Volume 295, number 1,2,3, 35–38 FEBS 10500 December 1991

Structural properties of connectin studied by ultraviolet resonance Raman spectroscopy and infrared dichroism

Kiyoshi Uchida[1], Issei Harada[1], Yuni Nakauchi[2] and Koscak Maruyama[2]

[1]*Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan* and [2]*Department of Biology, Faculty of Science, Chiba University, Chiba 260, Japan*

Received 24 September 1991; revised version received 15 October 1991

Ultraviolet resonance Raman spectra of solubilized connectin indicated the presence of β-sheets and hydrogen-bonded irregular structures. Some Trp and Tyr sidechains are located in hydrophobic environments and some NHs of mainchain amides and Trp indoles are not easily reached by solvent water, suggesting the presence of folded structures constructed of the β- and irregular parts. Infrared spectra showed an abundance of β-sheets in a connectin fiber, some of which were aligned with their mainchain axes parallel to the fiber axis. Thus, the β-spiral structure proposed for elastin is improbable in connectin. This conclusion is also supported by their different amide III frequencies in the visible Raman spectra. A possible filamentous structure of repeated domains, consisting of β-sheets and irregular parts, is discussed.

Connectin; β-Sheet and irregular structure; Ultraviolet resonance Raman spectroscopy; Trp residue; Tyr residue; Infrared dichroism; Visible Raman spectroscopy

1. INTRODUCTION

Connectin (or titin) is a protein constituting very long and flexible fine filaments of vertebrate striated muscle, each connectin molecule spanning from an M-line to a Z-line in a myofibril [1–3]. Its molecular weight (ca. 2.8 MDa) is so large that its length in a fully extended form would be about 6–2 times longer than the M–Z distance at rest and at extreme stretch, respectively [1]. Therefore, connectin filaments must be folded in a sarcomere [1]. In order to understand the elastic nature of connectin, it is important to clarify the secondary and tertiary structures. The secondary structure has been studied by CD spectroscopy for rabbit skeletal muscle connectin [4] and chicken breast muscle connectin [5] with contradictory results, namely random coil [4] and β-sheets [5].

Ultraviolet resonance Raman (UVRR) spectroscopy is a powerful technique for probing the protein structure, in very dilute solution regardless of the molecular weight, because vibrational scattering from the mainchain and aromatic side-chains in a protein may be selectively enhanced by choosing appropriate excitation wavelengths [6,7]. We observed the UVRR spectra of solubilized connectin for the first time. Infrared dichroism and visible Raman spectra were also used to study a fibrous sample. The protein in solution contains both β-sheets and hydrogen-bonded (H-bonded) irregular structures. Some Trp and Tyr sidechains are in hydrophobic environments and some NHs of the mainchain amides and Trp indole are hardly reached by the solvent water, indicating the presence of folded structures constructed of the β- and irregular segments. Some of the β-sheets are susceptible to 1% SDS and converted into α-helices with Trp and Tyr sidechains exposed to the solvent. In the fiber, the β-sheets tend to partly align with their mainchain axes parallel to the fiber axis. The β-spiral structure was not identified and a possible structure of filamentous connectin is discussed.

Correspondence address: I. Harada, Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan. Fax: (81) (22) 263 9205.

2. MATERIALS AND METHODS

Connectin was prepared in the partly proteolyzed β-form (2.1 MDa) from chicken breast muscle as described in a previous paper [8]. Deuterated connectin was obtained by repeated dialyses (at 4°C) against 0.3 M NaCl (pD 7.6 or 6.6) or 0.15 M sodium phosphate (pD 6.6) in D_2O (D-content ≥99.8%). Treatment with SDS was made by adding 0.1 vol. of 10% SDS aqueous solution to connectin solution (4.1 mg/ml, 0.15 M sodium phosphate, pH(D) 6.6) and keeping the mixture at 50°C for 15 min. UVRR spectra excited at 240 nm with an H_2-Raman-shifted pulsed Nd:YAG laser (average power, 0.6–1.0 mW) were obtained for the solutions in a spinning quartz cell at room temperature [9]. A fibrous sample for infrared measurements was prepared by pressing out the solubilized connectin (2.3 mg/ml in 0.3 M NaCl, pH 7.0) from a Pasteur pipette onto a ZnSe plate and drying it. Polarized infrared spectra were recorded on a Jasco FT/IR-7000 spectrophotometer equipped with a wire grid polarizer. Visible Raman spectra of the fiber were obtained with 488-nm excitation.

