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Hydrodynamic Properties of Bovine Cardiac Troponin C†

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ABSTRACT: The size and shape of bovine cardiac troponin C (TN-C) in solution have been examined by gel filtration, ultracentrifugation, and viscosity in the presence and absence of Ca^{2+}. Cardiac TN-C ($-Ca^{2+}$) has an intrinsic sedimentation coefficient, $s^0_{20,w}$, of 1.87 S and a Stokes radius, R_S, of 26.3 Å as determined by gel chromatography on Sephacryl S-300. In 2 mM Ca^{2+}, $s^0_{20,w}$ is increased to 2.04 S and R_S is decreased to 24.3 Å, indicating a conformational change to a more compact structure. Furthermore, the intrinsic viscosity of TN-C in the absence of Ca^{2+} (6.4 mL/g) is reduced to 5.4 mL/g when Ca^{2+} is added. Sedimentation equilibrium studies indicate that the effects of Ca^{2+} are not due to changes in the molecular weight of the protein. The hydrodynamic data suggest that TN-C is a moderately asymmetric protein with an axial ratio of 4–6.

The Ca^{2+} regulation of striated muscle occurs at the level of the thin filament via the troponin–tropomyosin complex [for recent reviews, see Adelstein & Eisenberg (1980); McCubbin & Kay (1980)]. Troponin consists of three nonidentical subunits: TN-C (the Ca^{2+}-binding subunit), TN-I (the inhibitory subunit), and TN-T (the tropomyosin-binding subunit). The signal for muscle contraction apparently involves the binding of Ca^{2+} to TN-C although subsequent events, which ultimately lead to activation of the actomyosin adenosinetriphosphatase, are not understood in molecular detail. It is clear that any refined model of thin filament regulation must include some structural knowledge of the regulatory proteins involved. This knowledge can be obtained either from X-ray analysis or from optical and hydrodynamic studies in solution.

Most of the current information about the structure and function of troponin has been obtained with the proteins from rabbit skeletal muscle. However, troponin subunits from cardiac muscle appear to be qualitatively similar to their skeletal counterparts, with minor differences being attributable to the particular requirements of cardiac muscle regulation (McCubbin & Kay, 1980). The most extensively studied cardiac subunit, bovine cardiac TN-C, has been sequenced (van Eerd & Takahashi, 1975) and has a molecular weight of 18 459. This TN-C contains two cysteine and three tyrosine residues but no tryptophan or histidine. Like rabbit skeletal TN-C, this protein has two high-affinity ($K_a \sim 10^7$ M^{-1}) Ca^{2+}-binding sites, which can also bind Mg^{2+} (Hincke et al., 1978; Holroyde et al., 1980). Cardiac TN-C differs, however, in that it contains only one Ca^{2+}-specific site ($K_a \sim 2 \times 10^5$ M^{-1}) (Leavis & Kraft, 1978; Holroyde et al., 1980). So far, no X-ray structural determination has been reported for TN-C from any source, although crystals suitable for this purpose have recently been obtained for chicken skeletal TN-C (Strasburg et al., 1980). In this paper, the structural properties of bovine cardiac TN-C in solution are investigated by hydrodynamic methods. The results indicate that TN-C is a moderately asymmetric molecule that undergoes a conformational change when Ca^{2+} is present.

Experimental Procedures

Protein Preparation. Bovine cardiac TN-C was initially isolated from crude troponin (Tsukui & Ebashi, 1973) by chromatography on DEAE-Sephadex[1] in 6 M urea as previously described (Burtnick et al., 1975). Pooled fractions containing TN-C were dialyzed against 50 mM Tris-HCl, 2 mM EGTA, and 0.5 mM DTT (pH 7.5) and applied to a DEAE-Sephadex A25 column (2 × 20 cm) equilibrated with the same buffer. TN-C was eluted with a linear 0–0.8 M NaCl gradient at 4 °C. The dominant peak eluting at ~0.4 M NaCl was pure TN-C, as judged by $NaDodSO_4$–polyacrylamide gel electrophoresis (Weber & Osborn, 1969).

