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THE AMINO ACID SEQUENCE OF THE CALMODULIN OBTAINED FROM SEA ANEMONE (*Metridium senile*) MUSCLE

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Summary

The amino acid sequence of the calmodulin obtained from sea anemone muscle was determined. The calmodulin was composed of 148 amino acid residues and its amino terminal was blocked. When compared with bovine brain calmodulin, the number of amino acid residues per molecule was the same and there were 3 replacements at residues 99 (Tyr → Phe), 143 (Gln → Lys) and 147 (Ala → Ser).

Introduction

The calcium-dependent modulator protein (calmodulin) was first dicovered in vertebrates (1,2). Recently, calmodulin is found widely in invertebrates and plants (3 - 12), suggesting that this protein is ubiquitous among the eucaryotes.

The amino acid sequences of calmodulins isolated from bovine brain (13), rat testis (14) and bovine uterus (15) have been already reported and the sequences are very similar. We isolated a calmodulin from marine invertebrate, sea anemone (16), and attempt was made to establish the amino acid sequence of the sea anemone muscle calmodulin.

Materials and Methods

Sea anemone (*Metridium senile*) was obtained from the Sea of Okhotsk. The purification procedure of this calmodulin was described in detail in the previous paper (16). Tryptic digestion of calmodulin was performed in 0.1 M NH_4HCO_3 at 37°C for 7 h without any prior modification of the calmodulin. Tryptic peptides were fractionated by gel filtration of Sephadex G-25 (superfine) and were further separated using the two-dimensional peptide mapping (1st dimension, high voltage paper electrophoresis at pH 5.5; 2nd

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dimension, descending paper chromatography using the solvent (n-butanol/pyridine/acetic acid/water = 15/10/3/12)). Some of the peptides were further digested by thermolysin or *Staphylococcal* V-8 protease and the resulting peptides were separated by the peptide mapping. Overlapping peptides were obtained by the cyanogen bromide cleavage of calmodulin. Isolation procedures of the peptides include Sephadex G-50 chromatography and the peptide mapping.

The amino acid analysis was performed by Hitachi 835-50 single column system amino acid analyzer after acid hydrolysis.

The amino acid sequence of the peptides was determined by dansyl-Edman method (17).

Results and Discussion

The peptides produced by tryptic digestion of sea anemone muscle calmodulin were separated to three peaks by gel filtration as shown in Fig. 1. The first peak components were further divided to early (tube number 48-54) and later (tube numbers 55-67) eluting fractions. Peptides included in the four separated fractions were further isolated by the peptide mapping, and the amino acid sequences of the isolated peptides were determined primarily by the procedure as described in Materials and Methods. The amino acid sequence of the calmodulin was deduced from

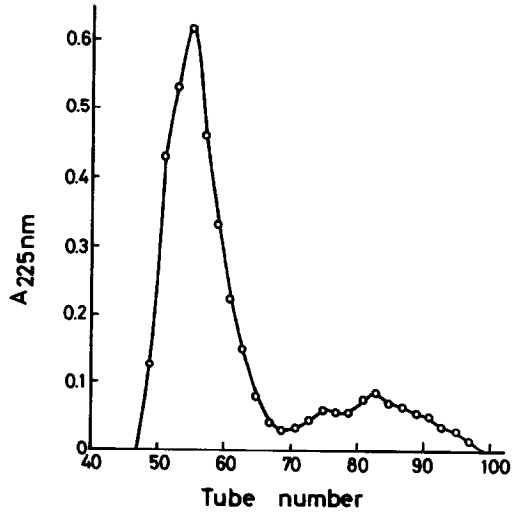

Fig. 1 Chromatography of tryptic digest of sea anemone calmodulin. The digest (ca. 11 mg in 0.1 M NH_4HCO_3) was applied to a column (2.5 x 140 cm) of Sephadex G-25 equibrated with 0.05 M NH_4HCO_3 and eluted with the same buffer at a rate of 12 ml/hr.

analyses of these isolated tryptic peptides combined with those of overlapping peptides. The calmodulin was composed of 148 amino acid residues, which was the same number of amino acid residues of bovine brain (13), rat testis (14) and bovine uterus calmodulins (15). The amino terminal was blocked by an unidentified group. Some residues of glutamic acid were determined by the specific cleavage by V-8 protease, and several of glutamic acid, glutamine, aspartic acid and asparagine were determined by the mobility of the peptides on paper electrophoresis. As shown in Fig. 2, the sequence was established unequivocally except for the amino terminal tripeptide and the amide assignment of nearly one half of aspartic and glutamic acids. A detailed description of the determination of complete amino acid sequence of sea anemone muscle calmodulin will be published elsewhere.

The amino acid sequence of bovine brain calmodulin was compared with the sequence of this sea anemone muscle calmodulin and the amino acid residues different from those of sea anemone calmodulin are shown under the primary structure in Fig. 2 in parentheses. Without regard to

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                                   10                                  20
X(Ala,Asx,Glx)Leu-Thr-Glx-Glx-Glx-Ile-Ala-Glu-Phe-Lys-Glu-Ala-Phe-Ser-Leu-Phe-Asp-

                                   30                                  40
Lys-Asp-Gly-Asx-Gly-Thr-Ile-Thr-Thr-Lys-Glu-Leu-Gly-Thr-Val-Met-Arg-Ser-Leu-Gly-

                                   50                                  60
Gln-Asn-Pro-Thr-Glu-Ala-Glu-Leu-Glx-Asx-Met-Ile-Asx-Glu-Val-Asx-Ala-Asx-Gly-Asx-

