THE AMINO ACID SEQUENCE OF BOVINE CARDIAC TROPONIN-C. COMPARISON WITH RABBIT SKELETAL TROPONIN-C

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Summary. The amino acid sequence of bovine cardiac troponin-C has been determined. The protein chain is composed of 161 amino acid residues and its amino terminal is acetylated. There are 55 replacements and 2 additional amino acids when compared with rabbit skeletal troponin-C. Cardiac troponin-C probably contains 3 calcium binding sites, one less than rabbit skeletal troponin-C. The difference in amino acid sequence is largely due to the difference in tissue, not to the difference in species.

Introduction. Troponin is a protein located on the thin filaments of muscle (1). It is composed of 3 subunits, one of which (troponin-C) has a strong affinity for calcium ions (2). The binding of calcium ions induces a large conformational change in troponin-C (3) and initiates a sequence of events resulting in muscular contraction.

Recently the almost complete amino acid sequence of rabbit skeletal troponin-C has been reported although experimental details have not yet been published (4). It was shown that rabbit skeletal troponin-C is homologous to parvalbumins. Parvalbumins form a group of low molecular weight calcium binding proteins which were thought to occur only in lower vertebrates such as fish and amphibians, but which recently also were found in rabbit and human skeletal muscle (5). The exact location of the calcium binding sites in a parvalbumin of carp has been determined by X-ray diffraction analysis (6). By comparing the amino acid sequence of this parvalbumin with the amino acid

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sequence of rabbit skeletal troponin-C, Collins et al. (7) tentatively indicated 4 calcium binding sites per molecule in rabbit skeletal troponin-C.

Tsukui and Ebashi recently published a method to obtain pure cardiac troponin in good yield (8). Further, Ebashi has described a method to separate troponin into 3 components (9) and with a slight modification this method can also be applied for the separation of cardiac troponin-C into its components.

In this paper the amino acid sequence of bovine cardiac troponin-C is presented. Only 3 likely calcium binding sites can be indicated, in agreement with the observation of Ebashi who has found 2.4 calcium binding sites per molecule cardiac troponin (10). The difference in amino acid composition between bovine cardiac troponin-C and rabbit skeletal troponin-C is 35%. It indicates that the proteins are more distantly related than the difference in species would suggest. The difference in amino acid sequence is thought to be mainly a reflection of the difference in tissue.

A detailed description of the determination of the amino acid sequence of bovine cardiac troponin-C will be published separately (manuscript in preparation).

Material and methods

Bovine cardiac troponin was a gift from professor S. Ebashi. It was prepared according to the method of Tsukui and Ebashi (8). The calcium binding component was isolated according to the method of Ebashi (9) with a slight modification.

Cyanogen bromide peptides of reduced carboxymethylated troponin-C were isolated by a combination of gel filtration on Sephadex G-50, ion-exchange chromatography on Dowex 50-X2 or DEAE-Sephadex A-25, and high voltage paper electrophoresis. Large peptides were further fractionated after tryptic or chymotryptic digestion.

Overlapping peptides were isolated from a tryptic digest of reduced carboxymethylated and citraconylated (11) troponin-C. Isolation procedures included Sephadex G-50 and DEAE-Sephadex A-25 chromatography.

The amino acid sequences of the peptides were determined by a modified Edman degradation (12) and carboxypeptidases A and B digestion. Identification of released and converted PTH-amino acids was performed by gas chromatography (13) and thin layer chromatography (14). PTH-Leu and PTH-Ile were differentiated by amino acid analysis after HI hydrolysis (15).

The N-acetyl group was determined by hydrazinolysis followed by dansylation of the acetyl-hydrazide (16).

Results and discussion

Amino acid sequence of bovine cardiac troponin-C

The complete amino acid sequence is presented in Fig. 1. There is one single

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                                        10                                  20
Ac-Met-Asp-Asp-Ile-Tyr-Lys-Ala-Ala-Val-Glu-Gln-Leu-Thr-Glu-Glu-Gln-Lys-Asn-Glu-Phe-

                                        30                                  40
  Lys-Ala-Ala-Phe-Asp-Ile-Phe-Val-Leu-Gly-Ala-Glu-Asp-Gly-Cys-Ile-Ser-Thr-Lys-Glu-

                                        50                                  60
  Leu-Gly-Lys-Val-Met-Arg-Met-Leu-Gly-Gln-Asn-Pro-Thr-Pro-Glu-Glu-Leu-Gln-Glu-Met-

                                        70                                  80
  Ile-Asp-Glu-Val-Asp-Glu-Asp-Gly-Ser-Gly-Thr-Val-Asp-Phe-Asp-Glu-Phe-Leu-Val-Met-

                                        90                                  100
  Met-Val-Arg-Cys-Met-Lys-Asp-Asp-Ser-Lys-Gly-Lys-Ser-Glu-Glu-Glu-Leu-Ser-Asp-Leu-