Analytical solutions were prepared by dissolving lyophilized TN-C in 0.2 M KMED buffer (0.2 M KCl, 50 mM Mops, 1 mM EGTA, and 1 mM DTT) at pH 7.2, followed by dialysis against this buffer for at least 20 h (48 h for densitometry or sedimentation equilibrium studies). Buffers representing the

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[1] Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; BSA, bovine serum albumin; Mops, 3-(*N*-morpholino)propanesulfonic acid; DTT, dithiothreitol; 0.2 M KMED buffer, 0.2 M KCl, 50 mM Mops, 1 mM EGTA, and 1 mM DTT; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane; $NaDodSO_4$, sodium dodecyl sulfate; NMR, nuclear magnetic resonance.

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+Ca^{2+} state also contained 2 mM $CaCl_2$. Prior to analytical work, all protein samples were clarified by centrifugation (20 min, 50000*g*) on a Beckman Model L centrifuge.

Protein concentrations were determined on a Cary 118C spectrophotometer using an $E^{1\%}_{276nm}$ of 2.3. This value was established by the refractometric method of Babul & Stellwagen (1969), assuming 4.1 fringes are equivalent to 1 mg/mL. The extinction coefficient reported here is in excellent agreement with that determined by amino acid analysis or calculated from the tyrosine content.

Sedimentation Analyses. Ultracentrifugation studies were performed at 20 °C on a Beckman Spinco Model E analytical ultracentrifuge equipped with an RTIC temperature-control unit and electronic speed control. Aluminum or charcoal-filled Epon centerpieces and sapphire windows were used for all experiments. Photographic plates were analyzed on a Nikon Model 6C microcomparator.

Molecular weights were measured by low-speed sedimentation equilibrium with Rayleigh interference optics (Richards et al., 1968). Double-sector centerpieces (12 mm) were employed in these experiments, and concentration determinations were carried out in duplicate with synthetic boundary cells. Equilibrium photos were taken at periodic intervals after 24 h until no further change was apparent.

Sedimentation coefficients were measured at 60 000 rpm with cup-type synthetic boundary cells and schlieren optics. For a given protein concentration, data were collected for both the plus and minus Ca^{2+} states in a single run with two cells and a 1° positive-wedge window. Solutions for these experiments were prepared by adding water or 0.2 M $CaCl_2$ (5 μL) to 0.5-mL TN-C samples in 0.2 M KMED buffer (pH 7.2) prior to the run. Sedimentation coefficients were calculated by the maximum ordinate method from schlieren photos taken at 16-min intervals. These values were corrected to the standard conditions of water at 20 °C ($s_{20,w}$) according to Svedberg & Pedersen (1940). The Stokes radius ($R_{S,sed}$) was calculated from the extrapolated value of $s_{20,w}$ at infinite dilution ($s^0_{20,w}$) by the relationship:

$$R_{S,sed} = M_r(1 - \bar{v}\rho)/(6N\pi\eta_0 s^0_{20,w}) \quad (1)$$

where M_r is the molecular weight, $\bar{v}$ is the partial specific volume, ρ is the solvent density, N is Avogadro's number, and η_0 is the solvent viscosity in poise.

Densitometry. Density measurements of TN-C and buffer solutions were carried out at 20 °C with a digital density meter (DMA 60 and DMA 601; Anton Paar) calibrated with dry air and water. Temperature control was maintained to within 0.01 °C with a Lauda/Brinkmann K-2/R circulating water bath. Density data were used to calculate partial specific volume ($\bar{v}$) according to Kratky et al. (1973). Instrument performance was checked with standard solutions of sucrose and bovine serum albumin (BSA). The value of $\bar{v}$ for TN-C was also calculated from the amino acid composition (van Eerd & Takahashi, 1975) as described by Cohn & Edsall (1943).

