Biological activities of bovine cardiac-muscle troponin C C-terminal peptide (residues 84–161)

Nataliya V. BARSKAYA and Nikolai B. GUSEV

Department of Biochemistry, School of Biology, M. V. Lomonosov State University, Moscow 117234, U.S.S.R.

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1. Bovine cardiac-muscle troponin C was digested at cysteine residues 35 and 84, and the C-terminal peptide (residues 84–161) was isolated. 2. The C-terminal peptide contains two Ca^{2+} -binding sites. These sites bind Ca^{2+} with a binding constant of $2.0 \times 10^8 \text{ M}^{-1}$. In the presence of 2 mM-Mg^{2+} the binding constant for Ca^{2+} is decreased to $3.7 \times 10^7 \text{ M}^{-1}$. The corresponding constants for native troponin C are $5.9 \times 10^7 \text{ M}^{-1}$ and $2.9 \times 10^7 \text{ M}^{-1}$ respectively. 3. Electrophoretic mobility of the C-terminal peptide is increased in the presence of 0.1 mM-CaCl_2 as compared with the mobility in the presence of 2 mM-EDTA . The same phenomenon was observed when electrophoresis was performed in the presence of 6 M-urea or 0.1% sodium dodecyl sulphate. 4. When saturated with Ca^{2+} , the C-terminal peptide forms complexes with bovine cardiac-muscle troponin I both in the absence and in the presence of 6 M-urea . This complex is dissociated on removal of Ca^{2+} . 5. The data suggest that the C-terminal peptide of troponin C contains two $\text{Ca}^{2+}/\text{Mg}^{2+}$ -binding sites and interacts with troponin I. Thus, despite the 30% difference in amino acid composition, the properties of bovine cardiac-muscle troponin C C-terminal peptide are similar to those of rabbit skeletal-muscle troponin C C-terminal peptide.

The contractility of skeletal and cardiac muscles is controlled by the troponin–tropomyosin complex (Perry, 1979; Adelstein & Eisenberg, 1980). The interaction of actin with myosin occurs only after saturation of definite troponin C Ca^{2+} -binding sites. The Ca^{2+} -binding properties of skeletal-muscle troponin C have been thoroughly investigated. Skeletal-muscle troponin C contains four Ca^{2+} -binding sites (Potter & Gergely, 1975). Two sites located in the N-terminal half of the protein molecule specifically bind Ca^{2+} (Leavis *et al.*, 1978; Nagy *et al.*, 1978), and two other sites located in the C-terminal half of troponin C bind both Mg^{2+} and Ca^{2+} (Leavis *et al.*, 1978). The Ca^{2+} -binding properties of cardiac-muscle troponin C have been investigated to a far smaller extent. Until recently the number and the cation-binding properties of Ca^{2+} -binding sites in cardiac-muscle troponin C were a matter of speculation (Burtnick & Kay, 1977; Stull & Buss, 1978; Kohama, 1979). However, Leavis & Kraft (1978) and Holroyde *et al.* (1980) postulated that cardiac-muscle troponin C contains two $\text{Ca}^{2+}/\text{Mg}^{2+}$ -binding sites and one Ca^{2+} -specific binding site. The considerable degree of homology of primary structure of

troponins C from different sources led Holroyde *et al.* (1980) to propose the hypothesis that two $\text{Ca}^{2+}/\text{Mg}^{2+}$ -binding sites are located in the C-terminal part of the protein molecule.

The aim of present work was to investigate the cation-binding properties of cardiac-muscle troponin C C-terminal peptide (residues 84–161) and the possible interaction of other troponin components with this part of the troponin C molecule.

Materials and methods

Bovine cardiac-muscle troponin was isolated by the method of Tsukui & Ebashi (1973), with some modifications (Barskaya & Gusev, 1981). The troponin components were isolated by ion-exchange chromatography in the presence of 8 M-urea (Perry & Cole, 1974; Wilkinson, 1974; Barskaya & Gusev, 1981). Bovine brain calmodulin was kindly supplied by Dr. V. A. Tkachuk (Department of Biochemistry, School of Biology, M. V. Lomonosov State University).

