Caldesmon

MOLECULAR WEIGHT AND SUBUNIT COMPOSITION BY ANALYTICAL ULTRACENTRIFUGATION*

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A wide range of values has been reported for the subunit and molecular weights of smooth muscle caldesmon. There have also been conflicting reports concerning whether caldesmon is a monomer or dimer. We attempted to resolve these uncertainties by determining the molecular weight of chicken gizzard smooth muscle caldesmon using the technique of sedimentation equilibrium in the analytical ultracentrifuge. Unlike previous methods that have been used to estimate the molecular weight of caldesmon, the molecular weight determined by equilibrium sedimentation does not depend upon assumptions about the shape of the molecule. We concluded that caldesmon in solution is monomeric with a molecular mass of 93 ± 4 kDa, a value that is much less than those previously reported in the literature. This new value, in conjunction with sedimentation velocity experiments, led to the conclusion that caldesmon is a highly asymmetric molecule with an apparent length of 740 Å in solution. The mass of a cyanogen bromide fragment, with an apparent mass of 37 kDa from sodium dodecyl sulfate-polyacrylamide gel electrophoresis, was determined to be 25.1 ± 0.6 kDa using sedimentation equilibrium. These results imply that the reported molecular weights of other fragment(s) of caldesmon have also been overestimated. We have determined an optical extinction coefficient for caldesmon (ε280 nm = 3.3) by determining its concentration from its refractive index which was measured in the analytical ultracentrifuge. From the above values of the molecular weight and the extinction coefficient, we redetermined that the caldesmon molecule has two cysteines and recalculated the stoichiometric molar ratio of actin/tropomyosin/caldesmon in the smooth muscle thin filament to be 28:4:1.

Muscle contraction, which is regulated by Ca2+, is due to the interaction of the actin thin filament with the myosin thick filament during the hydrolysis of ATP by this actomyosin complex. In smooth muscle there appears to be a dual system of Ca2+ regulation. Ca2+ binding to calmodulin activates the phosphorylation of one of the light chains of myosin in the thick filament and triggers the actomyosin ATPase activity, leading to muscle contraction (see Ref. 2 for a review). It has been suggested that the thin filament is switched on by the binding of Ca2+ to calmodulin which in turn binds to actin-bound caldesmon, resulting in the release of caldesmon’s inhibition of the actomyosin ATPase activity (3–8). Since actin-bound tropomyosin is necessary for full regulation by caldesmon (7, 9–13), tropomyosin, and caldesmon may act in concert in thin filament regulation, possibly via a direct interaction between them (14–16). Caldesmon may also regulate the binding of myosin to actin (17–19), possibly via a direct interaction with myosin (18, 19). In order to understand the molecular mechanisms underlying thin filament regulation, it is necessary to determine the binding parameters of caldesmon’s interaction with these various proteins and to correlate them with caldesmon’s effect on actomyosin ATPase activity. For these and other studies, it is necessary to know caldesmon’s subunit composition, molecular weight, and concentration.

A wide range of values has been reported for caldesmon’s subunit and molecular weights. The subunit mass of chicken gizzard caldesmon has been reported to be between 120–150 kDa (3, 7, 20–22), and the reported molecular mass of the caldesmon molecule has ranged from 120–310 kDa (3, 20, 22). These results have been determined using methods which can be influenced by the amount of sodium dodecyl sulfate (SDS)1 bound and/or by the asymmetry of the molecule. It is also uncertain whether caldesmon exists as a monomer or dimer (3, 20, 22–25). In addition, there has been no reliable optical extinction coefficient reported for concentration determination (20, 22). We set out to determine the native molecular weight and subunit composition of caldesmon by means of sedimentation equilibrium in the analytical ultracentrifuge, a method which depends neither on the shape of the molecule nor on the molecule’s ability to bind SDS. The analytical ultracentrifuge was also used to determine an optical extinction coefficient for caldesmon.

MATERIALS AND METHODS

Caldesmon, from fresh (Mayflower Poultry Co., Cambridge, MA) or frozen (Pel-Freeze, Rogers, AK) chicken gizzards, was prepared by the method of Bretscher (20) except that chromatography on the gel filtration column was omitted. Caldesmon was further purified by chromatography on a calmodulin-Sepharose 4B affinity column where caldesmon was retained in the presence of 1 mM Ca2+ and was eluted with a buffer containing 5 mM EDTA, a procedure similar to that described by Sobue et al. (3). The calmodulin affinity column was prepared by reacting calf brain calmodulin with CNBr-activated Sepharose 4B according to manufacturer’s instructions (Pharmacia

1 The abbreviations used are: SDS, sodium dodecyl sulfate; Mops, 4-morpholinepropanesulfonic acid; DTT, dithiothreitol; Hepes, N-2-hydroxyethylpipерazine-N'-2-ethanesulfonic acid; Nbs, 5,5'-dithiobis(2-nitrobenzoic acid).