Gel Chromatography. Analytical gel-filtration experiments were performed at room temperature (21 ± 2 °C) in a 75 × 1.1 cm column packed with Sephacryl S-300 (Pharmacia). Elution volumes were determined gravimetrically, followed by collection of 0.4-mL fractions in the region of interest. Protein concentrations were measured by absorbance at 276 nm, and peak positions were estimated by interpolation (Andrews, 1965). The following gel standards and Stokes radii (R_S) were used: catalase, 52 Å (Siegel & Monty, 1966); lactate dehydrogenase, 41 Å (de Riel & Paulus, 1978); bovine serum albumin, 35 Å (Siegel & Monty, 1966); ovalbumin, 28.4 Å (Henn & Ackers, 1969; Martenson, 1978; Pharmacia calibration kit); α-chymotrypsinogen, 21.9 Å (Dedman et al., 1977; Pharmacia calibration kit); myoglobin, 19.8 Å (Henn & Ackers, 1969; Dedman et al., 1977); cytochrome *c*, 17.2 Å (Siegel & Monty, 1966; Henn & Ackers, 1969). Since discrepancies exist in the published values of R_S for many proteins, the numerical average was used. The void volume (V_0) and total included volume (V_T) were measured with Blue dextran and potassium chromate, respectively. All TN-C solutions and gel markers were run individually, and elution volumes were reproducible to within 0.2 mL. The partition coefficient (σ) was calculated from the elution volume (V_e) by the relationship:

$$\sigma = (V_e - V_0)/(V_T - V_0) \quad (2)$$

The Stokes radius of TN-C ($R_{S,gel}$) was then calculated from a standard curve of log R_S vs. σ as in Siegel & Monty (1966). Experiments in the presence of Ca^{2+} were done by equilibrating the same column in 0.2 M KMED buffer (pH 7.2) with 2 mM Ca^{2+} and recalibrating with the protein standards. Ca^{2+} produced no significant change in the partition coefficients of these standards.

Viscosity. Viscosity experiments were performed at 20 ± 0.01 °C with a Cannon-Manning semimicroviscometer (water flow time about 530 s). Measurements were made on protein samples (0.5 mL) obtained by sequential dilution of the most concentrated sample. The data were treated according to the equation:

$$(t - t_0)/(t_0 c) = [\eta]_u + k'[\eta]_u^2 c \quad (3)$$

where t_0 and t are the flow times of the solvent and solution, c is the protein concentration in milligrams per milliliter, $[\eta]_u$ is the uncorrected intrinsic viscosity, and k' is the dimensionless Huggins coefficient. Several time readings, reproducible to within 0.2 s, were taken for each solution, and the value of $t - t_0$ at the lowest protein concentration was about 8 s. The values of $[\eta]_u$ obtained were corrected for the difference in density between solvent and protein solution (Tanford, 1955) to give the true intrinsic viscosity $[\eta]$. This correction amounts to an increase in $[\eta]$ of about 0.3 mL/g.

Hydrodynamic Treatment. Translational frictional ratios (f/f_{min}) were calculated from the experimental Stokes radii obtained by gel filtration ($R_{S,gel}$) or sedimentation velocity ($R_{S,sed}$) by using the relationship:

$$f/f_{min} = R_S/R_0 = R_S/[3M_r\bar{v}/(4\pi N)]^{1/3} \quad (4)$$

where R_0 is the Stokes radius of the equivalent unhydrated sphere of molecular weight M_r and partial specific volume $\bar{v}$. The frictional ratio due to asymmetry (f/f_0) was calculated by separating the contribution of particle hydration according to

$$f/f_{min} = (f/f_0)[1 + \omega/(\bar{v}\rho)]^{1/3} \quad (5)$$

where ω is the degree of hydration expressed as grams of water bound per gram of protein. The degree of hydration was estimated as 0.48 g of H_2O/g of TN-C, employing the method of Kuntz & Kautzmann (1974) from the amino acid composition. Axial ratios for both prolate and oblate ellipsoids were generated from frictional ratio estimates by using tabulated data (Schachman, 1959). These tables were also used to relate the axial ratio to the viscosity increment due to shape alone (ν_a) according to

$$[\eta] = \nu\bar{v} = \nu_a[1 + \omega/(\bar{v}\rho)]\bar{v} \quad (6)$$

where ν is the experimental viscosity increment.

