THE AMINO ACID SEQUENCE OF ACTIN FROM CHICKEN SKELETAL MUSCLE ACTIN AND CHICKEN GIZZARD SMOOTH MUSCLE ACTIN

Joël VANDEKERCKHOVE* and Klaus WEBER
Max-Planck-Institute for Biophysical Chemistry, Postfach 968, D-3400 Göttingen, FRG

Received 28 March 1979

1. Introduction

There is now firm evidence from direct amino acid sequence data that higher mammals express at least 6 different actins in a tissue specific manner. These actins are: two cytoplasmic actins typical of non-muscle tissue; two smooth muscle actins and two striated muscle actins, i.e., cardiac muscle actin and skeletal muscle actin [1]. Complete amino acid sequences are currently available only for rabbit skeletal muscle actin [2] and for the two mammalian cytoplasmic actins [3]. Sequence data on smooth muscle actins however are still incomplete [1,4]. Among the smooth muscle tissues currently studied (see [1]), chicken gizzard has a rather simple actin composition with one type of smooth muscle actin (γ-like) expressed preferentially [5–7].

Here we complete our studies in [1,4] and show that the major chicken gizzard actin polypeptide differs by 6 conservative amino acid replacements from rabbit skeletal muscle actin taken as reference. That these 6 amino acid replacements reflect tissue rather than species specificity is documented by the fact, also reported here, that a complete screening of chicken skeletal muscle actin revealed no amino acid replacements by comparison to rabbit skeletal muscle actin. We also discuss the possible evolutionary relationship of different muscle tissue specific actins.

* Present address: Laboratorium voor Histologie en Genetika, State University of Ghent, Ledeganckstraat, 35, B-9000 Ghent, Belgium

2. Materials and methods

Actins were purified from acetone powders of chicken breast muscle and chicken gizzards using standard procedures [8]. In agreement with previous isoelectric focusing studies, chicken skeletal muscle actin revealed the typical α-species and chicken gizzard actin showed as major product the typical γ-like species [5–7].

All relevant procedures used in the screening of the complete amino acid sequence of the actins have been documented in detail ([1,3], J.V., K.W., in preparation). Briefly, actins are processed through performic acid oxidation followed by digestion with trypsin. The mixture of soluble tryptic peptides was further cleaved with the glutamoyl-specific protease of *Staphylococcus aureus* and the insoluble tryptic 'core' peptides were further hydrolyzed with thermolysin. The resulting two sets of fully soluble and relatively small peptides (average size 6.5 residues) were resolved in individual peptides by a preparative two-dimensional fingerprint system as in [3]. Peptides were detected with a dilute fluorescamine stain [9] and aliquots hydrolyzed. All peptides were characterized for total charge according to [10]. Peptides were positioned in the actin polypeptide chain by homology with the rabbit skeletal muscle actin sequence [2]. The exact location of the amino acid exchanges was obtained either by direct sequencing using the dansyl-Edman degradation procedure or by cleaving the primary peptide with a second protease followed by sequencing of the secondary peptide which covered the amino acid exchange.

The screening procedure outlined above covers almost the complete actin polypeptide chain. The

peptide comprising the 3 N-terminal residues of chicken gizzard actin and the peptide comprising the 4 N-terminal residues of chicken skeletal muscle actin, which form a cluster of very acidic residues [1,3,4] migrate from the paper under the electrophoretic conditions used for optimal separation of the other peptides. These residues can however be studied easily as a part of the N-terminal tryptic peptide covering the 17 (gizzard) or 18 (skeletal muscle) N-terminal residues [1].

3. Results

3.1. *Chicken gizzard smooth muscle actin*

Table 1 shows the amino acid compositions of those peptides from chicken gizzard actin which differ in composition from the corresponding rabbit skeletal muscle actin peptides [2,3]. The positioning of the amino acid substitutions were made as follows:

Peptide 2–18: The sequence of this peptide was documented in detail [1]. It is: X–Glu–Glu–Glu–Thr–Thr–Ala–Leu–Val–Cys–Asp–Asn–Gly–Ser–Gly–Leu–Cys–Lys (X stands for a N-terminal blocking group which is most likely, as in other actins, an acetyl group; see [2,11]).