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was dialyzed overnight versus 200 volumes of the buffer of interest at 1 mM DTT always present to prevent intra- and intermolecular disulfide cross-linking (22). SDS slab-gel electrophoresis was performed on 15 or 50 mM acrylamide in the presence of 0.5 mM DTT, according to Laemmli (26), on caldesmon or its fragments and on molecular weight standards purchased from Sigma.

The amino acid composition of caldesmon and its 37-kDa cyanogen bromide fragment were determined on a Beckman 115/119CL analyzer after protein hydrolysis in vacuo in 6 M HCl at 110 °C for 22 h. The cysteine content of caldesmon was determined in two different ways. In the first method, the protein (3.1 mg/ml) in 50 mM Hepes, 0.1 M KCl, 5 mM EDTA, pH 7.5, was first reduced with 100 mM DTT at the desired temperature, followed by dialysis against two changes of 500 volumes of a buffer containing 25 mM Hepes, 0.1 M KCl, pH 7.5. An aliquot of the reduced caldesmon or the second dialysate (used as a reference) was then reacted with 1 mM Nbs⁻ and the reaction allowed to continue for 3 h. This Nbs⁻-modified caldesmon was then exhaustively dialyzed versus 0.1 M NaCl, 5 mM Mops, 0.5 mM EDTA, pH 8.0, and the absorbance at 280 nm, extinction coefficient determined below. In the second method, caldesmon (1 mg/ml) in 5 mM potassium HCl, 0.1 M NaCl, 5 mM Mops, 0.5 mM EDTA, pH 7, was reduced for 2 h with 5 mM DTT, Guanidine HCl was present to denature the caldesmon in order to expose any buried cysteine residues. Since it has been reported that removal of DTT from caldesmon by dialysis may result in intra- and intermolecular disulfide formation (22), leading to a value for the cysteine content which would be too low, Nbs⁻ (13 mM) was added in excess of the DTT, the pH adjusted to 7.5 with 0.5 M Mops, pH 8.0, and the reaction allowed to continue for 3 h. This Nbs⁻-modified caldesmon was then exhaustively dialyzed versus 0.1 M NaCl, 5 mM Mops, 0.5 mM EDTA, pH 7. An aliquot of Nbs⁻-modified caldesmon was then reacted with 1 mM Nbs⁻ and the absorbance at 421 nm measured to determine the released Nbs⁻ which was equivalent to protein sulfhydryl content. This absorbance did not change if we added 0.2% SDS in order to denature the protein completely to avoid any further disulfide formation or precipitation. High speed equilibrium ultracentrifugation runs were performed on a Beckman model E analytical ultracentrifuge equipped with Rayleigh optics and an externally adjustable Rayleigh mask assembly (30). A helium-neon laser light source was used in the continuous mode. Sapphire cell windows were used and the camera was focused at the 2/3 plane of the cell (31-33). External loading 6-channel, 12-mm centerpiece were used (34). Rayleigh interferograms were taken on a Beckman model-E analytical ultracentrifuge equipped with Rayleigh optics with an externally adjustable Rayleigh mask assembly on a Reticon photodiode array similar to the one described by Laue and Yphantis (35). Fringe displacements were obtained using the Fourier transform method of DeRosier et al. (36) employing code written in assembly language for the 8080A system. Fringe displacement data were analyzed using the Apple Macintosh-II microcomputer together with the techniques and computer programs described by Yphantis (31), Roark and Bloomfield (41). Sedimentation velocity runs were performed at 20°C on a model E ultracentrifuge equipped with an UV photoelectric scanner at a wavelength of 280 nm. Sedimentation coefficients were reduced to water at 20°C by standard corrections.

Translational frictional coefficients for various models were calculated using the computer program GENTRA of Garcia de la Torre and Bloomfield (41).