                                        110                                 120
  Phe-Arg-Met-Phe-Asp-Lys-Asn-Ala-Asp-Gly-Tyr-Ile-Asp-Leu-Glu-Glu-Leu-Lys-Ile-Met-

                                        130                                 140
  Leu-Gln-Ala-Thr-Gly-Glu-Thr-Ile-Thr-Glu-Asp-Asp-Ile-Glu-Glu-Leu-Met-Lys-Asp-Gly-

                                        150                                 160
  Asp-Lys-Asn-Asn-Asp-Gly-Arg-Ile-Asp-Tyr-Asp-Glu-Phe-Leu-Glu-Phe-Met-Lys-Gly-Val-

  Glu-OH
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Fig. 1. The complete amino acid sequence of bovine cardiac troponin-C.

peptide chain consisting of 161 amino acid residues. The calculated molecular weight is 18,459. This is in good agreement with the molecular weight estimated by SDS gel electrophoresis (8). The amino terminal is acetylated, a property which it shares with all structural muscle proteins sequenced so far. The protein is strongly acidic and has an excess of 30 negatively charged groups. There are only 2 cysteine, 2 proline and 3 tyrosine residues. Tryptophan and histidine are absent.

Comparison with rabbit skeletal troponin-C

In Fig. 2 is compared the amino acid sequence of bovine cardiac troponin-C with the amino acid sequence of rabbit skeletal troponin-C (17). There are 55 amino acid replacements and the bovine cardiac chain contains 2 amino acids more. In other words 35% of the amino acid sequence is different. In the case of cytochrome c the difference in amino acid sequence between the bovine form and the rabbit form is only 4%, and in the case of the alpha chain of haemoglobin 18% (18). Demaille et al. (19) estimated that the mutation rate for par-

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                                    10                                  20
Ac-Met-Asp-Asp-Ile-Tyr-Lys-Ala-Ala-Val-Glu-Gln-Leu-Thr-Glu-Glu-Gln-Lys-Asn-Glu-Phe-
   Ac(Asp,Thr,Gln,Gln)Ala-Glu  "  Arg-Ser-Tyr  "  Ser  "  "  Met-Ile-Ala  "  "

                                    30                                  40
Lys-Ala-Ala-Phe-Asp-Ile-Phe-Val-Leu-Gly-Ala-Glu-Asp-Gly-Cys-Ile-Ser-Thr-Lys-Glu-
 "  "  "  "  "  Met  " [Asp-----Ala-Asp-Gly-Gly  "  Asp  "  "  Val  "  " ]

                                    50                                  60
Leu-Gly-Lys-Val-Met-Arg-Met-Leu-Gly-Gln-Asn-Pro-Thr-Pro-Glu-Glu-Leu-Gln-Glu-Met-
 "  "  Thr  "  "  "  "  "  "  "  Thr  "  "  Lys  "  "  "  Asp-Ala-Ile

                                    70                                  80
Ile-Asp-Glu-Val[Asp-Glu-Asp-Gly-Ser-Gly-Thr-Val-Asp-Phe-Asp-Glu]Phe-Leu-Val-Met-
 "  Glu  " (Val[Asp,Glu,Asp,Gly,Ser,Gly,Thr)Ile  "  "  Glu  " ]  "  "  "  "

                                    90                                  100
Met-Val-Arg-Cys-Met-Lys-Asp-Asp-Ser-Lys-Gly-Lys-Ser-Glu-Glu-Glu-Leu-Ser-Asp-Leu-
 "  "  "  Gln  "  "  Glu  "  Ala  "  "  "  "  "  "  "  "  Ala-Glu-Cys

                                    110                                 120
Phe-Arg-Met-Phe[Asp-Lys-Asn-Ala-Asp-Gly-Tyr-Ile-Asp-Leu-Glu-Glu]Leu-Lys-Ile-Met-
 "  "  Ile  " [  "  Arg  "  "  "  "  "  "  "  Ala  "  " ]  "  Ala-Glu-Ile-

                                    130                                 140
Leu-Gln-Ala-Thr-Gly-Glu-Thr-Ile-Thr-Glu-Asp-Asp-Ile-Glu-Glu-Leu-Met-Lys-Asp-Gly-
Phe-Arg  "  Ser  "  "  His-Val  "  Asp-Glu-Glu  "  "  Ser  "  "  "  "  "

                                    150                                 160
[Asp-Lys-Asn-Asn-Asp-Gly-Arg-Ile-Asp-Tyr-Asp-Glu]Phe-Leu-Glu-Phe-Met-Lys-Gly-Val-
[  "  "  "  "  "  "  "  "  "  Phe  "  " ]  "  "  Lys-Met  "  Glu  "  "

Glu-OH
Gln-OH
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Fig. 2. Comparison of the amino acid sequence of bovine cardiac troponin-C (upper line) with the amino acid sequence of rabbit skeletal troponin-C (lower line). Only amino acid replacements and uncertainties in the sequence are indicated. [ ] indicate tentative calcium binding sites.

