PHYLOGENETIC DIVERSITY OF TROPONIN SUBUNIT-C AMINO ACID COMPOSITION

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1. Introduction

Troponin, a muscle regulatory complex located on thin filaments, consists of 3 protein subunits [1]. One of these, troponin-C, has M_r 18 000 and binds Ca^{2+} [1]. Vertebrate troponin-C is a very acidic protein having a characteristically high content of aspartic and glutamic acid residues [2]. In [3] we found the apparent acidity of scallop striated muscle troponin-C to be lower than that of its vertebrate counterpart, indicating that troponin-C may not be a highly conserved protein. Here, we isolated troponins-C from several phyla and compared their amino acid composition and electrophoretic mobility, to test for possible phylogenetic diversity.

2. Materials and methods

Troponins-C were prepared from rabbit striated muscle, from striated muscle of several arthropods including the rock crab *Cancer irroratus*, the lobster *Homarus americanus*, and the horseshoe crab *Limulus polyphemus*, from body-wall muscle of several marine worms, including two annelids, the blood worm *Glycera dibranchiata* and the rag worm *Nereis virens*, the sipunculid *Golfingia gouldi*, and the nemertine worm, *Cerebratulus lacteus*, as well as from the translucent adductor muscle of the oyster *Crassostrea virginica*. Rabbit and arthropod troponins-C were prepared from purified troponin as in [1] and [4], respectively. All other troponins-C were isolated from native thin filaments as in [3].

The purity and electrophoretic mobility of the troponins-C were ascertained by SDS– and alkaline–urea polyacrylamide gel electrophoresis as in [5] and [6], respectively (fig.1,2). Amino acid composition of 24 h hydrolysates were determined using a Beckman 119C1 amino acid analyzer.

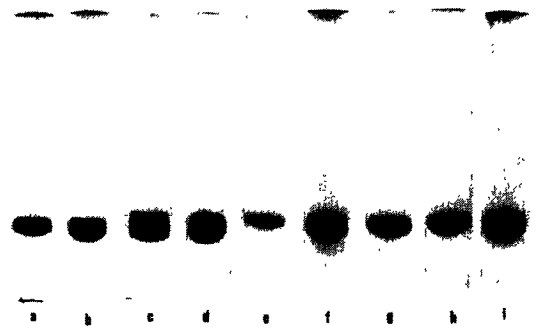

Fig.1. SDS–polyacrylamide gel electrophoresis of troponin-C preparations. Gels (10% acrylamide) were stained with Coomassie brilliant blue R. Source of troponin-C: (a) rabbit; (b) *Limulus*; (c) *Cancer*; (d) *Homarus*; (e) *Nereis*; (f) *Glycera*; (g) *Golfingia*; (h) *Crassostrea*; (i) *Cerebratulus*.

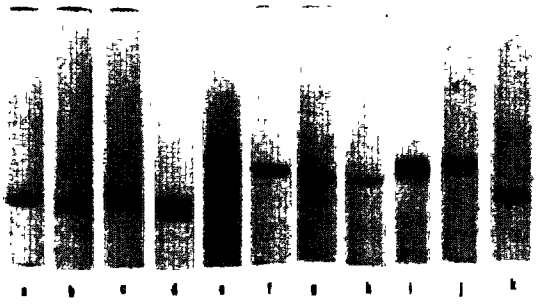

Fig.2. Alkaline 6 M urea–polyacrylamide-gel electrophoresis of troponin-C preparations. Gels (8% acrylamide) were stained with Coomassie brilliant blue R; all gel samples used contain 5 mM EGTA. Source of troponin-C; (a) rabbit; (b) *Limulus*; (c) *Cancer*; (d) *Homarus*; (e) *Nereis*; (f) *Glycera*; (g) *Golfingia*; (h) *Crassostrea*; (i) *Aequipecten*; (j) *Cerebratulus*; (k) rabbit. Two types of migration patterns are observed; troponins-C from higher animals (a–d,k) migrate more rapidly than those from lower invertebrates (e–j). *Homarus* troponin-C (d) consistently shows 3 closely spaced bands on this gel system.

Table 1
mol amino acid/mol troponin-C

Amino acid	Source of troponin-C							
	Rabbit[a]	*Limulus* (2)	*Cancer* (2)	*Homarus* (2)	*Glycera* (5)	*Aequipecten*[b]	*Crassostrea* (4)	*Cerebratulus* (2)
Asx	23	23.1	23.7	25.6	28.4	23.0	26.8	29.0
Thr	5	9.9	8.4	9.5	5.5	7.1	9.7	6.9
Ser	7	8.5	7.4	8.2	7.6	8.7	10.3	8.6
Glx	31	31.0	27.1	29.1	30.4	26.9	30.6	23.9
Pro	1	0.7	2.9	1.0	1.3	2.2	1.7	1.9
Gly	13	12.5	14.9	12.8	11.9	11.8	12.8	12.0
Ala	13	11.4	10.2	10.8	10.3	10.8	8.8	9.5
Val	7	6.3	6.7	8.4	5.6	10.4	4.5	6.4
Cys	1	0.7	0.7	0.8	0.4	1.1	–	–
Met	10	6.8	5.6	4.6	5.7	3.9	4.6	6.0
Ile	10	8.1	10.9	9.6	6.8	7.0	4.9	7.6
Leu	9	15.6	16.3	15.0	13.6	16.0	14.1	14.1
Tyr	2	2.1	2.4	1.9	1.9	1.6	1.4	2.3
Phe	10	8.7	8.8	9.6	6.2	7.3	8.5	7.1
Lys	9	6.3	8.8	7.4	16.3	16.2	12.7	18.1
His	1	0.8	0.3	1.2	0.7	0.7	1.9	0.4
Arg	7	7.7	6.5	5.6	6.3	6.1	6.5	4.6
$\frac{\text{Asx + Glx}}{\text{Lys + Arg}}$	3.4	3.9	3.3	4.2	2.6	2.2	3.0	2.3

[a] From sequence data in [7]; [b] From [3]; Values in parentheses indicate number of preparations analyzed

3. Results and discussion

Amino acid composition of a number of troponins-C are given in table 1. In common with vertebrate troponin-C, the troponins-C analyzed have a high Phe/Tyr ratio and a low proline, cysteine, and histidine content, however the Asx + Glx/Lys + Arg ratio for the troponins-C vary substantially. This ratio in vertebrates and in higher invertebrates (arthropods) is >3.0, whereas in other invertebrates tested the ratio is ≤3.0. To a large extent, this difference reflects the increased lysine content in the lower invertebrate troponins-C, and suggests that these proteins may be less acidic than the troponin-C of higher animals. Since the values for asparagine and glutamine have not been determined, we cannot calculate the Asp + Glu/Lys + Arg ratio directly. However, the apparent difference in acidity is confirmed by alkaline-urea gel electrophoresis (fig.2), showing rabbit and arthropod troponins-C migrating more rapidly than the lower invertebrate troponins-C.

This dissimilarity in amino acid composition may give rise to different Ca^{2+}-binding or subunit interaction properties of the different troponins-C. However, the significance of these results obviously cannot be deduced from amino acid composition data alone. Information regarding the amino acid sequence of the various troponins-C and their structures will give further insight into the evolution of the protein.

Acknowledgements

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