THE AMINO ACID SEQUENCE OF TROPONIN C FROM CHICKEN SKELETAL MUSCLE

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1. Introduction

Troponin C is the component of the troponin complex which binds Ca^{2+} and thereby triggers the activation of the actomyosin ATPase and hence the onset of contraction. The amino acid sequence of troponin C from rabbit fast skeletal muscle has been determined by Collins et al. [1] and that from bovine cardiac muscle by van Eerd and Takahashi [2]. These proteins have been shown to be homologous not only with the calcium binding parvalbumins but also with both the DTNB and alkali light chains of myosin [3]. Based on the homology with the parvalbumins, four binding sites for Ca^{2+} have been proposed for rabbit troponin C and a three dimensional structure has been predicted by Kretsinger and Barry [4].

Van Eerd and Takahashi [2] have shown that the amino acid sequence of bovine cardiac troponin C differs by 35% from that of rabbit fast muscle troponin C and they have ascribed this to tissue difference rather than species difference on the basis of the mutation rate of parvalbumins, there being no such data available for troponin C. They have also shown that the sequence of one of the proposed Ca^{2+} binding sites contains 7 amino acid replacements out of 14 residues compared with a maximum of 2 replacements in the other three sites. They have suggested, on the basis of this sequence information, that the cardiac protein has lost the Ca^{2+} binding site nearest to the N-terminus of the protein and requires only three for its activity.

Chicken troponin is similar to that from rabbit muscle both in its subunits and its activity [5,6], with the exception that there are different troponin T components present in breast and leg muscle [7]. Hirabayshi and Perry [5] have shown that chicken troponin C isolated from a mixture of breast and leg muscle is a single antigen and hence, by inference the troponin C components present in both tissues are very similar if not identical. The present paper reports the amino acid sequence of chicken troponin C and discusses its relationship with rabbit troponin C to which it is very similar. No evidence of heterogeneity in the sequence has been found.

2. Experimental

Troponin C was prepared from mixed breast and leg muscle and purified by chromatography on DEAE-cellulose as described by Perry and Cole [7].

Troponin C, in which the cysteine residue had been alkylated with iodo[^{14}C]acetic acid was digested with trypsin and the resulting peptides separated on a column of Sephadex G50 in 50 mM NH_4HCO_3. The largest peptide was eluted as a single component from the column while the others were purified by high voltage paper electrophoresis. The tryptic peptides were sequenced by the dansyl-Edman technique and in some cases they were subdigested with thermolysin, pepsin or the protease from the V8 strain of *Staphylococcus aureus* [8]. Overlapping information was obtained from the CNBr peptides which were separated in a manner similar to that used for the tryptic peptides. The methods of enzymic and CNBr digestion, amino acid analysis, high voltage electrophoresis and the dansyl-Edman technique have been described previously by Grand et al. [9].

3. Results and discussion

The amino acid sequence of chicken troponin C is

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shown in fig.1. The sequence contains 162 amino acid residues and is thus three residues longer than rabbit troponin C, these three residues being at the N-terminal end. The amino terminus is blocked, presumably by an acetyl group, and it has not been possible to determine the order of the four N-terminal residues. For reasons which are not clear, it was not possible to cleave the methionine in the N-terminal tetrapeptide with CNBr. All other methionine residues cleaved as expected giving rise to the appropriate CNBr fragments. All the expected tryptic peptides, with the exception of that from residue 12–13 were isolated and thus, with the exception of this region, overlap data was obtained for the complete protein. All the peptides obtained could be positioned by homology with the sequence of rabbit troponin C