Additional comparison was made by using the Scheraga–Mandelkern β function (Scheraga & Mandelkern, 1953). β

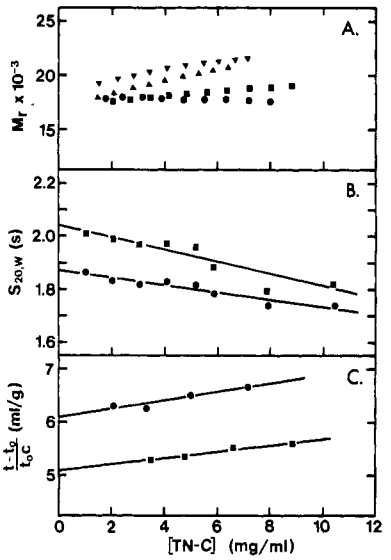

FIGURE 1: (A) Effect of Ca^{2+} on the molecular weight of cardiac TN-C. Sedimentation equilibrium experiments were performed in 0.2 M KMED buffer (pH 7.2) at an initial TN-C concentration of 4 mg/mL (0.1 mL) and a rotor speed of 18 000 rpm. (B) Effect of protein concentration on the sedimentation coefficient of cardiac TN-C. (C) Viscosity of cardiac TN-C. The values of $[\eta]_u$ (eq 3) are 6.1 mL/g ($-Ca^{2+}$) and 5.1 mL/g ($+Ca^{2+}$). Calcium concentratioins are (●) no Ca^{2+}, (■) 2 mM Ca^{2+}, (▲) 10 mM Ca^{2+}, and (▼) 25 mM Ca^{2+}.

is a function only of the axial ratio for prolate and oblate ellipsoids. Its value is calculated from

$$\beta = [N/(16200\pi^2)]^{1/3}\nu^{1/3}/(f/f_{min}) \quad (7)$$

where N is Avogadro's number and ν is the viscosity increment. The longest semiaxis (a) for an ellipsoidal model was evaluated from

$$a = R_0(r_1^2 r_2)^{1/3} \quad (8)$$

where r_1 is the ratio of the longest axis to the intermediate axis and r_2 is the ratio of the intermediate axis to the shortest axis. For a prolate ellipsoid, r_1 is the axial ratio and r_2 is unity, while for an oblate ellipsoid, r_2 is the axial ratio and r_1 is unity.

Results

In order to interpret the effects of Ca^{2+} on the hydrodynamic properties of cardiac TN-C, it is first necessary to establish whether this cation influences the molecular weight behavior of the protein. The sedimentation equilibrium of TN-C in 0.2 M KMED buffer (pH 7.2) was therefore studied at various Ca^{2+} concentrations (Figure 1A). The apparent weight-average molecular weight (M_w) of TN-C in the absence of Ca^{2+} was 17 500, independent of protein concentration across the cell. This value is in fairly good agreement with the sequence molecular weight of 18 459 (van Eerd & Takahashi, 1975). In the presence of 2 mM $CaCl_2$ (about 1 mM free Ca^{2+}), there was no significant increase in M_w at concentrations below 4 mg/mL, although a slight increase was observed at higher protein concentrations (Figure 1A). Addition of larger amounts of Ca^{2+} (up to 25 mM) resulted in a more pronounced increase in M_w to values greater than 20 000. However, even at high Ca^{2+} and protein concentrations, the apparent molecular weight of TN-C did not approach the dimer value of 36 000. It is concluded from these experiments that Ca^{2+}, at concentrations below 2 mM, has little influence on the monomeric tendency of cardiac TN-C.

Due to the importance of the partial specific volume term in molecular weight and hydrodynamic calculations, the value of this parameter was measured by densitometry for TN-C in the presence and absence of Ca^{2+}. The average partial specific volume ($\bar{v}$) from five separate determinations for TN-C in 0.2 M KMED buffer ($-Ca^{2+}$) was 0.701 ± 0.003 mL/g. In the same buffer plus 2 mM Ca^{2+}, the value was identical: 0.701 ± 0.002 mL/g (three determinations). Thus, it appears that the partial specific volume of TN-C is unaffected by Ca^{2+}.