3. RESULTS

3.1. *Connectin solution*

Fig. 1A shows a 240-nm excited UVRR spectrum of

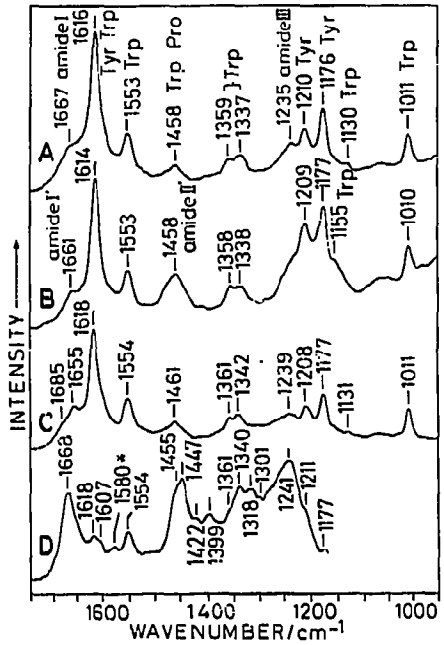

Fig. 1. Ultraviolet resonance Raman spectra of solubilized connectin and a visible Raman spectrum of a connectin fiber in (A) 0.3 M NaCl-H_2O, 3.1 mg/ml, pH 6.4. (B) 0.3 M NaCl-D_2O, 3.1 mg/ml, pD 7.5 (a broad band due to D_2O overlaps around 1208 cm^{-1} and (C) in 0.14 M sodium phosphate buffer (1% SDS) after incubation at 50°C for 15 min, 3.7 mg/ml, pH 6.6. See Materials and Methods for preparation of the fiber. * in D is an overlap of a Trp band and a plasma line.

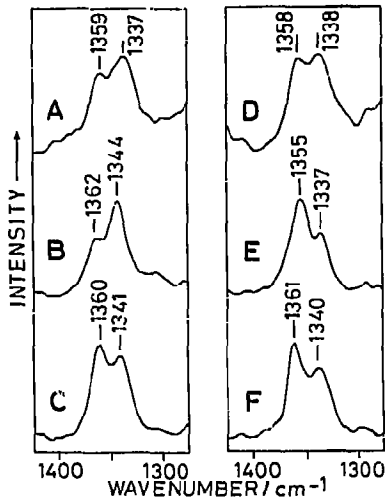

Fig. 2. Ultraviolet resonance Raman spectra (1400–1300 cm^{-1} region) of connectin in (A) 0.3 M NaCl-H_2O and (D) 0.3 M NaCl-D_2O and *N*-acetyl-L-tryptophan ethyl ester in (B) 2 mM in H_2O, (C) 10 mM in CH_2Cl_2, (E) 2 mM in D_2O and (F) 10 mM of deuterated-NAcTrpEE in CH_2Cl_2.

connectin in H_2O solution. Scattering from the mainchain and Trp and Tyr sidechains dominates the spectrum and assignments of most of the Raman bands are straightforward [6,7]. The 1235-cm^{-1} amide III (a coupled NH bend and CN stretch) band, whose intensity tends to decrease in D_2O solution (Fig. 1B), is due to β-sheet parts of the molecule and the tail in the higher-frequency side is ascribed to H-bonded irregular (other than β-sheet and α-helical) parts. (The amide III scattering from α-helices with 240-nm excitation was found to be very weak (vide infra).) The compositions of the β and irregular parts, however, could not be estimated from the spectrum, because of unknown factors of resonance enhancement for the two structures [10]. The broad amide I (a C=O stretch) band, which peaked around 1667 cm^{-1}, is consistently assigned to the two coexisting structures. Based on the intensities of the residual amide III and a newly appeared amide II′ band in the D_2O solution, deuteration of the mainchain amides is not completed at room temperature, indicating that some amide NHs are buried in the interior of the molecule.

A pair of Trp bands around 1360 and 1340 cm^{-1} arise from Fermi resonance between a fundamental and one or two combination bands of the indole ring, and the intensity ratio I(1360)/I(1340) with 240-nm excitation is sensitive to the ring environment: it is higher in hydrophobic environments than in hydrophilic ones [11–13]. As shown in Fig. 2, the ratio for connectin (Fig. 2A, 0.8) is higher than that for a model compound *N*-acetyl-L-tryptophan ethyl ester (AcTrpEE) dissolved in H_2O (Fig. 2B, 0.6), but lower than that in CH_2Cl_2 (Fig. 2C, 1.2). This observation indicates that some Trp sidechains are located in hydrophobic environments. When the indole N_1 site is deuterated, the intensity ratio increases. For N_1-deuterated AcTrpEE, the ratio is 1.7 in D_2O (Fig. 2E) and 1.4 in CH_2Cl_2 (Fig. 2F). The ratio for connectin in D_2O (1.0, Fig. 2D), however, is much lower than those for the N_1-deuterated model compound. Accordingly, some Trp indole NHs in the protein molecule are located in positions that are not reached by the solvent D_2O. Even after incubation of the D_2O solution at 50°C for 15 min, the Trp doublet does not become similar to those in Fig. 2E and F, showing that some indole NHs remain undeuterated under such heat treatment.