Peptide 85–91: This peptide was isolated in relatively low yield as a peptide containing a performic acid oxidized tryptophan moiety. In addition the same amino acid exchange was covered by a chymotryptic peptide obtained in high yield. This peptide covered residues 87–91 and showed the following composition: Ser, 0.91; Tyr, 0.81; Phe, 1.00; His, 1.68. The threonine (skeletal muscle actin) to serine (gizzard actin) exchange was located at position 89 by direct sequence analysis.

Peptide 291–311: Since this peptide was too long for complete direct sequencing using the dansyl-Edman

Table 1
Amino acid compositions of chicken smooth muscle actin peptides, which show an amino acid exchange when compared with the corresponding peptides of chicken muscle actin

Amino acids	Chicken gizzard smooth muscle actin				Chicken skeletal muscle actin			
	Peptide composition (mol amino acid/mol peptide)							
	2–18	85–91	291–311	356–358	1–18	85–91	291–311	356–358
Cysteic acid	1.9				1.0			
Aspartic acid	2.0		3.8		3.9		4.1	
Methionine sulfone			1.0				1.9	
Threonine	1.8		1.8		1.9	1.0	1.8	1.0
Serine	0.9	0.9	0.9	1.0	1.0		0.8	
Glutamic acid	2.9		0.1		1.8		0.2	
Proline			1.1				1.0	
Glycine	1.9		3.2		2.2		3.1	
Alanine	1.0		2.0		1.0		2.0	
Valine	1.0		0.8		2.1		1.0	
Isoleucine		0.8	0.9	1.0		0.8	1.0	1.0
Leucine	1.8		1.9		1.9		0.8	
Tyrosine		0.8	1.8			0.7	1.7	
Phenylalanine		1.0				1.0		
Lysine	0.9		0.1	0.9	0.9			1.0
Histidine		1.8				1.7		
Arginine			1.1				1.1	
Tryptophan		+				+		

Peptides are indicated by the position of the amino acid residues in the actin polypeptide chain using rabbit skeletal muscle actin [2] as a reference. Amino acid differences between chicken gizzard and skeletal muscle actin are underlined. The presence of tryptophan in the performic acid oxidized peptides was deduced from the fluorescence of the spots under ultraviolet light (+) (detailed in [3])

procedure, it was further digested with chymotrypsin. The methionine to leucine exchange was located in the secondary chymotryptic peptide covering residues 294–299 (Asp, 2.11; Ala, 1.00; Val, 0.94; Leu, 0.97). The peptide was fully sequenced as Ala–Asx–Asx–Val–Leu, with the two Asx residues being asparagine, since the peptide behaves neutral at pH 6.5. The methionine (skeletal muscle actin) to leucine (gizzard actin) substitution is therefore placed at position 298.

Peptide 356–358: The direct sequence of this peptide (Ile–Ser–Lys) reveals the exact position of the threonine (skeletal muscle actin) to serine (gizzard actin) exchange at position 357.

A summary of the 6 amino acid exchanges which distinguish chicken gizzard actin from the skeletal muscle actins is presented in table 2.

3.2. *Chicken skeletal muscle actin*

The N-terminal tryptic peptide containing residues 1–18 was sequenced completely in [1]. In addition the complete actin polypeptide has now been screened by amino acid analysis of the *Staphylococcus aureus* protease and thermolysine peptides as in section 2. Using this approach no amino acid differences were observed between skeletal muscle actins from chicken and rabbit.

Table 1 lists only those peptides of chicken skeletal muscle actin, which are of special interest, because they comprise those regions where chicken gizzard smooth muscle actin shows amino acid replacements (see above).

4. Discussion

Our amino acid sequence results on chicken breast muscle and chicken gizzard muscle extend our knowledge about actin divergence during evolution and muscle differentiation in the following aspects.

(1) The full amino acid sequence of chicken skeletal muscle revealed no amino acid substitution by comparison to rabbit skeletal muscle actin, emphasizing the potential lack of species specificity of actins among higher vertebrates also implied [3] in the case of several mammalian cytoplasmic (i.e., non-muscle) actins.

