence of Ca^{2+} these proteins were hardly degraded (table 2). Similar Ca^{2+}-sensitive degradation of α-actinin was also observed when this protein was used as an exogenous substrate. During 48 h incubation almost all added α-actinin was degraded by purified myofibrils (fig.3). Degradation of α-actinin, MHC and actin was diminished by several inhibitors of proteolytic enzymes (table 3). The effect of thiol protease inhibitors was strongest. Several inhibitors of both trypsin- and chymotrypsin-like proteases also inhibited the splitting of myofibrillar proteins considerably in the presence of Ca^{2+}. Pepstatin, an inhibitor of carboxyl proteases, has no influence on this degradation (tabe 3), suggesting lack of cathepsin D activity.

Fig.2. Two-dimensional gel electrophoresis of purified myofibrils. α-Actinin (α-A).

Table 1

Content of protein and trichloroacetic acid-soluble products in myofibrils after autolysis

	Control	Autolysis	
		1 mM EDTA	2 mM $CaCl_2$
Protein (mg/g wet muscle wt)	218	180	143
Trichloroacetic acid-soluble products[a] (μg/mg total protein)	2.1	7.8	17.8

[a] Myofibrils after autolysis and myofibrils without incubation (control) were centrifuged for 10 min at 600 × *g*. The supernatant was mixed with 1 vol. of 10% cold trichloroacetic acid and after centrifugation for 10 min at 1000 × *g* the content of trichloroacetic acid-soluble products were measured [20]

4. DISCUSSION

As shown above, Ca^{2+} is necessary for degradation of α-actinin, MHC and actin during autolysis of myofibrils (table 2, fig.1). The effect of protease inhibitors (table 3) points to the action of thiol and serine proteases during this degradation and suggests the complex character of this process. The action of thiol proteases could be rather important for the degradation of α-actinin (table 3). Among the known muscle thiol endopeptidases, CAF seems to be responsible for proteolysis under the conditions used. Calcium dependence, total inhibition by EDTA (table 2) and the activity of this enzyme at neutral pH [1] strongly support this suggestion. The degradation of α-actinin by muscle cathepsins B, L and H [11,12,21,22] seems impossible. The above-mentioned cathepsins are active in the presence of EDTA, not activated by Ca^{2+}, and an acid pH is optimal for their activity. α-Actinin is not degraded by cathepsin B [11], and the effect of cathepsin H is unknown. Only cathepsin L splits α-actinin within a very low pH range [23]. This enzyme is, however, very susceptible to alkaline pH medium [21] and should be inactivated under the conditions used for homogenization.

As shown here α-actinin, MHC and actin are split to a similar degree during autolysis of myofibrils, in spite of their very different susceptibilities to cleavage by proteolytic enzymes [1,9–12], suggesting that the same factor is a requisite for the splitting of all these proteins. For example, splitting of α-actinin, or another myofibrillar protein, following the CAF action could be a 'switching on' factor. It is known that myofibrillar α-actinin, but not purified α-actinin,

Table 2

Content of proteins in myofibrils after autolysis expressed as % of control value

Autolysis	α-Actinin		Actin		Myosin	
	%	*N* (*n*)	%	*N* (*n*)	%	*N* (*n*)
EDTA (1 mM)[a]	100	8 (4) n.s.	98	8 (5) n.s.	99	8 (4) n.s.
$CaCl_2$ (2 mM)[b]	72	18 (0) $p < 0.001$	78	18 (0) $p < 0.001$	67	18 (0) $p < 0.001$

Amount of individual proteins eluted from gels and measured [16]. Protein content of discs corresponding to individual myofibrillar protein compared in pairs. Samples from myofibrils incubated with EDTA compared with controls, samples incubated in the presence of Ca^{2+} compared with those incubated with EDTA. The results were statistically evaluated by sign test [26]. *N*, number of pairs used for comparison; *n*, number of pairs in which content of protein was higher: [a] in control samples vs those incubated with EDTA, [b] in samples incubated with Ca^{2+} vs those incubated with EDTA