Caldesmon concentration, for extinction coefficient determinations, was determined by measuring the difference in the refractive index between the protein solution and its dihydrate by the synthetic boundary method using interference optics in the analytical ultracentrifuge. This refractive index difference is proportional to protein concentration. We assumed a proportionality constant (refractive index increment, δn/δc) at 5461 Å of 0.185 ml/g ± 5% which is essentially invariant for a wide variety of proteins (42). We used a 12-mm synthetic boundary cell in the model E at 3000 rpm. The refractive index dispersion was measured with both the AH-6 high pressure mercury arc lamp (5461 Å) and the He-Ne laser light source (6328 Å). The value of 0.165 chosen at 5461 Å was corrected to 0.182 ml/g at 6328 Å using the Perlmann-Longsworth equation (42). Protein samples were dialyzed exhaustively against buffer and transferred to the cell at low temperature to minimize evaporation. A caldesmon solution was first dialyzed versus 0.1 M NaCl, 5 mM Mops, pH 7, 0.5 mM EDTA, 0.01% NaN₃, 0.5 mM DTT. An aliquot of this solution and the dihydrate were used to determine protein concentration in the ultracentrifuge, and the remainder of the solution was used to measure the absorbance at 278, 280, and 290 nm versus the dihydrate. The absorbance at 290 nm was subtracted from that at 278 and 280 nm to correct for light scattering. We then re-determined these values at 23 mM NaCl, since the refractive index measurement is more accurate at lower salt due to possible mismatch of buffer components.
either from evaporation or from incomplete equilibration during dialysis.

RESULTS

Molecular Weight and Subunit Composition—In equilibrium centrifugation runs, caldesmon in 0.1 M NaCl, 0.5 mM EDTA, 1 mM DTT, 5 mM Mops, pH 7, behaved as a single, monodisperse, nonideal component with a molecular mass of 93 ± 4 kDa (Fig. 1). Fig. 2A shows SDS gels on a sample before and after a run indicating that no significant proteolytic degradation had occurred during the run. The apparent molecular mass on SDS gels was 140 kDa (Fig. 2B) in agreement with previous observations (3, 7, 20, 21). Sedimentation velocity experiments, in the same buffer, showed a single component with a sedimentation coefficient of $s_{20,w} = 2.65 ± 0.05$ S (Fig. 3), again in agreement with previous measurements (20, 22). No appreciable concentration dependence of $s_{20,w}$ was observed over the low concentration range studied

![Figure 1](image1)

**FIG. 1.** Equilibrium sedimentation of caldesmon in 0.1 M NaCl, 0.5 mM EDTA, 1 mM DTT, 5 mM Mops, pH 7.0, at 4 °C; speed = 20,000 rpm. A, plot of the point-by-point weight average molecular weight, $M_w$, as a function of the local cell concentration at three different cell loading concentrations: curve A, 0.1 mg/ml; curve B, 0.3 mg/ml; and curve C, 1.0 mg/ml. B, plot of the reciprocal of $M_w$, $M_m$, and $M_s$ as a function of local cell concentration at a cell-loading concentration of 1.0 mg/ml from the same cell as shown in A.

![Figure 2](image2)

**FIG. 2.** A, SDS-Polyacrylamide (7.5%) gel electrophoresis of: lane A, caldesmon (CaD) before sedimentation equilibrium; lane C, caldesmon after sedimentation equilibrium; lane B, molecular weight standards, a = myosin (205 kDa), b = galactosidase (116 kDa), c = phosphorylase b (97.4 kDa), and d = bovine serum albumin (66 kDa). B, semilog plot of molecular weight versus mobility for standards (C). Caldesmon (b) migrates as 140 kDa.

![Figure 3](image3)

**FIG. 3.** Sedimentation velocity centrifugation of caldesmon. An ultraviolet photoelectric scanner trace showing that caldesmon migrates with a single symmetrical boundary. Path length is 12 mm; concentration = 1.3 mg/ml in 0.1 M NaCl, 0.5 mM EDTA, 1 mM DTT, 5 mM Mops, pH 7.0, at 19.4 °C and 52,000 rpm.

(≤1.6 mg/ml). A value for the Stokes radius was calculated from the molecular weight and sedimentation coefficient and was found to be 87 Å. This number is also in good agreement with previously measured values (20, 22). From these results we conclude that the caldesmon we have prepared is the same as that used in previous investigations, and that caldesmon in solution is a monomer with a molecular mass of 93 kDa.

A recent report concludes that there is a caldesmon monomer-dimer equilibrium which is a function of ionic strength (25). These authors reported that at 60 mM KCl, 2 mM MgCl₂ caldesmon is completely monomeric, whereas at 150 mM KCl, 2 mM MgCl₂ 40% of the caldesmon is dimeric. Therefore, we performed sedimentation equilibrium experiments on caldesmon under a variety of ionic conditions, i.e. in 0.15 M NaCl, 0.3 M NaCl, 0.1 M NaCl, 5 mM MgCl₂, and 0.15 M NaCl, 2 mM MgCl₂, and in a caldesmon concentration range which included that used in the previous work (25). The molecular weight distributions which we obtained under all of these conditions were essentially the same as that shown in Fig. 1 for caldesmon in 0.1 M NaCl, that is, we found the presence
of only 93-kDa monomers and no indication of dimers. Another report indicates that caldesmon aggregates upon freezing and thawing (23). Therefore, we stored some caldesmon frozen at -20 °C, thawed it, and then ran an equilibrium sedimentation experiment. We found the presence of only 93-kDa monomers as we found for fresh caldesmon. We also prepared caldesmon from frozen gizzards and compared its sedimentation equilibrium runs with caldesmon prepared from fresh gizzards and found only 93-kDa monomers for both preparations. These results lead to the conclusion, in agreement with earlier reports (20, 22), that caldesmon is a monomer and not a dimer or in a monomer-dimer equilibrium as suggested by others (3, 23-25).