(2) The complete amino acid sequence of the major gizzard actin provides the first sequence for a smooth muscle actin and clearly emphasizes that smooth muscle actins are very closely related to skeletal muscle actin (6 replacements; see table 2) and quite different from cytoplasmic actins (18 replacements; see [3]). In addition the data prove our hypothesis in [1,4] that the close similarity of gizzard actin and γ-cytoplasmic

Table 2
Summary table of the amino acid differences between chicken gizzard smooth muscle actin, skeletal muscle actin and bovine cardiac actin

Actin type	Residue number						
	1	2	3	17	89	298	357
Skeletal muscle actin (rabbit, bovine, chicken)	Asp	Glu	Asp	Val	Thr	Met	Thr
Cardiac muscle actin (bovine)		Asp	Glu			Leu	Ser
Smooth muscle actin (chicken gizzard)	absent		Glu	Cys	Ser	Leu	Ser

The reference sequence is that of rabbit skeletal muscle actin [2] with the minor modifications proposed in [3]. Residues which are not given in the table are the same as in rabbit skeletal muscle actin [2,3]. Data on the two other skeletal muscle actins are from this study or [1,4]. Data on cardiac actin are taken from [1,4]. The chicken gizzard smooth muscle actin is shorter by the very first N-terminal residues. In order to align this actin with other muscle actins, chicken gizzard actin starts with residue 2 rather than with residue 1 (see [1,4]). The amino acid exchange at position 357 in partial amino acid sequence studies on human and bovine heart actin has been reported [12]

actin deduced from isoelectric focusing studies [5–7] is purely fortuitous and is due to the sequence identity in the amino-terminal 4 amino acid residues rather than the 2 actins being coded for by the same gene. This result cautions against attempts to prove actin relationship and identities solely on grounds of isoelectric focusing studies (detailed in [1]).

(3) Our results together with studies on cytoplasmic actins, heart muscle actins and skeletal muscle actins ([3,12], J.V., K.W., in preparation) establish that actins from the various differentiated muscle tissues are more closely related to each other than they are to cytoplasmic actin. The number of amino acid exchanges indicates that skeletal muscle actin is the furthest removed actin species and that smooth muscle actin is still the closest to cytoplasmic actin. Heart muscle actin lies between smooth and skeletal muscle actin. This evolutionary relation of the various muscle actins is indicated by the amino acid exchange patterns in positions 17, 298 and 357 (table 2) in which there is a various degree of coincidence with cytoplasmic actins. As argued previously the amino acid exchanges in residues 1–6 are too difficult to evaluate in this respect. The unique change in position 89 typical for gizzard actin but also found in preliminary studies of the major smooth muscle actin from bovine aorta (J.V., K.W., unpublished observation) could indicate an evolutionary drift of smooth muscles independent of the other actin evolution.

Acknowledgements

J. Vandekerckhove is Bevoegdverklaard navorser at the Belgian National Fund for Scientific Research (NFWO).

References

[1] Vandekerckhove, J. and Weber, K. (1978) J. Mol. Biol. 126, 783–802.
[2] Collins, J. H. and Elzinga, M. (1975) J. Biol. Chem. 250, 5915–5920.
[3] Vandekerckhove, J. and Weber, K. (1978) Eur. J. Biochem. 90, 451–462.
[4] Vandekerckhove, J. and Weber, K. (1978) Proc. Natl. Acad. Sci. USA 75, 1106–1110.
[5] Rubenstein, P. A. and Spudich, J. A. (1977) Proc. Natl. Acad. Sci. USA 74, 120–123.
[6] Izant, J. G. and Lazarides, E. (1977) Proc. Natl. Acad. Sci. USA 74, 1450–1454.
[7] Zechel, K. and Weber, K. (1978) Eur. J. Biochem. 89, 105–112.
[8] Spudich, J. A. and Watt, S. (1971) J. Biol. Chem. 246, 1866–1871.
[9] Vandekerckhove, J. and Van Montagu, M. (1974) Eur. J. Biochem. 44, 279–288.
[10] Offord, R. E. (1966) Nature 211, 591–593.
[11] Gaetjens, E. and Bárány, M. (1966) Biochim. Biophys. Acta 117, 176–183.
[12] Lu, R. C. and Elzinga, M. (1977) Biochemistry 16, 5801–5806.