Circular dichroism spectra show abundance of β-sheet structure in connectin, a muscle elastic protein

Koscak Maruyama, Yoshiharu Itoh and Fumio Arisaka+

Department of Biology, Faculty of Science, Chiba University, Chiba 260 and +*Department of Biochemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan*

Received 9 May 1986

Circular dichroism spectra of native connectin from chicken breast muscle strongly suggested the abundant presence of β-sheet structure, as much as 70% in 0.5 M KCl and 50 mM phosphate buffer, pH 7.5. α-Helix was not detected. These results are in contradiction with the conclusion that native connectin from rabbit skeletal muscle consists entirely of random coil [(1984) J. Mol. Biol. 180, 331–356].

Connectin (Muscle elastic filament) β-Structure CD

1. INTRODUCTION

Connectin (also called titin) is a very long, flexible filament of vertebrate skeletal muscle that links myosin filaments to Z lines in myofibrils ([1], reviews [2,3]). Connectin has been isolated in a native form from chicken breast [4], rabbit skeletal [5,6] and porcine cardiac muscles [7]. The physicochemical properties of these native connectins are very similar. Trinick et al. [6] reported that rabbit skeletal muscle connectin consists entirely of random coil as judged from its circular dichroism (CD) spectra. Our preliminary data suggested the presence of β-structure in addition to random coil [3]. Therefore, a further study has been attempted to clarify this situation, since the secondary structure of a connectin filament is of vital importance to the understanding of its elastic nature ([8], cf. [3]).

2. MATERIALS AND METHODS

Native connectin was prepared from chicken breast muscle as described [4,9] and purified by hydroxyapatite chromatography [7]. The purity was checked by SDS gel electrophoresis using 3% polyacrylamide gels, and any preparations contaminated with other proteins such as myosin, C-protein and actin were not used for this study. The native connectin solution was dialyzed against a salt solution containing 0.5 M KCl and 50 mM potassium phosphate buffer, pH 7.5 [6]. Protein concentration ranged from 0.2 to 0.6 mg/ml.

CD spectra were recorded on a Jasco J-500A spectropolarimeter at 20°C. Usually, a quartz cell of 1 mm light path was used. In the case of guanidine-HCl solution, a cell of 0.5 mm light path was employed. The CD spectra were analyzed to estimate the secondary structure of the protein according to Provencher and Glöckner [10]. The computer program used was provided by Dr Provencher.

3. RESULTS

3.1. *CD spectra of native connectin*

Typical CD spectra at 190–250 nm are presented in fig.1. A negative mean residue ellipticity (-3000 degree·cm^2·dmol^{-1}) was observed at 210 nm. From the values of mean residue ellipticity at 190–240 nm, the percentages of α-helix, β-structure and the rest were calculated according to Provencher and Glöckner [10]. The results showed that connectin filaments consist of approx. 0% (α-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

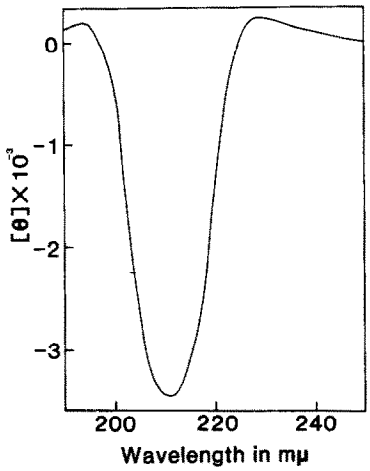

Fig.1. CD spectra of native connectin from chicken breast muscle. Conditions: 0.5 M KCl, 0.05 M phosphate buffer (pH 7.5); 20°C. θ, mean residue ellipticity, expressed in degree·cm^2·dmol^{-1}. The mean residue weight of connectin was calculated to be 125 based on the amino acid composition [4].

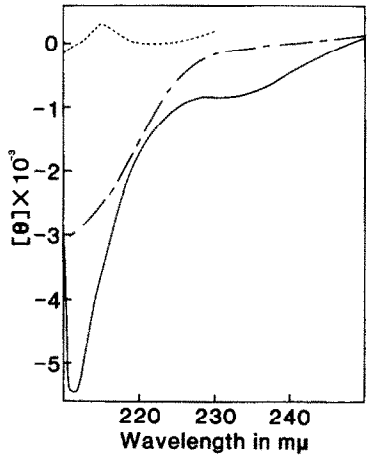

Fig.2. CD of denatured and renatured connectin from chicken breast muscle. Native connectin was treated with 6 M guanidine-HCl overnight at 4°C (——), and dialyzed against 0.5 M KCl and 0.05 M phosphate buffer overnight at pH 7.5 and 4°C (-·-·-). For comparison, a part of the CD spectra of native connectin rabbit skeletal muscle [6] is plotted (– – –).

helix), 69% (β-structure) and 31% (remainder). The calculations suggested that the rest (31%) consisted of 21% β-turn and 10% random coil. These estimations are in sharp contradiction with those of Trinick et al. [6] that connectin is comprised of 100% random coil.