The cleavage of troponin C at cysteine residues was performed by the methods of Jacobson *et al.* (1973) and Degani & Patchornik (1974). Troponin C (2 mg/ml) dissolved in buffer A (40 mM-Tris/HCl

Abbreviation used: SDS, sodium dodecyl sulphate.

buffer, pH 8.0, containing 6M-urea and 2mM-EDTA) was reduced with dithiothreitol at a final concentration of 0.3 mM (30 min, 37°C). Then a solution of 5,5'-dithiobis-(2-nitrobenzoic acid) in buffer A was added to the protein [final concentration of 5,5'-dithiobis-(2-nitrobenzoic acid) 2 mM]. The mixture was incubated for 30 min at 37°C. An aqueous solution of KCN, to give a final concentration of 40 mM, was added to the modified protein, and the mixture was incubated overnight at 37°C. The peptides were desalted on a column of Sephadex G-50 (superfine grade) equilibrated with 50 mM-NH₄HCO₃. The peptide mixture obtained was concentrated in a rotor evaporator and was then subjected to chromatography on a column (2.6 cm × 90 cm) of Sephadex G-50 (superfine grade). Eight fractions were collected after gel filtration of the peptide mixture (Fig. 1); the final, eighth, fraction contained no peptide material and was strongly retarded on the column, presumably owing to the presence of degradation products of 5,5'-dithiobis-(2-nitrobenzoic acid). Further purification of the fourth and seventh fractions was achieved by chromatography on a column (1.6 cm × 90 cm) of Sephadex G-50 (superfine grade) in 10 mM-HCl. The amino acid composition was determined with an LKB 3201 amino acid analyser after peptide hydrolysis with 6M-HCl *in vacuo* (110°C, 24 h).

SDS/polyacrylamide-gel electrophoresis was performed in a Weber & Osborn (1969) system. The electrode buffers and gels contained either 0.1 mM-CaCl₂ or 2 mM-EDTA. The samples were dissolved in a buffer containing 1% (w/v) SDS and 1% (v/v) mercaptoethanol and were heated in a boiling-water bath for 2 min.

Urea/polyacrylamide-gel electrophoresis was performed by the method of Schibeci & Martonosi (1980) with the aim of direct determination of ⁴⁵Ca²⁺ binding to troponin C and its peptide. After electrophoresis the cylindrical gels (diam. 6 mm) were soaked in a 10-fold volume of solution containing 25 mM-Tris/192 mM-glycine buffer, pH 8.3, and 1–2 μCi of ⁴⁵Ca²⁺/l (specific radioactivity 0.8–1.5 μCi/μmol; The Radiochemical Centre, Amersham, Bucks., U.K.) for 3 h. The gels were then washed in a large volume of water for 10–12 h at 3°C and cut into 2 mm-thick discs; each disc was dried on a square (2 cm × 2 cm) of Whatman 3MM paper. The radioactivity of each sample was determined in an Intertechnique SL-4000 liquid-scintillation counter as described previously (Dobrovolskii *et al.*, 1981). The interaction of the peptide with troponin components was studied by polyacrylamide-gel electrophoresis both in the presence and in the absence of 6M-urea (Schaub & Perry, 1969). The electrode buffers and gels contained

Fig. 1. Chromatography of the peptide mixture obtained after hydrolysis of cardiac-muscle troponin C at cysteine residues. The hydrolysate (25 mg) was loaded on a 2.6 cm × 90 cm column of Sephadex G-50 (superfine grade) equilibrated with 50 mM-NH₄HCO₃. The flow rate was 9 ml/h, and the fraction volume was 5 ml. For full experimental details see the text.

either 0.1 mM-CaCl₂ or 2 mM-EDTA. The gels were stained with Coomassie Brilliant Blue R250 as described by Weber & Osborn (1969), and after destaining were scanned either in a Pye Unicam SP.1700 spectrophotometer or in a Carl Zeiss (Jena) ERI-65 M densitometer.

The Ca²⁺-binding properties of troponin C and its peptide were determined by measuring the dependence of tyrosine fluorescence on Ca²⁺ concentration. A solution of troponin C (0.025 mg/ml) or a peptide solution (0.02 mg/ml) in 50 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/KOH buffer, pH 7.0, containing 2 mM-EGTA was titrated with CaCl₂, and tyrosine fluorescence was measured. The concentration of Ca²⁺ was determined in accordance with the Imai & Takeda (1967) formula:

$$\text{pCa} = 6.7 + \log \left(\frac{[\text{EGTA}]}{[\text{Ca}^{2+}]} - 1 \right)$$

When it was impossible to use Ca²⁺/EGTA buffer, the peptide solutions were passed through a Chelex-100 column pre-equilibrated with 50 mM-Hepes buffer, pH 7.0. The fluorescence was excited at 280 nm and measured at 300 nm.