In order to see the effect of detergent on the structure of the protein, the aqueous solutions were incubated in the presence of 1% SDS. After the incubation of the H_2O solution, the amide I band lies at 1655 cm^{-1} and the amide III region is broad towards higher frequency with a decreased intensity around 1235 cm^{-1}, suggesting the formation of α-helices at the expence of β-sheets (Fig. 1C). It should be noted that the amide III scattering from α-helices (1300–1265 cm^{-1} region) is very weak. Many Trp indole N_1 sites are exposed to the solvent, because a broad singlet at 1357 cm^{-1} with a low-frequency shoulder appears in D_2O (not shown) similar to that of N_1-deuterated AcTrpEE (Fig. 2E). In addition, the intensities of Tyr bands at 1208 and 1177 cm^{-1} relative to that of Trp 1011-cm^{-1} band decreased

by 30% upon the SDS treatment (trace C vs. A in Fig. 1). Possibly, the environments of many Tyr sidechains are hydrophilic, because the Tyr scattering in H_2O is expected to be a half of that in hydrophobic environments on the basis of data on acetyltyrosine ethyl ester in H_2O and in chloroform (E. Kurashiki et al., unpublished results). These observations can be consistently explained by the assumption that the folded β-sheets become partly dissociated upon addition of SDS and are converted into isolated α-helices with their Trp and Tyr sidechains exposed to the solvent.

3.2. *Connectin fiber*

The connectin solution was so dilute that nonresonant visible Raman spectra could not be observed. For a connectin fiber, however, a good quality spectrum was obtained as shown in Fig. 1D. The broad amide I and amide III bands lie at 1668 and 1241 cm^{-1}, respectively, in agreement with the coexistence of β-sheets and irregular structures. α-Helices are known to give a strong amide I band around 1655–1645 cm^{-1} with visible excitation [6]. Since no such band is identified in the spectrum the content of α-helices must be low, if present. (The amide III band shape and the peak frequency are different from those in the UVRR spectrum (Fig. 1A), partly because the intensity enhancement with the UV excitation is larger for β-sheets than for the other structures [10].) It is interesting to note that the amide III frequency is different from that (1254 cm^{-1}) of elastin [14].

Fig. 3 shows the infrared spectra of the connectin fiber. In the amide I region of the non-polarized spectrum (Fig. 3D), the main peak lies at 1638 cm^{-1} with a shoulder at 1685 cm^{-1}. This pattern is characteristic

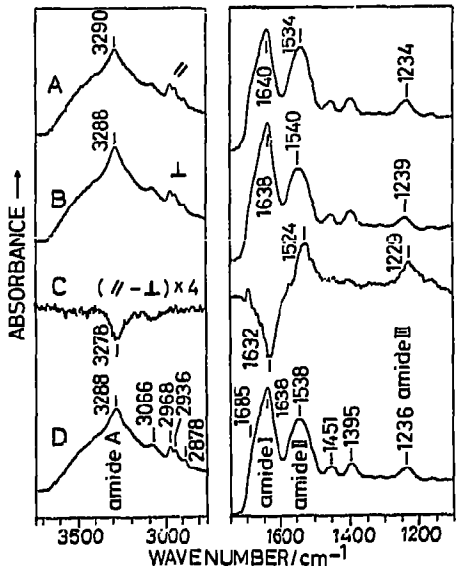

Fig. 3. Infrared spectra of connectin fiber with polarization parallel (A) and perpendicular (B) to the fiber axis; (C) the difference spectrum (A–B) × 4; (D) the spectrum without polarization.

of antiparallel β-sheets, although the main-peak frequency is slightly higher than the typical value (1632 cm^{-1}) [15]. The difference can be due to the presence of irregular structures or/and α-helices. In the amide III region, however, the band at 1236 cm^{-1} is assignable to β-sheets and/or irregular structures but not to α-helices (expected around 1260–1280 cm^{-1}) [16,17]. Hence, the absence of α-helices in the fiber is confirmed. Further, β-sheets are dominant with a lower content of irregular structures, which is consistent with the solution structure suggested by a CD study [5].