It should be noted here that a preparation contaminated with myosin consisted of 5% α-helix, 60% β-structure, and 35% remainder (cf. [3]). The α-helix must have been derived from contaminated proteins such as myosin.

3.2. *Effect of denaturing agents*

Based on the above observations that native connectin has β-structure, the effects of denaturing agents such as guanidine-HCl (6 M) and SDS (0.1%) were investigated to determine whether the β-structure is unfolded by denaturation. As shown in fig.2, the negative value of molecular ellipticity greatly increased from -3000 to -5000 degrees at 212 nm suggesting that the β-structure of native connectin was effectively unfolded in the presence of 6 M guanidine-HCl. Removal of guanidine-HCl by dialysis largely restored the CD spectra to the original form.

On the other hand, in the presence of SDS, the CD spectra could not be obtained below 240 nm largely due to orientation effects. This was not surprising, because a large number of straight filaments were observed in the presence of SDS under an electron microscope [11].

4. DISCUSSION

Our results conflict with the report of Trinick et al. [6]. Part of their data is plotted in fig.2 for comparison. As mentioned by Trinick et al. [6], the value of molecular ellipticity at 208 nm was only -1000 degree·cm^2·dmol^{-1} as compared with the present value (-3000 degree·cm^2·dmol^{-1}) for unknown reasons. Results very similar to ours have also been obtained with native connectin isolated from porcine cardiac muscle [7].

Although it is generally difficult to estimate precisely the β-structure content from CD spectra, the method of Provencher and Glöckner [10] has been successfully applied to proteins including those rich in β-structure. Our data simultaneously obtained with lysozyme and myoglobin led to the following values: α-helix, 33% (41%), β-sheet, 35% (26%); and α-helix, 81% (77%), β-sheet, 0% (2%), respectively. The values in parentheses are taken from [10]. The agreement is satisfactory. Thus, although quantitative assessment of β-

structure is difficult, native connectin appears to be a β-structure-rich protein.

The presence of β-structure in abundance in native connectin suggests the possibility that the β-spiral structure proposed by Urry [12] may exist in native connectin. If this kind of structure is proved to be the case, it would be easier to explain the elasticity of connectin in a sarcomere.

ACKNOWLEDGEMENTS

We thank Dr K. Imahori, Director of Mitsubishi-Kasei Institute of Life Sciences, who first suggested the presence of β-structure in connectin. We are also indebted to Dr S.W. Provencher of the European Molecular Biology Laboratory, Heidelberg, for kindly supplying the computer program. This work was supported by grants from the Ministry of Education, Science and Culture, Ministry of Health and Welfare, and Muscular Dystrophy Association.

REFERENCES

[1] Maruyama, K., Yoshioka, T., Higuchi, H., Ohashi, K., Kimura, S. and Natori, R. (1985) J. Cell Biol. 101, 2167–2172.
[2] Wang, K. (1985) in: Cell and Muscle Motility (Shay, J.W. ed.) vol.6, pp.315–369, Plenum, New York.
[3] Maruyama, K. (1986) Int. Rev. Cytol. 104, 81–114.
[4] Kimura, S. and Maruyama, K. (1983) J. Biochem. 94, 2083–2085.
[5] Wang, K., Ramirez-Mitchell, R. and Palter, D. (1984) Proc. Natl. Acad. Sci. USA 81, 3685–3689.
[6] Trinick, J., Knight, P. and Whiting, A. (1984) J. Mol. Biol. 180, 331–356.
[7] Itoh, Y., Kimura, S., Suzuki, T., Ohashi, K. and Maruyama, K. (1986) J. Biochem., in press.
[8] Higuchi, H., Yoshioka, T., Umazume, Y., Kimura, S., Ohashi, K. and Maruyama, K. (1986) Biomed. Res., in press.
[9] Kimura, S., Yoshidomi, H. and Maruyama, K. (1984) J. Biochem. 96, 1947–1950.
[10] Provencher, S.W. and Glöckner, J. (1981) Biochemistry 20, 33–37.
[11] Maruyama, K., Kimura, S., Yoshidomi, H., Sawada, H. and Kikuchi, M. (1984) J. Biochem. 98, 1423–1433.
[12] Urry, D.W. (1984) J. Protein Chem. 3, 403–434.