Peptide and protein concentrations were determined as described by Spector (1978), with bovine cardiac-muscle troponin C as a standard.

Results

Cleavage of bovine cardiac-muscle troponin C at cysteine residues

In order to investigate the nature and location of Ca²⁺-binding sites of cardiac-muscle troponin C it seems sensible to isolate peptides containing an unmodified Ca²⁺-binding loop and two α -helices that flank this loop on both sides. Bovine cardiac-muscle troponin C contains two cysteine residues per molecule (van Eerd & Takahashi, 1976). Hence a complete hydrolysis at these residues will produce three peptides. Fig. 2 shows that the first *N*-terminal peptide (residues 1–34) contains only part of the first Ca²⁺-binding loop. Moreover, according to van Eerd & Takahashi (1976), the first potential Ca²⁺-binding site of bovine cardiac-muscle troponin C cannot

bind Ca²⁺ owing to the replacement of certain acidic amino acids involved in Ca²⁺ co-ordination by neutral amino acids. Therefore the *N*-terminal peptide will not bind Ca²⁺. The second peptide (residues 35–83) contains a site that is homologous with the second Ca²⁺-binding site of rabbit skeletal-muscle troponin C. This peptide includes an unaltered Ca²⁺-binding loop and two α -helices located on both sides of this loop. According to the hypothesis proposed by Holroyde *et al.* (1980) this peptide should contain a single Ca²⁺-specific binding site of bovine cardiac-muscle troponin C. And, finally, the third peptide (residues 84–161) contains two sites that are homologous with the third and fourth Ca²⁺-binding sites of rabbit skeletal-muscle troponin C. Hence this peptide should contain two Ca²⁺-binding sites and appears to be large enough to be investigated for interaction with other troponin components.

Isolation of N-terminal (residues 1–34) and C-terminal (residues 84–161) peptides of cardiac-muscle troponin C

As mentioned above, gel filtration of the peptide mixture obtained by cleavage of troponin C at cysteine residues results in seven peptide fractions (Fig. 1). This complicated elution pattern is due to strong interaction between the peptides and to incomplete hydrolysis at both cysteine residues (Degani & Patchornik, 1974). The most-retarded peptide fraction (fraction VII; Fig. 1) was further purified on a Sephadex G-50 column (1.6 cm $\times$ 90 cm) in 10 mM-HCl. A homogeneous peptide obtained by this method (peptide 7-1) has an amino acid composition identical with that of the *N*-terminal peptide (residues 1–34) of cardiac-muscle troponin C (Table 1).

The peptide material eluted with the fourth fraction (Fig. 1) was further purified by a twofold gel chromatography on a Sephadex G-50 column (1.6 cm $\times$ 90 cm) in 10 mM-HCl. The peptide obtained (peptide 4-3-2) was homogeneous by the criterion of SDS/polyacrylamide-gel electrophoresis. The amino acid composition of this peptide is similar to that of the *C*-terminal peptide (residues 84–161) of cardiac-muscle troponin C (Table 1).

Fig. 2. Schematic diagram of cardiac-muscle troponin C

■, Ca²⁺-binding loops proper; ▨, putative flanking α -helices. The four regions are indicated by Roman numerals. Arrows indicate sites of cleavage at cysteine residues 35 and 84. For full explanation see the text.

Table 1. Amino acid compositions of the peptides isolated after hydrolysis of bovine cardiac-muscle troponin C at cysteine residues

Amino acid	Amino acid content (residues/molecule)			
	Peptide 7-1	<i>N</i> -Terminal peptide (residues 1-34)*	Peptide 4-3-2	<i>C</i> -Terminal peptide (residues 84-161)*
Asp	5.0	5	14.5	16
Thr	0.9	1	3.0	3
Ser	0	0	2.5	3
Glu	7.0	7	13.0	13
Gly	2.0	2	6.5	6
Ala	4.5	5	2.9	2
Val	2.0	2	1.3	1
Met	0	0	3.7	5
Ile	2.0	2	5.3	5
Leu	2.1	2	8.1	7
Tyr	1.4	1	1.9	2
Phe	3.5	3	4.3	4
Lys	2.2	3	7.0	8
Arg	0	0	2.2	2

* Based on sequence determined by van Eerd & Takahashi (1976).

