Salt-Induced Conformational Changes in Skeletal Myosin Light Chains, Troponin-C, and Parvalbumin†

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ABSTRACT: Evidence for salt-induced changes in myosin light chains [dissociated by treatment with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB)], troponin-C (TnC), and parvalbumin was obtained from chymotryptic digestion, circular dichroism, fluorescence, and difference absorption studies. High salt (0.6 M NaCl) protects the DTNB light chain from proteolysis, increases its α-helical content, and quenches the tryptophan fluorescence. These effects are similar to the changes induced by Ca^{2+} but smaller in magnitude. TnC is affected by monovalent cations in a similar manner. Changes in the α-helical content resemble the effect of Ca^{2+}. The enhancement of tyrosine fluorescence reflects conformational changes in the Ca^{2+}–Mg^{2+} binding sites. The increase in the fluorescence of dansylaziridine-labeled TnC suggests perturbation of Ca^{2+}-specific sites by salt. Cancellation of this effect by Mg^{2+} binding to the high-affinity sites is indicative of site–site interactions. In Whiting parvalbumin, salt induced a perturbation of tryptophan absorption similar in nature to the Ca^{2+} effect.

It is generally accepted that the calcium regulation of skeletal muscle contraction is mediated primarily through troponin-C (TnC).[1] When the concentration of Ca^{2+} around the thick and thin filaments of muscle is high, Ca^{2+} binds to TnC and contraction occurs. When the concentration of Ca^{2+} is low, TnC looses the bound Ca^{2+} and the muscle relaxes. TnC has four divalent cation binding sites, which have been identified by sequence homology with the Ca^{2+} binding sites of parvalbumin (Collins, 1976). The three-dimensional structure of parvalbumin has been resolved, to an atomic resolution, by X-ray crystallography (Kretsinger & Nockolds, 1973). Recently, the nature of the four divalent cation binding sites of TnC has been resolved by studying the Ca^{2+} binding to its proteolytic fragments (Leavis et al., 1978; Sin et al., 1978). These studies have identified two high-affinity sites, referred to as Ca^{2+}–Mg^{2+} sites, and two low-affinity sites, the Ca^{2+}-specific sites. Binding of Ca^{2+} to the Ca^{2+}–Mg^{2+} sites produces a large conformation change in TnC (van Eerd & Kawasaki, 1972; Murray & Kay, 1972; Leavis et al., 1978). Relatively small changes are induced with Ca^{2+} saturation of the low-affinity sites (Leavis et al., 1978; Johnson & Potter, 1978; Hincke et al., 1978). These sites are presumably required for the regulation of contraction.

Evidence from several laboratories suggests the existence of a second control mechanism in skeletal muscle contraction involving the binding of Ca^{2+} to the myosin DTNB light chain (Werber et al., 1972; Morimoto & Harrington, 1974; Margossian et al., 1975; Haselgrove, 1975; Lehman, 1977). Such a control mechanism could regulate the release of myosin cross-bridges from the backbone of the thick filament. When the isolated DTNB light chain binds Ca^{2+}, conformational changes similar to those in TnC are observed. Binding of Ca^{2+} to the DTNB light chain in the intact myosin molecule apparently affects the hydrodynamic properties of thick filaments (Morimoto & Harrington, 1974). The clearest manifestation of the Ca^{2+}-induced changes in the thick filament comes from the elegant digestion studies of Weeds & Pope (1977). Chymotryptic proteolysis of polymeric myosin (low salt) in the absence of Ca^{2+} results in the production of subfragment 1. A similar digestion conducted in the presence of Ca^{2+} or Mg^{2+} ions leads to the production of heavy meromyosin (Weeds & Pope, 1977).

It is tempting to correlate the Ca^{2+}-mediated switch in the products of digestion with the conformational changes or structural transitions in the S-2 region of myosin. However, we must bear in mind that a similar shift in the digestion products of myosin, from subfragment 1 to heavy meromyosin, can be achieved in the absence of Ca^{2+} by raising the salt concentration in the digestion reaction. The shift in digestion products may result from salt-induced changes in the organization of myosin. Alternatively, salt may influence myosin in a manner similar to the Ca^{2+} effect (in low salt); both Ca^{2+} and monovalent cations may stabilize and protect the structure of the DTNB light chain. Protection of the light chain can, in turn, reduce the chymotryptic susceptibility at the subfragment 1 site, leading to digestion at the heavy meromyosin site (Weeds & Pope, 1977; S. Oda and E. Reisler, unpublished experiments). Such interpretation (masking of the subfragment 1) places more emphasis on specific changes in the DTNB light chain rather than on overall structural or organizational changes in the myosin molecule. The effect of salt on the DTNB light chain, if detected, would not be without precedence among the Ca^{2+}-binding proteins. Sodium cations have been shown to bind to parvalbumin (Grandjean et al., 1977) and to increase its thermostability (Fillimonov et al., 1978). NaCl apparently also affects the Ca^{2+}-dependent regulatory protein calmodulin (Wolf et al., 1977).