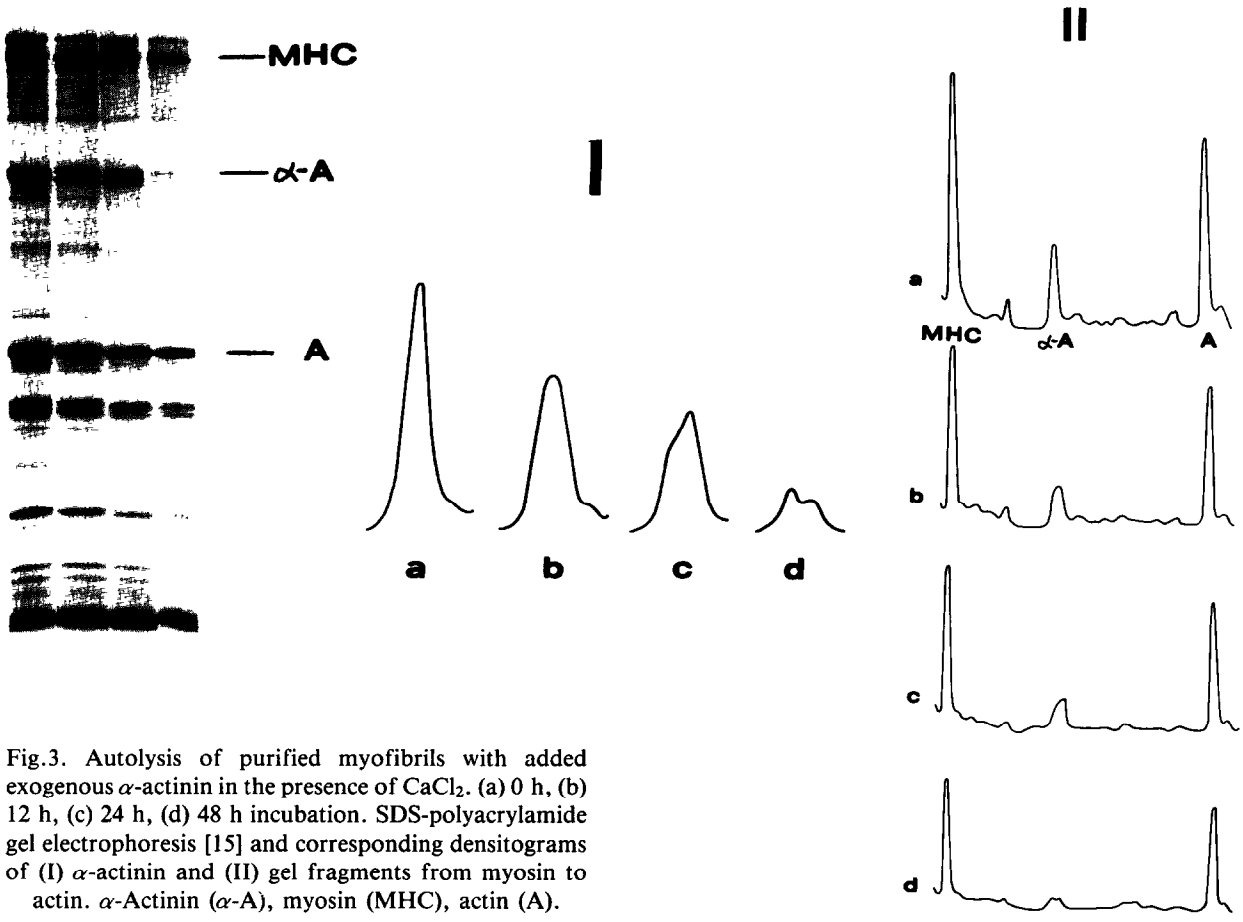

Fig.3. Autolysis of purified myofibrils with added exogenous α-actinin in the presence of $CaCl_2$. (a) 0 h, (b) 12 h, (c) 24 h, (d) 48 h incubation. SDS-polyacrylamide gel electrophoresis [15] and corresponding densitograms of (I) α-actinin and (II) gel fragments from myosin to actin. α-Actinin (α-A), myosin (MHC), actin (A).