Effect of Ca²⁺ on the electrophoretic mobility of the C-terminal peptide of troponin C

SDS/polyacrylamide-gel electrophoresis of the peptide mixture obtained after chemical cleavage of troponin C at cysteine residues in the presence of 2 mM-EDTA produces two protein bands (Fig. 3). The mobility of the first band corresponds to that of non-hydrolysed troponin C. The second band, having a lower molecular weight, probably contains all peptides of troponin C that were badly resolved in 10% polyacrylamide gel. When the electrophoresis was performed in the presence of 0.1 mM-CaCl₂, a splitting of the peptide band took place. In addition to the non-hydrolysed troponin C, two peptide bands appeared on the gel (Fig. 3b). This suggests that the electrophoretic mobility of one (or several) troponin C peptides depends on the degree of their saturation with Ca²⁺. As might be expected, the electrophoretic mobility of the *N*-terminal peptide of troponin C (residues 1-34) was independent of Ca²⁺ concentration. On the other hand, the electrophoretic mobility of the *C*-terminal peptide (residues 84-161) was increased in the presence of Ca²⁺ in comparison with the mobility in the presence of EDTA (Figs. 3c and 3d). Among all Ca²⁺-binding proteins, only bovine brain calmodulin has hitherto been reported to show Ca²⁺-concentration-dependent changes in its electrophoretic mobility on SDS/polyacrylamide-gel electrophoresis (Burgess *et al.*, 1980). This suggests that both *C*-terminal peptide or troponin C and calmodulin retain their Ca²⁺-binding properties even in the presence of SDS.

When the electrophoresis was performed in the

presence of 6 M-urea, the electrophoretic mobility of the *C*-terminal peptide of troponin C in the presence of Ca²⁺ was also increased. In order to obtain direct evidence for Ca²⁺-binding to the *C*-terminal peptide, we used the method of Schibeci & Martonosi (1980). After electrophoresis in the presence of urea the gels were soaked in a solution containing ⁴⁵Ca²⁺, after which the distribution of the label along the gel was determined (see the Materials and methods section). Both native troponin C and its *C*-terminal peptide were able to bind ⁴⁵Ca²⁺ under the given conditions. Thus in the presence of urea or of SDS the *C*-terminal peptide of cardiac troponin C can bind Ca²⁺.

Investigation of the Ca²⁺-binding properties of the C-terminal peptide of troponin C

To determine the Ca²⁺-binding properties of this peptide we measured the dependence of its tyrosine fluorescence on Ca²⁺ concentration. This method seems to be a promising approach, since tyrosine residues 111 and 150 are both located in the third and fourth Ca²⁺-binding loops of the peptide (van Eerd & Takahashi, 1976). When the *C*-terminal peptide freed of Ca²⁺ by passage through a Chelex-100 column was titrated with Ca²⁺, the increase in fluorescence was maximal when 1.8 mol of Ca²⁺/mol of peptide was bound. This indicates that the peptide virtually contains two Ca²⁺-binding sites and that the fluorescence of both tyrosine residues located in different Ca²⁺-binding sites is dependent on Ca²⁺ concentration. A 1.4-1.5-fold increase in intrinsic fluorescence of the *C*-terminal peptide was observed

Fig. 3. SDS/polyacrylamide-gel electrophoresis of the peptide mixture obtained after hydrolysis of cardiac-muscle troponin C at cysteine residues (traces a and b) and of the C-terminal peptide of cardiac-muscle troponin C (traces c and d)

The electrophoresis was performed in the presence of 2 mM-EDTA (traces a and c) or 0.1 mM- CaCl_2 (traces b and d). Arrows indicate the position of Bromophenol Blue. For full experimental details see the text.