We have investigated the effect of salt on the DTNB light chain in order to clarify its role in determining the proteolytic cleavage position on myosin. Chymotryptic digestions of the DTNB light chain, circular dichroism, and fluorescence studies have revealed a significant salt effect on the conformation of this protein. We have also observed similar salt-induced conformational changes in troponin-C and parvalbumin. In

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[1] Abbreviations used: DTNB light chain, 19000 molecular weight subunit of myosin dissociated by treatment with 5,5′-dithiobis(2-nitrobenzoic acid); TnC, troponin-C; DANZ, dansylaziridine; TnC_{DANZ}, TnC covalently labeled with DANZ; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate.

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all cases, the salt effect resembles structural changes caused by the binding of Ca^{2+} to these proteins.

Materials and Methods

Dansylaziridine was purchased from Molecular Probes (Plano, TX). EDTA and EGTA were Sigma products (St. Louis, MO). All other reagents were of analytical grade.

Preparation of Proteins. Myosin was prepared from rabbit skeletal muscle according to the procedure of Godfrey & Harrington (1970).

Light chains of myosin were prepared by their dissociation from myosin in 5 M guanidine hydrochloride, followed by ethanol precipitation of the heavy chains (Holt & Lowey, 1975). The soluble light chains were dialyzed against 10 mM KCl, 10 mM Bistris (pH 7.0), and 1 mM DTE and applied onto a 20 × 1.5 cm Affi-Gel Blue column (Toste & Cooke, 1979; E. Reisler, J. Liu, M. Mercola, and J. Horwitz, unpublished experiments). Only the DTNB light chain was retained on the gel and subsequently eluted with the same buffer system in the presence of 0.5 M KCl.

Troponin-C was a generous gift of Drs. J. Horwitz, B. Bullard, and D. Mercola. We are grateful to Dr. H. White for providing us with Whiting parvalbumin.

Modification of Troponin-C. Troponin-C was labeled with dansylaziridine (DANZ), following the procedure of Johnson et al. (1978). A stock solution of 0.01 M dansylaziridine in ethanol was used to modify a 2 mg/mL solution of TnC in 10 mM potassium phosphate buffer, 90 mM KCl, 2.5 mM Ca^{2+}, and 2 mM EGTA at pH 7.0. The reaction was conducted at a 1.5 molar excess of DANZ to protein and was allowed to proceed for 24 h at room temperature. The reacted sample was then dialyzed against 10 mM potassium phosphate (pH 7.0), 90 mM KCl, and 2 mM EGTA. The incorporation of the probe (0.7 DANZ/TnC) was calculated by using an ϵ_{350} of 3980 M^{-1} cm^{-1} for the dansyl fluorophore (Johnson et al., 1978).

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. Gel electrophoresis was carried out according to the procedure of Laemmli (1970). The resolving gel consisted of an upper phase of 10% acrylamide–0.27% bis(acrylamide) and a lower phase of 15% acrylamide–0.4% bis(acrylamide). The protein distribution among the different bands was quantitated by weighing densitometric tracings of the stained bands. The integrated stain density was assumed to be linearly proportional to the amount of protein.

Chymotryptic Digestion of the DTNB Light Chain. The DTNB light chains were digested for 30 s with α-chymotrypsin, at room temperature, in 10 mM sodium phosphate (pH 7.0), 0.1 mM NaN_3, and either 1.0 mM EDTA or 1.0 mM $CaCl_2$ (Weeds & Pope, 1977). The concentration of sodium chloride ranged in these experiments from 0.05 to 0.6 M. The digestions were terminated by the addition of phenylmethanesulfonyl fluoride to a final concentration of 1.5 mM. The concentrations of the DTNB light chain and chymotrypsin in the digestion reactions were 0.5 mg/mL and 5 μg/mL, respectively. The products of the digestion were monitored on $NaDodSO_4$–polyacrylamide gels.