                                   70                                  80
Gly-Thr-Ile-Asx-Phe-Pro-Glu-Phe-Leu-Thr-Met-Met-Ala-Arg-Lys-Met-Lys-Asx-Thr-Asx-

                                   90                                 100
Ser-Glx-Glx-Glu-Ile-Arg-Glu-Ala-Phe-Arg-Val-Phe-Asp-Lys-Asp-Gly-Asx-Gly-Phe-Ile-
                                                                   (Tyr)
                                  110                                 120
Ser-Ala-Ala-Glu-Leu-Arg-His-Val-Met-Thr-Asx-Leu-Gly-Glu-Tml-Leu-Thr-Asx-Glx-Glu-

                                  130                                 140
Val-Asp-Glu-Met-Ile-Arg-Glu-Ala-Asx-Ile-Asx-Gly-Asx-Gly-Glx-Val-Asx-Tyr-Glx-Glu-

Phe-Val-Lys-Met-Met-Thr-Ser-Lys-OH
      (Gln)          (Ala)
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Fig. 2 Amino acid sequence of sea anemone calmodulin. Tml shows ε-N-trimethyllysine. X indicates an unidentified blocking group. Residues which differ in bovine brain calmodulin (13) are shown underneath in parenthesis.

the amides left undetermined, only 3 amino acids substitution was recognized out of 148 amino acid residues; Phe at position 99 was substituted by Tyr in brain calmodulin, Lys 143 was by Gln, and Ser 147 was by Ala. Vanaman and Sharief reported that the residue at position 99 in calmodulin of *Renilla reniformis* was Phe (18). A preliminary experiment showed that the residue 147 in scallop calmodulin was also Ser (T. Honma and M. Yazawa, unpublished observation).

Comparing to the number of amino acid substitutions between bovine cardiac and rabbit skeletal muscle troponin C where 57 substitutions were found out of 161 amino acid residues (19), calmodulin is much more conservative protein than troponin C although they are regarded as a family of Ca^{2+}-binding proteins.

Bovine brain calmodulin contained 2 tyrosines at positions 99 and 138. Sea anemone calmodulin contained only one, ^{138}Tyr. Since an identical difference UV absorption sepctrum induced by Ca^{2+} was observed with the two calmodulins of equal weight (16), the conformation change corresponding to the difference spectrum around 280 nm should be attributed to the movement of ^{138}Tyr. If the EF hand model of parvalbumin (20) can be applied to calmodulin, one of the bound Ca^{2+} to calmodulin is coordinated by peptide carbonyl oxygen of ^{99}Tyr, which is involved in the third Ca^{2+}-binding region from the amino terminal of this protein. On the other hand, ^{138}Tyr located in between the residues 137 and 140 donating the ligands to coordinate Ca^{2+} at the fourth Ca^{2+}-binding region. Our results suggest that the phenol group of ^{99}Tyr does not move by the binding of Ca^{2+}, but that of ^{138}Tyr is displaced to more hydrophilic environment.

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References

1. Kakiuchi, S., Yamazaki, R., and Nakajima, H. (1970) Proc. Japan Acad. 46, 587-592
2. Cheung, W.Y. (1970) Biochem. Biophys. Res. Commun. 38, 533-538
3. Waisman, D., Stevens, F.C., and Wang, J.H. (1975) Biochem. Biophys. Res. Commun. 65, 975-982
4. Head, J.E., Mader, S., and Kaminar, B. (1978) J. Cell Biol. 80, 211-218
5. Waisman, D., Stevens, F.C., and Wang, J.H. (1978) J. Biol. Chem. 253, 1106-1113
6. Jones, H.P., Matthews, J.C., and Cormier, M.J. (1979) Biochemistry 18, 55-60
7. Jamieson, G.A., Vanaman, T.C., and Blum, J.J. (1979) Proc. Natl. Acad. Sci. USA 76, 6471-6475
8. Kumagai, H., Nishida, E., Ishiguro, K., and Morofushi, H. (1980) J. Biochem. 87, 667-670
9. Anderson, J.M., and Cormier, M.J. (1978) Biochem. Biophys. Res. Commun. 84, 595-602
10. Gomes, S.L., Mennucci, L., and Maia, J.C. daC. (1979) FEBS Lett. 99, 39-42
11. Charbonneau, H., and Cormier, M.J. (1979) Biochem. Biophys. Res. Commun. 90, 1039-1047
12. Grand, R.J.A., Nairn, A.C., and Perry, S.V. (1980) Biochem. J. 185, 755-760
13. Watterson, D.M., Sharief, F., and Vanaman, T. (1980) J. Biol. Chem. 255, 962-975
14. Dedman, J.R., Jackson, R.L., Schreiber, M.E., and Means, A.R. (1978) J. Biol. Chem. 253, 343-346
15. Grand, R.J.A., and Perry, S.V. (1978) FEBS Lett. 92, 137-142
16. Yazawa, M., Sakuma, M., and Yagi, K. (1980) J. Biochem. 87, 1313-1320
17. Gray, W.R. (1967) in Methods in Enzymology (Hirs, C.H.W Ed.) vol.XI, pp496-478, Academic Press, New York
18. Vanaman, T.C., and Sharief, F. (1979) Fed. Proc. 38, 788
19. van Eerd, J-P., and Takahashi, K. (1976) Biochemistry 15, 1171-1180
20. Krestinger, R.H., and Nockolds, C.E. (1973) J. Biol. Chem. 248, 3313-3326