Table 2. Determination of Ca^{2+} -binding parameters of the C-terminal peptide and native troponin C from bovine heart muscle

	C-Terminal peptide		Native troponin C	
	- Mg^{2+}	+ Mg^{2+}	- Mg^{2+}	+ Mg^{2+}
Concn. of Ca^{2+} at which half-maximal increase in fluorescence is observed (M)	4.9×10^{-9}	2.7×10^{-8}	1.7×10^{-8}	3.5×10^{-8}
Hill coefficient (<i>h</i>)	0.94	0.85	1.57	0.83

when the Ca^{2+} concentration was increased from pCa 9 to pCa 5 (Fig. 4a). A similar dependence was observed with native troponin C (Fig. 4b). An addition of Mg^{2+} (even in the absence of Ca^{2+}) increases tyrosine fluorescence both of troponin C and of its C-terminal peptide. Moreover, in the presence of Mg^{2+} the titration midpoint is achieved at a higher Ca^{2+} concentration than in the absence of Mg^{2+} (Fig. 4). Data in Table 2 demonstrate that Mg^{2+} competes with Ca^{2+} for the cation-binding sites of the C-terminal peptide. Hence the third and

fourth sites in cardiac-muscle troponin C are represented by the so-called high-affinity $\text{Ca}^{2+}/\text{Mg}^{2+}$ -binding sites. It may be mentioned that independently of the presence of Mg^{2+} there is no co-operativity in Ca^{2+} -binding by the C-terminal peptide of troponin C (the Hill co-efficient, *h*, is equal to 1.0). However, in the absence of Mg^{2+} a positive co-operativity in Ca^{2+} -binding is observed for native troponin C (*h* = 1.6), although an addition of Mg^{2+} abolishes this co-operative effect (Table 2).

Fig. 4. Dependence of the fluorescence of the C-terminal peptide of cardiac-muscle troponin C (a) and of cardiac-muscle troponin C (b) on Ca^{2+} concentration

The fluorescence was excited at 280 nm and registered at 300 nm. The peptide concentration was 0.02 mg/ml and that of troponin C was 0.025 mg/ml. The fluorescence was measured in 50 mM-Hepes buffer, pH 7.0, in the absence (●) and in the presence (▲) of 2 mM- $MgCl_2$. F and F_0 designate the intensity of fluorescence at a given concentration of Ca^{2+} and at $pCa = 9$ respectively. The constant concentration of Ca^{2+} was maintained by adding 2 mM- Ca^{2+} /EGTA buffer. For full experimental details see the text.

Fig. 5. Urea/polyacrylamide-gel electrophoresis of cardiac-muscle troponin I (a and b), of the C-terminal peptide of cardiac-muscle troponin C (c and d) and of the mixture of troponin I and C-terminal peptide of troponin C (e and f). The electrophoresis was performed in the presence of 2 mM-EDTA (a, c and e) or of 0.1 mM- $CaCl_2$ (b, d and f). Protein (30–50 μg) was loaded on the gel. Arrows indicate the position of Bromophenol Blue. For full experimental details see the text.

Interaction of the C-terminal peptide of troponin C with troponin I and troponin T

The interaction of the C-terminal peptide of troponin C with troponins I and T was studied by polyacrylamide-gel electrophoresis in the presence and in the absence of 6M-urea. Independently of Ca^{2+} concentration, bovine cardiac-muscle troponin I does not enter the gel under the given experimental conditions (Figs. 5a and 5b). The C-terminal peptide has a high electrophoretic mobility, which is Ca^{2+} -dependent. As already mentioned above, the mobility of the C-terminal peptide is increased in the presence of Ca^{2+} (Figs. 5c and 5d). When the troponin I/C-terminal peptide mixture was loaded on the gels containing 2mM-EDTA, only a peptide band was produced (Fig. 5e), indicating the absence of complex-formation at low concentrations of Ca^{2+} . In contrast, electrophoresis of the troponin I/C-terminal peptide mixture in the presence of 0.1M- CaCl_2 revealed several bands with rather a low mobility on the top of the gel. Either the intensity of the C-terminal peptide band was decreased or this band disappeared altogether, depending on the molar ratio of troponin I and the C-terminal peptide of troponin C (Fig. 5f). These data indicate that at high concentrations of Ca^{2+} formation of the troponin I-C-terminal peptide of troponin C complex occurs even in the presence of 6M-urea. When the interaction between troponin T and the C-terminal peptide of troponin C was studied, a very faint band (probably representing the troponin T-peptide complex) was observed only under very special conditions. This band, with an electrophoretic mobility lower than that of troponin T, was observed only when electrophoresis was performed at high Ca^{2+} concentrations in the absence of urea. Thus the complex, if any, between troponin T and the C-terminal peptide of troponin C is rather unstable and is formed only under very mild conditions.