The differences between the infrared spectra with polarization parallel and perpendicular to the fiber axis are small (Traces A and B, Fig. 3), because the sample was prepared only by pressing out the connectin solution from a Pasteur pipette and drying it. Nevertheless, the amide II (a coupled CN stretch and NH bend) and amide III bands are stronger in Fig. 3A than in Fig. 3B, while the reverse is observed for the amide A (the NH stretch) and amide I bands. Fig. 3C shows the difference spectrum (//−⊥) multiplied by 4, in which the dichroic nature is clearer with polarizations typical of antiparallel β-plated sheets aligned parallel to the fiber axis.

4. DISCUSSION

Whether the β-sheet parts take the β-spiral structure, proposed for elastin [18], or not is important in relation to the elastic nature of connectin. Since the present results strongly suggest the presence of ordinary β-sheets and H-bonded irregular structures, and the visible Raman amide III frequency is different from that of elastin, the β-spiral structure is improbable in connectin.

Recently, a regular pattern of two types of 100 residue motif (class I and II) has been encoded by partial connectin complementary DNA, the arrangement being -I-I-I-II-I-I-I-II-I-I-II- [19]. As connectin was previously suggested from electron microscopy to consist of a linear array of 43 Å globular domains, each containing approximately 100 residues [4,20], each motif has been considered likely to fold into a separate domain [19]. A possible model consistent with such findings, as well as the present observations, is the following: two or three domains rich in β-sheets and a joint domain rich in irregular structures are repeated, -β-β-β-R-β-β-β-R-β-β-R-, where β and R refer to domains rich in β-sheets and irregular structures, respectively. The β-structure may be conserved in aqueous solution but the domains may be randomly oriented due to increased flexibility of the irregular joints. They may partially align when the solution is pressed out from a Pasteur pipette.

It is tempting to speculate about the elasticity of this model molecule. Sliding and H-bond rearrangements of β-sheets may easily take place upon stretch of the connectin filament if the sheeted part contains short seg-

ments, but the β-domains themselves may not have strong restoring forces. At stretch, however, sizes of possible loop structures [21] as part of the irregular domains may be reduced and the repulsive forces within the loops increased. The increased repulsion within the loops may act as the restoring force when the stress is removed.

REFERENCES

[1] Maruyama, K. (1986) Int. Rev. Cytol. 104, 81–114.
[2] Furst, D.O., Osborn, M., Nave, R. and Weber, K. (1988) J. Cell. Biol. 106, 1563–1572.
[3] Funatsu, T., Higuchi, H. and Ishiwata, S. (1990) J. Cell. Biol. 110, 53–62.
[4] Trinick, J., Knight, P. and Whiting, A. (1984) J. Mol. Biol. 180, 331–356.
[5] Maruyama, K., Itoh, Y. and Arisaka, F. (1986) FEBS Lett. 202, 353–355.
[6] Harada, I. and Takeuchi, H. (1986) in: Spectroscopy of Biological Systems (Clark, R.J.H. and Hester, R.E. eds.) pp. 113–175, Wiley, New York.
[7] Spiro, T.G. and Strygon, C.A. (1988) J. Mol. Struct. 173, 79–90.
[8] Kimura, S. and Maruyama, K. (1983) J. Biochem. 94, 2083–2085.
[9] Takeuchi, H. and Harada, I. (1990) J. Raman Spectrosc. 21, 509–515.
[10] Copeland, R.A. and Spiro, T.G. (1987) Biochemistry 26, 2134–2139.
[11] Harada, I., Miura, T. and Takeuchi, H. (1986) Spectrochim. Acta 42A, 307–312.
[12] Miura, T., Takeuchi, H. and Harada, I. (1988) Biochemistry 27, 88–94.
[13] Harada, I., Yamagishi, T., Uchida, K. and Takeuchi, H. (1990) J. Am. Chem. Soc. 112, 2443–2445.
[14] Frushour, B.G. and Koenig, J.L. (1975) Biopolymers 14, 379–391.
[15] Miyazawa, T. and Blout, E.R. (1961) J. Am. Chem. Soc. 83, 712–719.
[16] Small, E.W., Fanconi, B. and Peticolas, W.L. (1970) J. Chem. Phys. 52, 4369–4379.
[17] Koenig, J.L. and Frushour, B. (1972) Biopolymers 11, 1871–1892.
[18] Urry, D.W. (1984) J. Protein Chem. 3, 403–436.
[19] Labeit, S., Barlow, D.P., Gautel, M., Gibson, T., Holt, J., Hsieh, C.-L., Francke, U., Leonard, K., Wardale, J., Whiting, A. and Trinick, J. (1990) Nature 345, 273–276.
[20] Whiting, A., Wardale, J. and Trinick, J. (1989) J. Mol. Biol. 205, 263–268.
[21] Leszczynski, J.F. and Rose, G.D. (1986) Science, 234, 849–855.