**Estimate of Maximum Degree of Dimerization**—We have shown that caldesmon behaves as a monomer of molecular mass 93 kDa in the analytical ultracentrifuge. However, although we have not observed any dimer formation (Fig. 1A), self-association can sometimes be masked by thermodynamic nonideality. Self-association would cause the apparent weight-average molecular weight to increase while nonideality would cause it to decrease with increasing concentration. A case in which the contribution from nonideality is greater than that from self-association would result in a decrease in molecular weight with increasing concentration, as we observed here (Fig. 1A) and could be misinterpreted as a simple monodisperse nonideal system. However, an estimate can be made of the nonideality of the caldesmon molecule, from which we can show that the maximum possible degree of dimerization present is insignificant.

In equilibrium runs, a significant amount of thermodynamic nonideality arising from excluded volume effects would be expected for a molecule having the apparent asymmetry of caldesmon. The excluded volume effect may make a contribution to the colligative second virial coefficient factor, B, which is defined by the following equations:

\[
\frac{1}{M_{z,\text{app}}} = \frac{1}{M_{z,\text{ideal}}} + \frac{1}{2BM_{z,\text{ideal}}} c
\]

(1)

\[
\frac{1}{M_{z,\text{app}}} = \frac{1}{M_{z,\text{ideal}}} + \frac{1}{2BM_{z,\text{ideal}}} c^2
\]

(2)

where \(M_z\) is the weight average molecular weight, \(M_t\) is the z molecular weight, and \(c\) is the protein concentration. For a monodisperse system \(M_{z,\text{ideal}}\) is a constant and is equal to \(M_t\), the monomer molecular weight. A theoretical value of \(BM_t\) for a rod having the hydrodynamically apparent dimensions (\(L = 740 \text{ Å}, d = 19 \text{ Å}\), see below) of caldesmon was calculated from the equation for a rod given by Tanford (43) \(BM_t = 0.73 \times 10^{-3} \text{ L/d} = 0.028 \text{ ml/mg}\). The experimental value was estimated from the slope of plots of 1/M\(_{z,\text{app}}\) and 1/M\(_{z,\text{app}}\) versus \(c\) in Fig. 1B and found to be \(BM_t = 0.013\) ml/mg in reasonable agreement with the calculated value. The maximum possible extent of dimerization in this system was estimated using the \(\beta\) parameter of Stafford (38), defined as \(\beta = BM_t/k_2\), where \(k_2\) is the dimerization constant. A plot of the data in Fig. 1A according to the standard curve in Fig. 1a of Stafford (38) gives a value of \(\beta\) which must be greater than about 0.5, putting an upper limit on the dimerization constant of about 0.06 liters/g or 6 \times 10^3 liters/mol. This corresponds to a possible maximum of about 6% dimer at 1 mg/ml. Plots of 1/M\(_{z,\text{app}}\), 1/M\(_{z,\text{app}}\), and 1/M\(_{z,\text{app}}\) versus \(c\) in Fig. 1B can also be used as a test for dimerization. 1/M\(_{z,\text{app}}\) is a molecular weight average, developed by Yphantis and Roark (44), that is insensitive to the nonideality since the second virial coefficient is eliminated and can be used to reveal self-association in a nonideal system such as this one. This moment is defined by using a combination of 1/M\(_{z,\text{app}}\) and 1/M\(_{z,\text{app}}\) so that

\[
1/M_{z,\text{ideal}} = 1/M_{z,\text{app}} = M_{z,\text{app}}/M_{z,\text{app}}\]

(3)

The lines shown in Fig. 1B are least squares fits to a linear equation. All three gave the same intercept of 93 ± 4 kDa. The three fits gave the following equations: (1) \(1/M_{z,\text{app}} = 0.0107 + (5.25 \times 10^{-4})c\) (R = 0.98); (2) \(1/M_{z,\text{app}} = 0.0108 + (2.66 \times 10^{-4})c\) (R = 0.98); and (3) \(1/M_{z,\text{app}} = 0.0108 + (0.23 \times 10^{-3})c\) (R = 0.15). The value for the slope of 1/M\(_{z}\) versus \(c\) is not statistically different from zero indicating no significant dimerization under these conditions. This moment is sensitive to self-association only if self-association exceeds about 7-8% at 1 mg/ml (45). Note that Equations 1 and 2 above are not linear relations for a self-associating system since 1/M\(_{z,\text{ideal}}\) would be concentration dependent with the y axis intercept equal to the monomer molecular weight. Thus the good fit to the linear equation further supports the contention that there is no significant self-association under these conditions.