Discussion

The results presented above suggest that the C-terminal peptide of cardiac-muscle troponin C contains two $\text{Ca}^{2+}/\text{Mg}^{2+}$ -binding sites. The electrophoretic mobility of the C-terminal peptide is increased in the presence of Ca^{2+} under both non-denaturing and denaturing (6M-urea or 0.1% SDS) conditions. Among all Ca^{2+} -binding proteins previously studied the electrophoretic mobility of bovine brain calmodulin alone is dependent on Ca^{2+} concentration in the presence of SDS (Burgess *et al.*, 1980). The specificity of Ca^{2+} -binding sites of calmodulin is not very well defined. According to Potter *et al.* (1977), all the four sites in calmodulin, having a roughly equal affinity for Ca^{2+} , specifically bind this cation. In contrast, the data of Wolf *et al.* (1977) suggest that Mg^{2+} competes with Ca^{2+} for

the cation-binding sites of calmodulin. If the second viewpoint is correct, one can speculate that all $\text{Ca}^{2+}/\text{Mg}^{2+}$ -binding sites have a very stable structure, which is not destroyed even by high concentrations of denaturing agents. Presumably the C-terminal peptide of rabbit skeletal-muscle troponin C (e.g. peptide TR-2) (Leavis *et al.*, 1978) will share the same property and will bind Ca^{2+} in the presence of SDS.

We cannot, however, exclude another possibility. It is known that calmodulin binds EGTA with a binding constant of about $60 \times 10^{-6}\text{M}$ (Haiech *et al.*, 1980). Presumably the C-terminal peptide of troponin C and calmodulin can bind EDTA with the same high value of the association constant. This binding could also change the electrophoretic mobility of both polypeptides even in the presence of SDS, although this possibility seems to be less probable.

When analysing the Ca^{2+} -binding properties of the C-terminal peptide, it should be emphasized that in the absence of Mg^{2+} the Ca^{2+} -binding constant of this peptide is 3–3.5 times that for native troponin C (Table 2). A similar phenomenon was described for the C-terminal peptide of skeletal-muscle troponin C (Leavis *et al.*, 1978). Therefore a removal of the N-terminal part of troponin C leads to an increase in the affinity of the C-terminal part of the molecule for Ca^{2+} . These findings are indicative of a strong interaction between two pairs of Ca^{2+} -binding sites. According to experimental (Reid *et al.*, 1981) and theoretical (Reid & Hodges, 1980) data the interaction of Ca^{2+} with troponin C starts from saturation of the third and fourth Ca^{2+} -binding sites. Ca^{2+} interaction with these sites causes significant conformational changes. Only as a result of these changes in protein structure does the interaction of Ca^{2+} with the second and first Ca^{2+} -binding sites become possible. If these assumptions are correct, the removal of the N-terminal part of the molecule will make the binding of Ca^{2+} to the C-terminal peptide thermodynamically more favourable. Therefore one can expect that the C-terminal peptide should have a higher Ca^{2+} -binding constant than native troponin C.

It appears that the C-terminal part of troponin C can become involved in the interaction with troponin I and to a smaller extent with troponin T. According to present-day data (Perry, 1979; Leavis *et al.*, 1978; Evans & Levine, 1980), two sites in the skeletal-muscle troponin C structure are responsible for the interaction with troponin I. These sites are located between the second and third Ca^{2+} -binding sites (residues 49–55) and between the third and fourth Ca^{2+} -binding sites (residues 89–96). It has already been mentioned above that there exists a strong homology between the structure of troponins C isolated from different sources. It may thus be expected that the peptide containing residues 91–98

of cardiac-muscle troponin C should interact with troponin I. Indeed, the C-terminal fragment containing this peptide interacts with troponin I. According to Perry (1979) and to Evans & Levine (1980), the C-terminal part of skeletal-muscle troponin C (residues 126–132) contains a site of troponin C interaction with troponin T. We can suppose that the homologous peptide of cardiac-muscle troponin C (residues 128–134) will interact with troponin T. Unfortunately, we failed to demonstrate this interaction unequivocally, although there are some indications of a very weak interaction, as revealed by electrophoresis in the absence of urea.

Hence, despite the 30% difference in the amino acid composition (van Eerd & Takahashi, 1976), the C-terminal part of cardiac-muscle troponin C retains all the biologically important properties of the C-terminal peptide of skeletal-muscle troponin C. The C-terminal part of both proteins contains two $\text{Ca}^{2+}/\text{Mg}^{2+}$ -binding sites and can interact with other troponin components.

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