Fluorescence Measurements. Fluorescence measurements were made in a corrected excitation–emission mode using a Farrand Model MKI spectrophotometer equipped with a Farrand autoprocessor 1. The tryptophan fluorescence of the DTNB light chain was monitored at 340 nm (excitation at 295 nm). The tyrosine fluorescence of TnC was monitored at 302.5 nm (excitation at 280 nm). Protein concentrations were 0.37 and 0.3 mg/mL for the DTNB light chain and TnC, respectively. These measurements were done in 50 mM Tris-HCl (pH 7.5), 10 mM NaCl, and 2 mM EDTA. (Titrations with salt or Ca^{2+}, performed in this buffer, did not affect the pH.) Small and constant volumes of different stock solutions of concentrated NaCl or KCl were added to the protein samples. When Ca^{2+} or Mg^{2+} ions were present in the titrated samples, their concentrations were 3 and 5 mM, respectively.

Emission spectra of troponin-C labeled with dansylaziridine (TnC_{DANZ}) were scanned between 250 and 750 nm (excitation at 340 nm). In titration experiments the fluorescence of TnC_{DANZ} was monitored at 520 nm, near the maximum emission wavelength (Table II). These measurements were made with 0.1 mg/mL solutions of TnC_{DANZ} in 10 mM KCl, 10 mM potassium phosphate, and 2 mM EDTA at pH 7.0.

Circular Dichroism. CD measurements were conducted with a modified Beckman CD spectrophotometer equipped with a Nicolet Model 527 signal averager (Horwitz & Heller, 1973). A 0.1-mm path for the far-UV spectra and 1-mm path cells for the single wavelength titrations were used. Unless stated otherwise, protein concentrations were the same as those in the fluorescence measurements and the same titration procedure was applied. Differential absorption data, ΔA, were converted to $\Delta\epsilon$ and to a mean residue ellipticity, $[\theta]$ (deg cm^2/mol), assuming the value of 110 for the mean residue molecular weight of amino acids. The α-helical content was calculated as described by Greenfield & Fasman (1969).

Difference Spectra. Difference absorption measurements were made with a Beckman Model 25 spectrophotometer. The spectra were recorded on an expanded scale (0.1 A) from 265 to 330 nm. The reference and sample cells contained identical 0.4 mg/mL parvalbumin solutions. In the titration experiment, small volumes of concentrated stock solutions of KCl were added to the sample cell and identical volumes of solvent were added to the reference cell. Appropriate corrections were made for the dilution effect.

The final titration data represented the salt-induced perturbation of the tryptophan signal at 293 nm.

Results

Previous digestion studies have shown that the site of proteolytic digestion on myosin and the state of the DTNB light chains are interdependent (Weeds & Pope, 1977). Subfragment 1 is obtained by chymotryptic proteolysis of myosin in low salt in the absence of divalent cations. The DTNB light chains are also digested under these conditions. Digestion in high salt (or in low salt but in the presence of divalent cations) yields heavy meromyosin with the DTNB light chains and the subfragment 1 site remaining uncleaved. Two explanations can be offered to account for these results. Either the presence of structurally different myosins (monomeric and filamentous forms) is responsible for the formation of different digestion products or salt increases the proteolytic resistance of the DTNB light chains and consequently of the subfragment 1 site. In order to resolve between these two possibilities, we have carried out chymotryptic digestions of the isolated DTNB light chain in different salt concentrations. The products of such digestions are shown on the gels and the corresponding densitometric tracings presented in Figure 1. The tracings demonstrate a decrease in the digestion of the DTNB light chain with increasing salt concentrations. This observation is better documented in Figure 2, in which we plot the relative protection of DTNB light chains from proteolysis as a function of the salt concentration. The protection levels are calculated from gel tracings. They relate the remaining, uncleaved DTNB light chain in each experiment to the control [i.e., the undigested protein which is considered to be fully (100%) protected]. Results of these calculations show an increasing