Table 3

Influence of protease inhibitors on degradation of myofibrillar proteins

Inhibitor	Final concentration	α-Actinin (% of activity)	Actin (% of activity)	Myosin (% of activity)
–		100	100	100
Iodoacetamide	10^{-3} M	30	38	42
NEM	10^{-3} M	3	38	13
DFP	2×10^{-4} M	58	66	65
Soya bean trypsin inhibitor	10 μg/mg	46	72	74
Ovomucoid	10 μg/mg	53	56	66
TLCK	10^{-3} M	38	53	60
TPCK	10^{-3} M	22	29	16
PMSF	10^{-3} M	107	107	113
Pepstatin	2×10^{-5} M	100	102	100

100% activity = amount of degraded proteins during incubation with Ca^{2+} without inhibitor

may be degraded by CAF [24,25]. Further degradation of myofibrillar proteins could be the result of serine protease(s) action. On the other hand, Ca^{2+}, in the used concentration, could influence myofibrillar proteins, making them susceptible to proteolytic cleavage. These problems are under investigation.

ACKNOWLEDGEMENTS

The authors wish to thank Dr J.V. Small and Dr B. Dahlmann for critical reading of the manuscript and for advice.

REFERENCES

[1] Dayton, W.R., Reville, W.J., Goll, D.E. and Stromer, M.H. (1976) Biochemistry 15, 2159–2167.
[2] Bush, W.A., Stromer, M.H., Goll, D.E. and Suzuki, A. (1972) J. Cell Biol. 52, 367–381.
[3] McNaughtan, A.F. (1978) J. Anat. 126, 7–20.
[4] Ishiura, S. (1981) Life Sci. 29, 1079–1087.
[5] Ishiura, S., Sugita, H., Nonaka, I. and Imahori, K. (1980) J. Biochem. 87, 343–346.
[6] Reddy, M.K., Etlinger, J.D., Rabinowitz, M., Fischmann, D.A. and Zak, R. (1975) J. Biol. Chem. 250, 4278–4284.
[7] Suzuki, A., Nonami, Y. and Goll, D.E. (1975) Agric. Biol. Chem. 39, 1461–1467.
[8] Koizumi, T. (1974) J. Biochem. 76, 431–439.
[9] Pemrick, S.M. (1981) Biochem. Biophys. Res. Commun. 102, 877–884.
[10] Yosogawa, N., Sanada, Y. and Katunuma, N. (1978) J. Biochem. 83, 1355–1360.
[11] Noda, T., Isogai, K., Hayashi, H. and Katunuma, N. (1981) J. Biochem. 90, 371–379.
[12] Schwartz, W. and Bird, J.W. (1977) Biochem. J. 167, 811–820.
[13] Jakubiec-Puka, A., Kulesza-Lipka, D. and Krajewski, K. (1981) Cell Tissue Res. 220, 651–663.
[14] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[15] Laemmli, U.K. (1970) Nature 227, 680–685.
[16] Jakubiec-Puka, A., Kulesza-Lipka, D. and Kordowska, J. (1982) Cell Tissue Res. 227, 641–650.
[17] O'Farrel, P.H. (1975) J. Biol. Chem. 72, 248–254.
[18] Langer, B.G. and Pepe, F.A. (1980) J. Biol. Chem. 255, 5429–5434.
[19] Gornall, A.G., Bardawill, C.J. and Dawid, M.M. (1949) J. Biol. Chem. 177, 751–766.
[20] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[21] Okitani, A., Matsukura, U., Kato, H. and Fujimaki, M. (1980) J. Biochem. 87, 1133–1143.
[22] Okitani, A., Nishimura, T. and Kato, H. (1981) Eur. J. Biochem. 115, 269–274.
[23] Matsukura, U., Okitani, A., Nishimuro, T. and Kato, H. (1981) Biochim. Biophys. Acta 662, 41–47.
[24] Dayton, W.R. and Schollmeyer, J.V. (1980) J. Mol. Cell. Cardiol. 12, 533–551.
[25] Ishiura, S., Sugita, H., Suzuki, K. and Imahori, K. (1979) J. Biochem. 86, 579–581.
[26] Conover, W.J. (1971) in: Practical Nonparametric Statistics, Wiley, New York.