The Stokes radius of TN-C in 0.2 M KMED buffer (pH 7.2) was determined by using a calibrated Sephacryl S-300 column. All TN-C samples eluted in a single symmetrical peak, and no effect of sample protein concentration on the elution volume was observed. In the absence of Ca^{2+}, the apparent Stokes radius ($R_{S,gel}$) of TN-C was 26.3 Å, which was decreased to 24.3 Å in the presence of 2 mM Ca^{2+}. These results suggest that TN-C undergoes a conformational change to a more compact shape when Ca^{2+} is added.

The hydrodynamic properties of TN-C were also investigated by sedimentation velocity. Schlieren photographs of TN-C in 0.2 M KMED buffer (pH 7.2) exhibited a single symmetrical boundary. The effect of protein concentration on $s_{20,w}$ is shown in Figure 1B. The extrapolated sedimentation coefficient, $s^0_{20,w}$, of TN-C in the absence of Ca^{2+} was 1.87 S, and the addition of 2 mM Ca^{2+} resulted in an increase in $s^0_{20,w}$ to 2.04 S. The Stokes radii ($R_{S,sed}$) calculated from $s^0_{20,w}$ were 26.2 ($-Ca^{2+}$) and 24.0 Å ($+Ca^{2+}$). These values are in excellent agreement with those obtained above from gel filtration (26.3 and 24.3 Å). This consistency strongly suggests that the Ca^{2+}-induced behavior of cardiac TN-C is due to a true conformational change and is not an artifact arising out of a particular method of analysis.

Viscosity was used to study independently the physical properties of TN-C in 0.2 M KMED buffer (pH 7.2). The results are presented in Figure 1C. The density-corrected intrinsic viscosity of TN-C in the absence of Ca^{2+} was 6.4 mL/g. This value was reduced to 5.4 mL/g in the presence of 2 mM Ca^{2+}, further indication of a conformational alteration. Using 10^{-4} M instead of 10^{-3} M Ca^{2+} did not significantly affect the intrinsic viscosity of TN-C ($+Ca^{2+}$) (data not shown). The Huggins coefficient (k') was 2.3 ($-Ca^{2+}$) and 2.1 ($+Ca^{2+}$), suggesting that TN-C behaves as a spherical or mildly asymmetric molecule in viscosity experiments (Bradbury, 1970).

Discussion

The binding of Ca^{2+} by TN-C is the key event in the regulation of striated muscle contraction. Thus, it is not surprising that so much effort has been devoted to the effects of Ca^{2+} on various properties of the TN-C subunit. For bovine cardiac TN-C, these effects include increased α-helical content (Burtnick et al., 1975), changes in the fluorescence of extrinsic probes (Johnson et al., 1980), and perturbation of the tyrosyl environment as monitored by fluorescence (Leavis & Kraft, 1978), difference spectroscopy (Hincke et al., 1978), and NMR techniques (Hincke et al., 1981a,b). In most cases, these structural alterations can be specifically associated with Ca^{2+} binding to either the low- or high-affinity binding sites of the TN-C molecule.

In the present study, we have demonstrated that the binding of Ca^{2+} also affects the overall hydrodynamic shape of cardiac TN-C. The structural parameters (summarized in Table I) indicate that TN-C in the absence of Ca^{2+} (f/f_{min} = 1.52) undergoes a conformational change to a more compact structure (f/f_{min} = 1.40) when Ca^{2+} is added. There is some evidence that a similar change occurs for rabbit skeletal TN-C on the basis of a Ca^{2+}-induced increase in $s^0_{20,w}$ (Mani et al., 1974) and intramolecular cross-linking (Gusev et al., 1980). This is also supported by a fluorescence study of the skeletal protein (van Eerd & Kawasaki, 1972), although no influence

Table I: Physical Parameters of Bovine Cardiac TN-C[a]

parameter	$-Ca^{2+}$	$+Ca^{2+}$ (2 mM)
M_w[b]	17 500	18 000
$\bar{v}$ (mL/g)	0.701	0.701
$R_{S,gel}$ (Å)	26.3	24.3
$s^0_{20,w}$ (S)	1.87	2.04
$R_{S,sed}$ (Å)	26.2	24.0
f/f_{min}[c]	1.52	1.40
$[\eta]$ (mL/g)[d]	6.4	5.4
β[e]	2.14×10^6	2.19×10^6

[a] All experiments performed with TN-C in 0.2 M KMED buffer (pH 7.2). [b] Weight-average molecular weight from Figure 1A where c is initial concentration. [c] From eq 4, using the average of $R_{S,gel}$ and $R_{S,sed}$. The value of R_0 is 17.25 Å. [d] Calculated with $[\eta]_u$ values obtained from Figure 1C. [e] Calculated from eq 7.