**Shape and Length**—Some idea of the shape of the caldesmon molecule can be obtained from the frictional ratio, \(f/f_0\), which can be calculated from the sedimentation coefficient and molecular weight. For caldesmon, \(f/f_0 = 2.52\), assuming hydration of 0.3 g H\(_2\)O/g caldesmon. Assuming caldesmon to be a prolate ellipsoid, one can then calculate from this value of \(f/f_0\) an apparent axial ratio, \(a/b = 35\), where \(a\) and \(b\) are major and minor semidiameters, respectively, (43). A rod-shaped molecule of length 2\(a\) and whose volume is equivalent to the prolate ellipsoid would have an axial ratio, i.e. length/diameter, L/d, of 43 (43). Thus with either model, it is obvious that caldesmon is a highly asymmetric molecule, in agreement with previous sedimentation and electron microscopic work (20, 22, 24).

The length of the caldesmon molecule can be calculated from its volume, \(V\), which can be estimated using the molecular weight, the partial specific volume (assumed to be 0.72 cm\(^3\)/g protein), and the water of hydration (assumed to be 0.3 g H\(_2\)O/g protein), and assuming a model for its shape. For the ellipsoidal model with \(a/b = 35\), the length is given by 2\(a\) and is 730 Å with a diameter of 2\(b\) = 20.8 Å. Assuming caldesmon to be a rod (L/d = 43), one calculates \(d = 17.2\) Å and \(L = 742\) Å. Finally, one can approximate caldesmon with a string of beads of the same volume having dimensions \(d = 20.4\) Å and \(L = 745\) Å. From this model one calculates the Stokes radius according to the method of Garcia de la Torre and Bloomfield (46) to have a value of 89.9 Å, in close agreement with the values which we and others have measured (see above). The string of beads model has nearly the same axial ratio as the ellipsoidal model; \(L/d = 36\). Therefore, the calculated dimensions of caldesmon in solution appear to be roughly 740 × 19 Å with an axial ratio of about 40:1, regardless of the model we choose.

**Cyanogen Bromide Fragment**—The molecular weight which we have determined for the intact caldesmon molecule implies that the molecular weight of some or all of its fragments (47, 48) have been overestimated by using gel electrophoresis. In support of this idea, we found that a cyanogen bromide fragment, which migrates with an apparent molecular mass of about 37 kDa on SDS gel electrophoresis (Fig. 4A) and which retains the ability to bind calmodulin and F-actin (27) as does a chymotryptic fragment of around 40 kDa (47, 48), has a molecular mass of 25.1 ± 0.6 kDa by sedimentation equilibrium (Fig. 4B). A similar reduction in molecular weight might also be expected for the chymotryptic fragment.

**Optical Extinction Coefficient**—The estimation of caldesmon concentration has generally been performed by a colori-
metric assay using a different protein as a standard. From this method an extinction coefficient of \( E_{275}^{275} = 3.0 \) was determined (20). A very different value of \( E_{275}^{275} = 4.9 \) was determined from the amino acid composition (22). Therefore, we redetermined the optical extinction coefficient by measuring the refractive index of a caldesmon solution in the analytical ultracentrifuge in order to calculate its concentration. From these measurements we found that \( E_{275}^{275} = 3.25 \pm 0.15 \) and \( E_{295}^{295} = 3.30 \pm 0.15 \) in both 25 and 100 mM NaCl. Within experimental error, the same value for the extinction coefficient was found at both wavelengths used to measure refractive index.

Cysteine Content—We determined the cysteine content of the caldesmon molecule using these newly measured values for the molecular weight and extinction coefficient and found that there were 2.0 cysteines/caldesmon molecule, regardless of which of the two methods we used (see “Materials and Methods”), regardless of whether the protein was native or denatured. This indicates that the cysteines are exposed and readily available for modification. Ngai and Walsh (21) reported a value of 6.6 cysteines/caldesmon molecule, assuming a molecular mass of 141 kDa and by determining protein concentration by a dye-binding assay. If this value is corrected to a molecular mass of 93 kDa, one calculates a value of 4.3 cysteines/caldesmon molecule. It is not possible to correct their cysteine content for protein concentration. Lynch et al. (22) found 2.0 cysteines/caldesmon molecule assuming a molecular mass of 140 kDa and using \( E_{275}^{275} = 4.9 \). If the value of Lynch et al. (22) is corrected for the lower molecular weight and extinction coefficient which we measured, then we calculate a value of 2.0 cysteines/caldesmon molecule, in agreement with our determination.