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valbumins lies between the mutation rates of cytochrome c and haemoglobin. Because troponin-C is homologous to parvalbumins its mutation rate is probably very similar. Therefore the difference in amino acid sequence of bovine cardiac troponin-C and rabbit skeletal troponin-C is largely a reflection of the difference in tissue and not a result of the difference in species.

Cardiac troponin-C has 2 cysteine residues, but none of the two corresponds with the single cysteine residue in skeletal troponin-C. The high mutation rate of these cysteine residues makes it unlikely for them to have a functionally important role. This was suggested before by Ebashi et al. (20).

Calcium binding sites

By comparison with the amino acid sequence of a parvalbumin of carp, Collins et al. (7) tentatively indicated 4 calcium binding sites in rabbit skeletal troponin-C. The amino acid sequences of 3 of the corresponding sites in bovine cardiac troponin-C are very similar to the corresponding sequences in rabbit skeletal troponin-C. the fourth one (residues 28 - 40) contains 7 amino acid replacements and one additional amino acid residue. Also the number of amino acids in this region that can provide suitable ligands for calcium binding (6) is reduced. Therefore in the case of bovine cardiac troponin-C this site probably has lost the ability to bind calcium and the total number of likely binding sites is reduced to 3, in rather good agreement with Ebashi's figure of 2.4 calcium binding sites per molecule (10).

It should be noted that the number of amino acid replacements in the first 40 residues is higher than in the rest of the sequence. Apparently the loss of the ability to bind calcium resulted in an increase of acceptable mutations. Furthermore it should be noted that the amino acid sequence at the likely calcium binding sites is more conservative than at other positions.

Cardiac troponin-C has a higher affinity for strontium than skeletal troponin-C (20). This could be explained by a slightly larger metal - oxygen distance in the case of cardiac troponin-C. In order to determine these bond distances however, it is necessary to know the three dimensional structure.

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References

1. Ebashi, S. and Endo, M. (1968) Prog. Biophys. Mol. Biol. 18, 123-183.
2. Hartshorne, D.J. and Pyun, H.Y. (1971) Biochim. Biophys. Acta 229, 698-711.
3. van Eerd, J-P. and Kawasaki, Y. (1972) Biochem. Biophys. Res. Commun. 47, 859-865.
4. Collins, J.H., Potter, J.D., Horn, M.J., Wilshire, G. and Jackman, N. (1973) FEBS Lett. 36, 268-272.

5. Lehky, P., Blum, H.E., Stein, E.A. and Fischer, E.H. (1974) J. Biol. Chem. 249, 4332-4334.
6. Kretsinger, R.H. and Nockolds, C.E. (1973) J. Biol. Chem. 248, 3313-3326.
7. Collins, J.H., Potter, J.D., Horn, M.J., Wilshire, G. and Jackman, N. (1974) Calcium binding proteins (Drabikowski, W., Strzelecka-Golaszewska, H. and Carafoli, E. Eds.), pp 51-63, Elsevier, Amsterdam and PWN-Polish Scientific Publishers, Warzawa.
8. Tsukui, R. and Ebashi, S. (1973) J. Biochem. 64, 456-477.
9. Ebashi, S. (1972) J. Biochem. 72, 787-790.
10. Ebashi, S. (1974) personal communication.
11. Atassi, M.Z. and Habeeb, A.F.S.A. (1972) Methods in Enzymology 25, (Hirs, C.H.W. and Timasheff, S.N. eds.), pp 546-553, Academic Press, New York and London.
12. Iwanaga, S., Wallen, P., Gröndahl, N.J., Henschen, A. and Blombäck, B. (1969) Eur. J. Biochem. 8, 189-199.
13. Pisano, J.J., VandenHeuvel, W.J.A. and Horning, E.C. (1962) Biochem. Biophys. Res. Commun. 7, 82-86.
14. Kulbe, K.D. (1974) Anal. Biochem. 59, 564-573.
15. Smithies, O., Gibson, D., Fanning, E.M., Goodfliesh, R.M., Gilman, J.G. and Ballantyne, D.L. (1971) Biochemistry 10, 4912-4921.
16. Schmer, G. and Kreil, G. (1969) Anal. Biochem. 29, 186-192.
17. Collins, J.H. (1974) Biochem. Biophys. Res. Commun. 58, 301-308.
18. Atlas of protein sequence and structure 5 (1972), (Dayhoff, M.O. ed.), pp D-8, D-53, National biomedical research foundation, Washington, D.C.
19. Demaille, J., Detruge, E., Capony, J.P. and Pechère, J.F. (1974) Calcium binding proteins (Drabikowski, W., Strzelecka-Golaszewska, H. and Carafoli, E. eds.), pp 643-677, Elsevier, Amsterdam and PWN-Polish Scientific Publishers, Warzawa.
20. Ebashi, S., Kodama, A. and Ebashi, F. (1968) J. Biochem. 64. 456-477.