Table II: Structural Parameters of Equivalent Ellipsoids of Revolution Based on Hydrodynamic Properties of Cardiac TN-C

	hydration, ω (g/g)	prolate axial ratio[a] calcd from R_S	prolate axial ratio[a] calcd from $[\eta]$	oblate axial ratio calcd from R_S	oblate axial ratio calcd from $[\eta]$
$-Ca^{2+}$	0	9.6 (156)[b]	7.4 (131)	11.6 (78)	11.6 (78)
	0.25	7.0 (126)	5.7 (110)	8.0 (69)	8.1 (69)
	0.50	5.2 (103)	4.6 (95)	5.9 (62)	6.0 (63)
	0.75	4.1 (88)	3.8 (84)	4.4 (56)	4.6 (57)
$+Ca^{2+}$	0	7.4 (131)	6.4 (119)	8.6 (71)	9.5 (73)
	0.25	5.2 (104)	4.9 (100)	5.8 (62)	6.5 (64)
	0.50	3.8 (84)	3.9 (85)	4.1 (55)	4.7 (58)
	0.75	2.8 (68)	3.1 (73)	2.9 (49)	3.5 (52)

[a] Axial ratio values were calculated from the Stokes radius (R_S) by using eq 4 and 5 or from the intrinsic viscosity ($[\eta]$) by using eq 6 as described in the text. [b] The longest axis ($2a$) in Å calculated by using eq 8 is shown in parentheses for each axial ratio value.

of Ca^{2+} on the size of the molecule determined by the fluorescence depolarization of an attached dansyl label was noted (Johnson et al., 1978). It should be emphasized that the apparent structural alteration observed here might not necessarily represent the in vivo response to Ca^{2+}. The regulatory trigger in the cardiac myofibril is believed to be the binding of Ca^{2+} to a single Ca^{2+}-specific site in TN-C (Johnson et al., 1980), which occurs over a more restricted range of Ca^{2+} concentration than that used in this study. Future experiments with physiological levels of Ca^{2+} will be required to determine how much of the observed conformational change is biologically relevant.

It is necessary to consider possible alternate explanations for the Ca^{2+}-induced behavior of cardiac TN-C. For example, the observed increase in $s^0_{20,w}$ when Ca^{2+} is added could also be caused by protein association. Indeed, rabbit skeletal TN-C has been shown to aggregate at Ca^{2+} concentrations above 10^{-4} M (Murray & Kay, 1972), apparently to a TN-C dimer (Stafford & Margossian, 1980). We have concluded, however, that aggregation of cardiac TN-C is not an important factor because sedimentation equilibrium studies showed little tendency toward higher molecular weight species in 2 mM Ca^{2+} (Figure 1A). Moreover, an increase in molecular weight is clearly incompatible with the observed Ca^{2+}-induced decrease in R_S determined by gel filtration. It is also apparent from Figure 1B,C that the sedimentation and viscosity concentration profiles in Ca^{2+} are consistent with a monomeric protein. Aggregation of skeletal TN-C probably occurs when very low affinity ($K_a \sim 10^3$ M^{-1}) Ca^{2+}-binding sites are filled at millimolar Ca^{2+} levels, resulting in a less negatively charged molecule. Although such nonspecific Ca^{2+}-binding sites are also found on cardiac TN-C (Holroyde et al., 1980), they must play a reduced role in the intermolecular association until higher Ca^{2+} concentrations are reached.

Due to the large net negative charge on TN-C at pH 7, the possibility of artifacts in gel-filtration experiments should also be considered. Although electrostatic interactions between proteins and the gel matrix are not usually observed at ionic strengths above 0.2 M (Martenson, 1978), this problem does appear to affect the characterization of calmodulin by gel filtration (Klee et al., 1980). However, the excellent agreement between R_S values obtained by gel filtration and sedimentation velocity (Table I) suggests that the role of such gel–protein interactions is minimal under the conditions used.