**DISCUSSION**

Caldesmon is a major calmodulin- and F-actin-binding protein that has been recently discovered in the thin filament of smooth muscle (3) and nonmuscle cells (49-59) and which is thought to play an important role in the Ca\(^{2+}\) regulation of smooth muscle contraction and nonmuscle motility. An understanding of the physical and chemical properties of the caldesmon molecule is essential to an elucidation of this role. However, there have been conflicting reports on a variety of properties of caldesmon, including the molecular weight, subunit composition, molecular length, extinction coefficient, and number of cysteine residues. In this study we have tried to resolve some of these controversies. We have found that, in solution, gizzard smooth muscle caldesmon is a monomer of molecular mass 93 kDa and is a highly asymmetric molecule with a length of about 740 Å. We have also measured an optical extinction coefficient with which we were able to determine a cysteine content of 2.0/molecule.

The molecular mass which we have determined for caldesmon, 93 kDa, is considerably smaller than that determined earlier by SDS-polyacrylamide gel electrophoresis, 135-150 kDa (3, 7, 20-22), or by calculation from hydrodynamic parameters, 120 kDa (20). The higher molecular weight of caldesmon and its fragment obtained by electrophoresis is apparently the result of anomalous migration in the presence of SDS, which is possibly due to the rather high proportion of Glx residues in caldesmon and its fragment (see Table I). Other proteins with a high Glx content also migrate anomalously slowly (60). An earlier work reported a molecular mass for caldesmon of about 120 kDa based on a Stokes radius of 91 Å, a sedimentation coefficient of 2.7 S, and assuming a partial specific volume of 0.725 cm\(^3\)/g for caldesmon (20). In light of the near agreement between these values of the sedimentation coefficient and Stokes radius and our values (see above), we recalculated a molecular mass from their values using the same equations and found a value of 101 kDa, indicating a possible miscalculation in the earlier study.

The value of 740 Å for the length of caldesmon obtained by sedimentation is about half the average value of 1400 Å observed in the electron microscope (22, 24). If the 1400 Å particles were monomers, one would have to postulate that caldesmon is unusually flexible in solution to account for its apparent length of 740 Å, since the Stokes radius is not very sensitive to even fairly large bending motions (41, 61). For example, using the method of Garcia de la Torre and Bloomfield (41), a 1400 Å monomer of caldesmon would have a Stokes radius of 151 Å for the corresponding rigid straight string of beads (1400 \( \times \) 12.7 Å; 111 beads) and 146 Å for a rod bent in the middle by 120°. It would seem that the 1400 Å monomer model does not fit the observed Stokes radius (87-91 Å) very well, even for the v-shaped 120°-bent model. Therefore, the length we have determined corresponds roughly to the contour length. It is also possible that the 1400 Å caldesmon particles seen in the electron microscope pictures are extended monomers and that caldesmon in solution exists
in a much more compact, and thus shorter, conformation. Another possibility, concluded by Furst et al. (24), is that the 1400 Å particles are end-to-end dimers and that the smaller population of shorter particles of 700–1100 Å seen in the electron microscope represents monomers. Under our conditions in solution we detected no dimers; however, it may be that the conditions used for microscopy favor dimer formation. Other plausible explanations for the observation of the shorter molecules by electron microscopy have been proposed (22). Thus, the difference in the apparent lengths determined by electron microscopy and by sedimentation remains to be resolved.

Our finding that the subunit and molecular masses of caldesmon are considerably smaller than the more generally used values of 120–150 kDa and 120–310 kDa, respectively, has several implications. First of all, the amount of caldesmon/actin in the actomyosin-domain thin filaments in vivo (7, 62) corresponds to about one caldesmon molecule/28 actin monomers or one caldesmon/four tropomyosin molecules, after correcting the earlier data (7) for differences in the dye binding ability of caldesmon and actin and using the new molecular mass for caldesmon of 93 kDa. Secondly, the full release of gizzard caldesmon's inhibition of actomyosin ATPase activity by Ca²⁺ requires the presence of a calmodulin/caldesmon molar ratio of between 2–10 (10–12). This rather high ratio raises questions as to whether calmodulin functions in this role in vivo. Our lower molecular weight for caldesmon results in a reduction of the calmodulin/caldesmon molar ratio necessary for release of inhibition. Finally, although caldesmon was first discovered in smooth muscle (3) it has since been identified in a variety of nonmuscle cells (49–59). The nonmuscle caldesmon subunit, from most tissues, migrates during SDS gel electrophoresis with an apparent mass of 70–80 kDa. The molecular weight for muscle caldesmon, which we have determined, suggests that the difference in molecular weight between muscle and nonmuscle caldesmons may be much smaller than originally thought, unless the nonmuscle caldesmon also migrates anomalously slowly during SDS-polyacrylamide gel electrophoresis. To resolve this, it will be necessary to determine the molecular weight of nonmuscle caldesmon by equilibrium sedimentation.