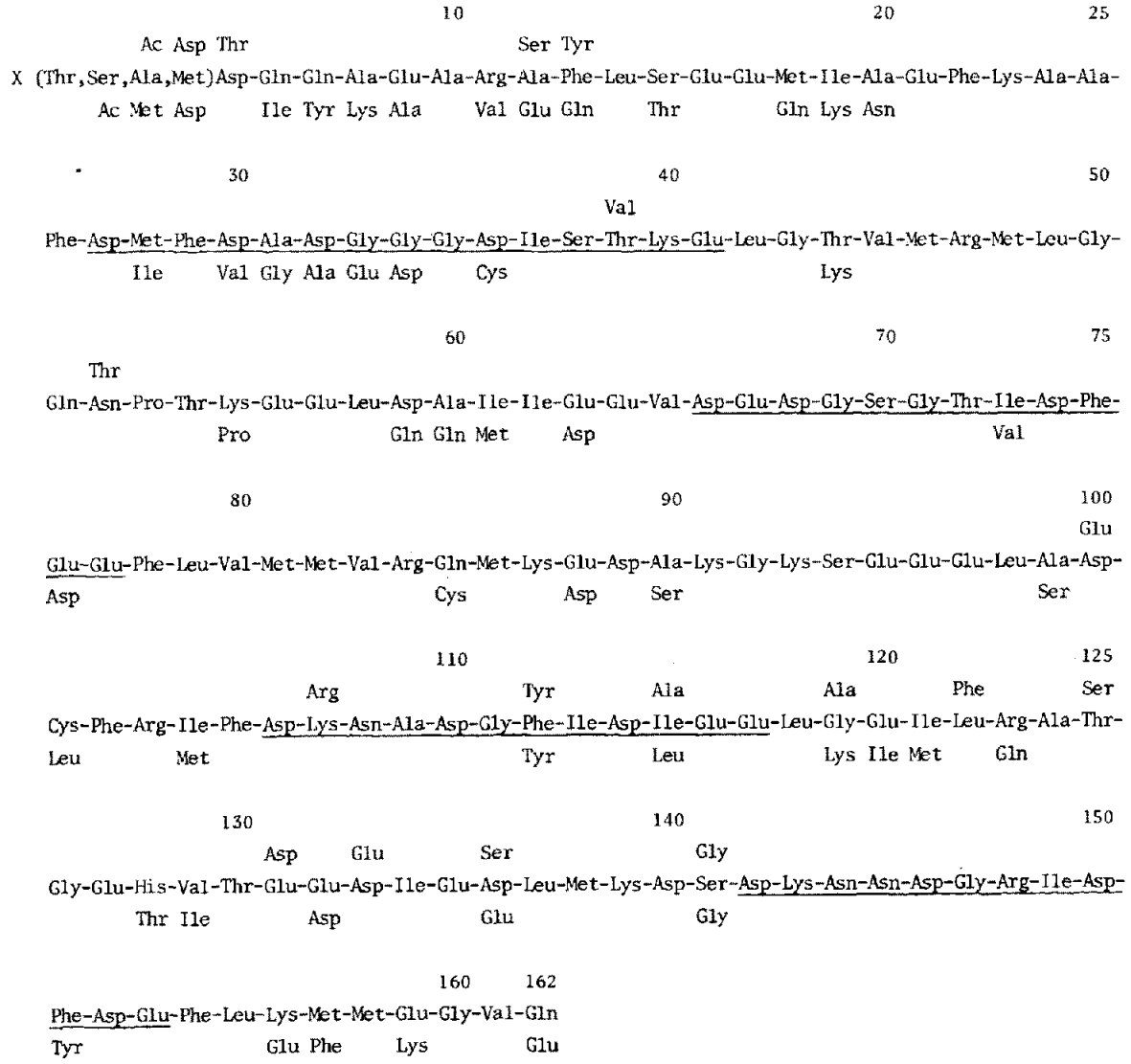

Fig.1. Amino acid sequence of chicken troponin C. Differences from the sequence of rabbit troponin C [1] are shown in the upper line. Differences from the sequence of bovine cardiac troponin *c* [2] are shown in the lower line. Residues underlined form the presumed Ca^{2+} binding sites [1]. X denotes the unknown blocking group.

and this was used as evidence for the sequence around methionine residue 18. No particular difficulties were encountered in determining the sequence. As indicated by the amino acid composition [5], the sequence contains no tyrosine or tryptophan and only one residue each of cysteine, histidine and proline.

As was expected from the immunological work of Hirabayshi and Perry [5] no evidence was found of polymorphism in the sequence. While it would be possible to miss some heterogeneity this seems unlikely in view of the good yield of all the peptides that was obtained. There is thus little doubt that there is only a single species of troponin C in the fast skeletal muscle of the chicken.

When compared with the sequence of troponin C from rabbit muscle (fig.1) there are substitutions at 17 positions giving a difference of 11%. For other complete sequences of chicken and rabbit proteins which are available [10], namely cytochrome *c*, insulin and the α chain of haemoglobin, the differences are 8%, 14% and 30% respectively. On this basis the rate of evolution of troponin C is of the same order as that of cytochrome *c* and insulin, and is rather low presumably due to the structural constraints inherent in the need to conserve the Ca^{2+} binding sites. The substitutions which occur are highly conservative almost without exception. Of the four Ca^{2+} binding sites proposed by Collins et al. [1], two, the second and fourth, are identical, the first contains one replacement and the third three. These replacements are all conservative and involve no change in charge. It thus seems likely that the overall structure of chicken troponin C is very similar to that of the rabbit protein.

When a comparison is made with the bovine cardiac troponin C sequence replacements are found at 50 positions, that is a difference of 31%. This is somewhat less than the 35% difference found between the rabbit skeletal and bovine cardiac proteins, but it is unlikely that this difference is significant. This is in agreement with the suggestion of van Eerd and Takahashi [2] concerning the tissue specificity of troponin C and suggests that there may be a significant difference in the mode of action of this protein in cardiac and skeletal muscle.

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References

[1] Collins, J. H., Potter, J. D., Horn, M. J., Wilshire, G. and Jackman, N. (1973) FEBS Lett. 36, 268–272.
[2] van Eerd, J-P. and Takahashi, K. (1975) Biochem. Biophys. Res. Commun. 64, 122–127.
[3] Collins, J. H. (1976) Nature 259, 699–700.
[4] Kretsinger, R. H. and Barry, C. D. (1975) Biochim Biophys. Acta 405, 40–52.
[5] Hirabayshi, T. and Perry, S. V. (1974) Biochim. Biophys. Acta 351, 273–289.
[6] Hitchcock, S. E. (1975) Eur. J. Biochem. 52, 255–263.
[7] Perry, S. V. and Cole, H. A. (1974) Biochem. J. 141, 733–743.
[8] Houmard, J. and Drapeau, G. R. (1972) Proc. Natl. Acad. Sci. USA 69, 3506–3509.
[9] Grand, R. J. A., Wilkinson, J. M. and Mole, L. E. (1976) Biochem. J., in the press.
[10] Dayhoff, M. O. (1972) Atlas of Protein Sequence and Structure, Vol. 5, pp. D8, D53, D186, National Biomedical Research Foundation, Washington, D.C.