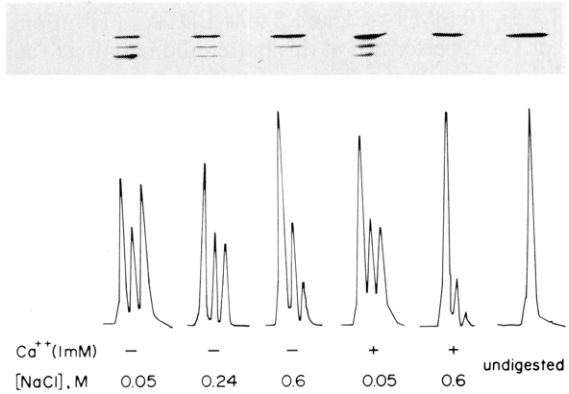

FIGURE 1: Salt effect on α-chymotryptic digestion of DTNB light chains. Representative $NaDodSO_4$–polyacrylamide gels and their corresponding densitometric tracings. The digestions were carried out for 30 s with 5 μg/mL α-chymotrypsin, at room temperature, on 0.5 mg/mL solutions of DTNB light chain.

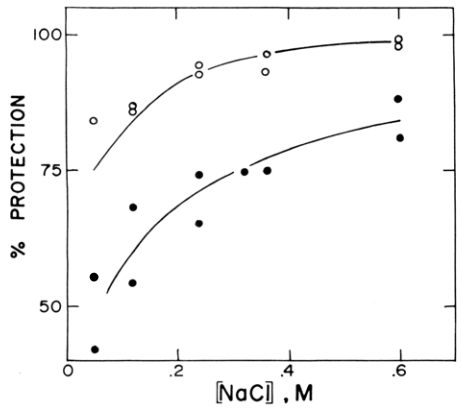

FIGURE 2: Salt effect on the relative protection of the DTNB light chains from proteolysis. 100% protection refers to the undigested control sample; zero protection corresponds to a complete disappearance of the original DTNB light chain band.

protection of the DTNB light chain from proteolytic digestion with increasing salt concentration (Figure 2). This effect is very pronounced in digestions carried out in the absence of divalent cations. In the presence of Ca^{2+}, the major protection effect is due to this cation (Weeds & Pope, 1977), although salt seems to supplement the Ca^{2+} effect. The absolute levels of protection depend on the particular proteolysis conditions such as the time of digestion and the concentrations of the DTNB light chain and α-chymotrypsin.

The observed effect of salt could be due either to a direct salt protection of the DTNB light chains or to a decreased activity of α-chymotrypsin. Chymotryptic digestions of bovine serum albumin have been performed in different salt concentrations to check the latter possibility. These studies have shown no change in the enzyme activity due to salt. Other studies have found a slight increase of α-chymotrypsin activity on synthetic substrates with increasing levels of salt (Kahana & Shalitin, 1974). Thus, we are led to the conclusion that salt indeed protects the DTNB light chain from digestion.

Calcium is known to produce considerable changes in the overall and local conformations of the DTNB light chains. This raises an interesting question: is the salt protection of the same nature as Ca^{2+} protection, affected by a similar structural change in the DTNB light chain? The induced transitions can be easily detected by CD, fluorescence, and hydrodynamic measurements (Alexis & Gratzer, 1978; Werber et al., 1972). We have employed these techniques to examine the effect of salt on the conformation of the DTNB light chain.

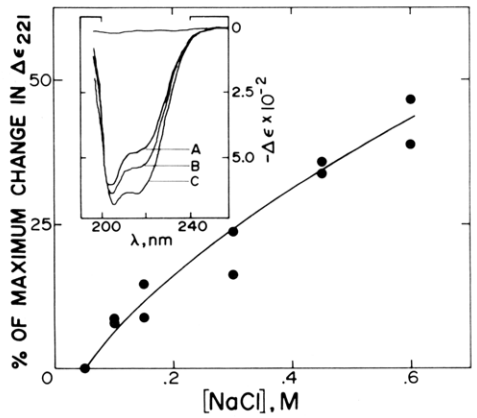

FIGURE 3: Relative changes in circular dichroism of DTNB light chains at 221 nm as a function of the salt concentration. The maximum change in Δε refers to the effect induced by Ca^{2+} (3 mM) in 10 mM NaCl, 50 mM Tris-HCl, and 2 mM EDTA, pH 7.5. Protein concentration was 0.37 mg/mL. Titrations were performed as described under Materials and Methods. Inset: circular dichroism spectra of DTNB light chains in far-UV. The scans were taken at a protein concentration of 1 mg/mL in 50 mM Tris-HCl and 2 mM EDTA, pH 7.5, and in the presence of (A) 50 mM KCl, (B) 0.5 M KCl, and (C) 0.5 M KCl and 3 mM Ca^{2+}.