The above stoichiometry suggests two possible arrangements for caldesmon on the thin filament, assuming that caldesmon's length on the thin filament is about 1400 Å, as suggested by electron microscopy, or about 740 Å, as we have determined in solution. If its length on the thin filament is 1400 Å, then it could be bound to both sides of the filament such that the repeating structural unit would be composed of 56 actin monomers (a span of 28 × 27.5 Å = 770 Å (63)), eight tropomyosin molecules, and two caldesmon molecules (Fig. 5A). If the conformation of caldesmon when bound to the actin filament is the same as it is in solution, then the following model, similar to one considered by Walsh (64), is suggested. Caldesmon could be bound to only one side of the thin filament, such that the repeating structural unit would be composed of 28 actin monomers (a span of 28 × 27.5 Å = 770 Å (63)), four tropomyosin molecules, and one caldesmon molecule (Fig. 5B). These models assume that caldesmon is elongated and lies along the thin filament. However, we cannot rule out other possible conformations or arrangements of caldesmon, in vivo.

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REFERENCES
1. Stafford, W. F., and Graceffa, P. (1988) Biophys. J. 53, 180 (abstr.)
2. Marston, S. B. (1983) Prog. Biophys. Mol. Biol. 41, 1–41
3. Sobue, K., Muramoto, Y., Fujita, M., and Kakibuchi, S. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 5652–5655
4. Sobue, K., Morimoto, K., Inui, M., Kanda, K., and Kakibuchi, S. (1982) Biomed. Res. 3, 186–196
5. Marston, S. B., Moody, C., and Smith, C. (1984) Biochem. Soc. Trans. 12, 945–948
6. Ngai, P. K., and Walsh, M. P. (1984) J. Biol. Chem. 259, 13656–13659
7. Marston, S. B., and Lehman, W. (1985) Biochem. J. 231, 517–522
8. Szpacenko, A., Wagner, J., Dabrowska, R., and Ruegg, J. C.