The observed partial specific volume of cardiac TN-C (0.701 mL/g) is significantly lower than the value calculated from the amino acid composition (0.727 mL/g). This discrepancy initially resulted in lower estimates of the frictional ratio calculated from sedimentation data (Byers & Kay, 1980). The low partial specific volume is undoubtedly the result of electrostriction of water around the numerous charged amino acid side chains of cardiac TN-C (Cohn & Edsall, 1943). When Ca^{2+} is added to TN-C, $\bar{v}$ remains constant, suggesting that if any further change in electrostriction occurs, it must be balanced by other factors such as excluded volume effects. Crouch & Klee (1980) have reported that bovine brain calmodulin also exhibits a lower than average $\bar{v}$ that is relatively insensitive to Ca^{2+}.

The translational frictional coefficient can be combined with viscosity data to give information about the structure of cardiac TN-C. However, the true size and shape of the subunit cannot be determined unless the contribution from particle hydration is known. Table II lists various combinations of hydration and axial asymmetry for TN-C derived from the experimental values of R_S and $[\eta]$. For TN-C in the absence of Ca^{2+}, the agreement between the two approaches is more consistent with the oblate model, although this may not be sufficient to unambiguously establish TN-C as oblate in character. For TN-C in 2 mM Ca^{2+}, no clear preference toward either ellipsoidal model is evident over all hydration values. By setting the degree of hydration (ω) to zero, the maximum possible asymmetry of the TN-C molecule can be obtained. A more reasonable estimate of ω, however, is the calculated value of 0.48 g/g of protein, which is consistent with average protein hydration values determined from hydrodynamic data and X-ray structure (Squire & Himmel, 1979). With this estimate of hydration, an axial ratio of 5–6 would be expected for TN-C in the absence of Ca^{2+} (Table II). This corresponds to a width of about 60 Å for an oblate ellipsoid or about 100 Å for a prolate ellipsoid. In 2 mM Ca^{2+}, these dimensions are decreased by 10–15%.

An alternate representation of these data can be made by using the hydrodynamic treatment of Scheraga & Mandelkern (1953). The value of β, which depends only on the axial ratio of the ellipsoid of revolution, is 2.14×10^6 for TN-C ($-Ca^{2+}$). This value is compatible with an oblate model but not with a prolate model of axial ratio greater than about 3. On the other hand, the estimate of β for TN-C in 2 mM Ca^{2+} (2.19×10^6) is slightly higher than that expected for an oblate ellipsoid but is consistent with a prolate ellipsoid with an axial ratio of about 4. Caution is necessary in making these comparisons because the effect of experimental error on the calculation of β is rather large. Moreover, the interpretation of hydrodynamic data in terms of ellipsoidal models should not be taken too literally. It should be kept in mind that TN-C is not designed to function free in solution and that constraints

of binding to other subunits on the thin filament could result in a rather irregular shape.

The arrangement of troponin subunits on the thin filament is slowly beginning to be elucidated. Using antitroponin antibodies and electron microscopy, Ohtsuki (1979) has demonstrated that the binding of skeletal TN-C and TN-I is centered about 130 Å from the C-terminal end of the tropomyosin molecule or approximately one-third of the period length from the tropomyosin overlap region. On the other hand, it was shown that TN-T binding occurs over the entire C-terminal third of the tropomyosin molecule. Troponin binding over an extended C-terminal portion of tropomyosin is also supported by the fragment studies of Pato et al. (1981). TN-T appears to be an elongated molecule containing a region rich in α helix that could interact with the tropomyosin coiled coil (Pearlstone & Smillie, 1977). Although analogous experiments have not been reported for cardiac troponin, hydrodynamic studies have indicated that this troponin is also asymmetric (Byers et al., 1979). If cardiac TN-C is indeed a flattened or elongated molecule with a maximum diameter of 60–80 Å, the asymmetry of the troponin complex in solution and along the thin filament would not be due to this subunit alone.

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