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**Fig. 5. Schematic representation of the stoichiometry of caldesmon binding to the thin filament, assuming caldesmon's length to be about 1400 Å in model A and 740 Å in model B.**
(1985) FEBS Lett. 192, 9–12
9. Smith, C. W. J., and Marston, S. B. (1985) FEBS Lett. 184, 115–119
10. Dabrowska, R., Goch, A., Galazkiewicz, B., and Osinska, H. (1985) Biochim. Biophys. Acta 842, 70–76
11. Sobue, K., Takahashi, K., and Wakabayashi, I. (1985) Biochem. Biophys. Res. Commun. 132, 645–651
12. Horiechi, K. Y., Miyata, H., and Chacko, S. (1986) Biochem. Biophys. Res. Commun. 136, 962–968
13. Chalovich, J. M., Cornelius, P., and Benson, C. E. (1987) J. Biol. Chem. 262, 5711–5716
14. Smith, C. W. J., Pritchard, K., and Marston, S. B. (1987) J. Biol. Chem. 262, 116–122
15. Graceffa, P. (1987) FEBS Lett. 218, 139–142
16. Graceffa, P. (1988) Biochim. Biophys. Acta 1275, 406–412
17. Lash, J. A., Sellars, J. R., and Hathaway, D. R. (1986) J. Biol. Chem. 261, 1655–1660
18. Hemric, M. E., and Chalovich, J. M. (1988) J. Biol. Chem. 263, 1878–1885
19. Ikebe, M., and Reardon, S. (1988) J. Biol. Chem. 263, 3055–3058
20. Bretscher, A. (1984) J. Biol. Chem. 259, 12873–12880
21. Ngai, P. K., and Walsh, M. P. (1985) Biochem. J. 230, 695–707
22. Lynch, W. P., Riseman, V. M., and Bretscher, A. (1987) J. Biol. Chem. 262, 7429–7437
23. Sobue, K., Takahashi, K., Tanaka, T., Kanda, K., Ashino, N., Kakiuchi, S., and Maruyama, K. (1985) FEBS Lett. 182, 201–204
24. Furst, D. O., Cross, R. A., De Mey, J., and Small, J. V. (1986) EMBO J. 5, 251–257
25. Cross, R. A., Cross, K. E., and Small, B. J. V. (1987) FEBS Lett. 219, 306–310
26. Laemmli, U. K. (1970) Nature 227, 680–685
27. Wang, C.-L. A., and Zhan, Q. (1987) Biophys. J. 51, 122a
28. Ellman, G. L. (1959) Arch. Biochem. Biophys. 82, 70–77
29. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. R. (1951) J. Biol. Chem. 193, 265–275
30. Stafford, W. F. (1978) Anal. Biochem. 88, 104–108
31. Yphantis, D. A. (1964) Biochemistry 3, 297–317
32. Richards, E. G., Teller, D., and Schachman, H. K. (1971) Anal. Biochem. 41, 189–214
33. Richards, E. G., Bell-Clark, J., Kirschner, M., Rosenthal, A., and Schachman, H. K. (1972) Anal. Biochem. 46, 295–331
34. Ansevin, A. T., Roark, D. E., and Yphantis, D. A. (1970) Anal. Biochem. 34, 237–261
35. Laue, T. M., and Yphantis, D. A. (1980) Fed. Proc. 39, 1602
36. DeRosier, D. J., Munk, P., and Cox, D. J. (1972) Anal. Biochem. 50, 139–153
37. Roark, D. E., and Yphantis, D. A. (1969) Ann. N. Y. Acad. Sci. 164, 245–278
38. Stafford, W. F. (1980) Biophys. J. 29, 149–166
39. Perkins, J. S. (1986) Eur. J. Biochem. 157, 169–180
40. Pilu, I. and Czerwenka, G. (1973) Die Makromolekulare Chemie 170, 185–190
41. Garcia de la Torre, J., and Bloomfield, V. A. (1981) Qu. Rev. Biophys. 14, 81–139
42. Doty, P., and Edsall, J. T. (1951) Adv. Protein Chem. 6, 55
43. Tanford, C. (1961) Physical Chemistry of Macromolecules, pp. 196, 327, 342. John Wiley & Sons, Inc., New York
44. Yphantis, D. A., and Roark, D. E. (1972) Biochemistry 11, 2925–2934
45. Stafford, W. F., and Yphantis, D. A. (1972) Biophys. J. 12, 1359–1365
46. Garcia de la Torre, J., and Bloomfield, V. A. (1980) Biochemistry 19, 5118–5123
47. Szpacenko, A., and Dabrowska, R. (1986) FEBS Lett. 202, 182–186
48. Fujii, T., Inai, M., Rosenfeld, G. C., and Bryan, J. (1987) J. Biol. Chem. 262, 2757–2763
49. Kakiuchi, R., Inui, M., Morimoto, K., Kanda, K., Sobue, K., and Kakiuchi, S. (1983) FEBS Lett. 154, 351–356
50. Fujita, H., Ishimura, K., Ban, T., Kurosuni, M., Sobue, K., and Kakiuchi, S. (1984) Cell Tissue Res. 237, 375–377
51. Owada, M. K., Hakura, A., Iida, K., Yahara, I., Sobue, K., and Kakiuchi, S. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 3133–3137
52. Sobue, K., Tanaka, T., Kanda, K., Ashino, N., and Kakiuchi, S. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 5025–5029
53. Ngai, P. K., and Walsh, M. P. (1985) Biochem. Biophys. Res. Commun. 127, 533–539
54. Bretscher, A., and Lynch, W. (1985) J. Cell Biol. 100, 1656–1663
55. Burgoyne, R. D., Cheek, T. R., and Norman, K.-M. (1986) Nature 319, 68–70
56. Dingus, J., Hwo, S., and Bryan, J. (1986) J. Cell Biol. 102, 1748–1757
57. Pho, D. B., Desbruyeres, E., Der Terrossian, E., and Olomucki, A. (1986) FEBS Lett. 202, 117–121
58. Litchfield, D. W., and Ball, E. H. (1987) Biophys. J. 52, 8056–8060
59. Ueki, N., Sobue, K., Kanda, K., Hada, T., and Higashino, K. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 9049–9053
60. Takano, E., Maki, M., Mori, H., Hatanaka, M., Marti, T., Titani, K., Kannagi, R., Ooi, T., and Murachi, T. (1988) Biochemistry 27, 1964–1972
61. Adler, A. J., Stafford, W. F., III, and Slayter, H. S. (1987) J. Biol. Chem. 262, 13198–13203
62. Lehman, W., Sheldon, A., and Madonia, W. (1987) Biochim. Biophys. Acta 914, 35–39
63. Squire, J. (1981) The Structural Basis of Muscle Contraction, Chap. 5. Plenum Publishing Co., New York
64. Walsh, M. P. (1987) in Regulation and Contraction of Smooth Muscle (Siegmam, M. J., Somloy, A. F., and Stephens, N. L., eds), pp. 119–141, Alan R. Liss, Inc., New York