Table I: Salt-Induced Changes in Fluorescence of DTNB Light Chains and TnC[a]

protein	% of the maximum effect of Ca^{2+}
DTNB light chain	25
TnC	30
TnC_{DANZ}	36

[a] The effect of salt refers to a change caused by increasing the salt concentration from 10 mM to 0.6 M NaCl (for DTNB light chain and TnC) or 0.6 M KCl (for TnC_{DANZ}). Fluorescence changes are relative to a maximum change induced by Ca^{2+} in the presence of 10 mM NaCl (DTNB light chain and TnC) or KCl (TnC_{DANZ}).

CD scans in far-UV reveal that high KCl concentrations significantly change the secondary structure of the DTNB light chain (Figure 3). The salt-induced change is of the same magnitude as the Mg^{2+} effect (not shown) but not as extensive as the structural transition caused by calcium binding to this protein. No salt effect is observed with Ca^{2+}-saturated DTNB light chain (the same far-UV scans have been recorded in the presence of Ca^{2+} in either low or high NaCl and KCl concentrations). The overall increase in the α-helical content of the DTNB light chain with the addition of Ca^{2+} is ~8% when measured in a 50 mM KCl solvent (from 22 to 30% α helix; curves A and C in Figure 3). The salt-induced change in the circular dichroism of the DTNB light chain, in the absence of divalent cations, has been titrated at 221 nm. The results of this titration are presented in Figure 3 and are relative to the maximum change induced by Ca^{2+} at this wavelength. At 0.5 M NaCl the salt-induced change approaches 40% of the Ca^{2+} effect. The transition in the CD signal and the corresponding α-helical content of the DTNB light chain does not saturate at 0.5 M NaCl. Obviously, the association of Na^+ with a protein is of a rather weak nature.

Local perturbations around the tryptophan group of the DTNB light chain have been followed by fluorescence measurements. We find that 0.6 M NaCl quenches the tryptophan fluorescence of the DTNB light chain by ~4%, which corresponds to 25% of the calcium-quenching effect (Table I). Similar quenching of the intrinsic fluorescence of light chains by bound Ca^{2+} has been observed by Alexis & Gratzer (1978).

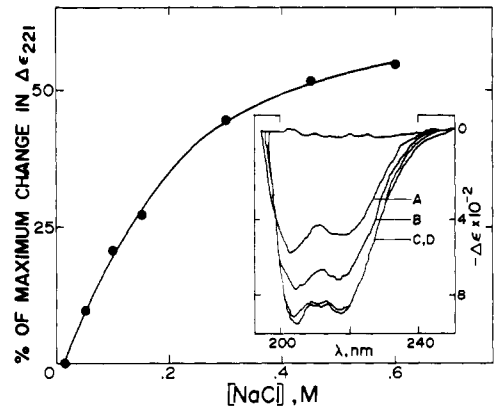

FIGURE 4: Circular dichroism titration of TnC at 221 nm as a function of the salt concentration. Maximum change in $\Delta\epsilon$ refers to the effect induced by 3 mM Ca^{2+} in 10 mM NaCl, 50 mM Tris-HCl, and 2 mM EDTA, pH 7.5. Inset: CD spectra of the TnC in far-UV. The scans were taken at a protein concentration of 0.3 mg/mL in 50 mM Tris-HCl and 2 mM EDTA, pH 7.5, and in the presence of (A) 10 mM NaCl, (B) 0.6 M NaCl, (C) 10 mM NaCl and 3 mM Ca^{2+}, and (D) 0.6 M NaCl and 3 mM Ca^{2+}.

Our results indicate that salt induces striking changes in the DTNB light chains. These changes are qualitatively comparable to changes due to the binding of Ca^{2+}. An analogy can be drawn between our results and the observations made by Leavis & Lehrer (1978) on TnC. These authors note that binding of H^+ to abnormally high pK carboxylic groups (pK_H' = 6.0) is responsible for conformational changes in TnC similar to those caused by Ca^{2+} or Mg^{2+}. These cations compete for the same site with the relative binding affinities $Ca^{2+} > H^+ > Mg^{2+}$. It appears now that Na^+ or K^+ acts in the same way as protons. Na^+ cations apparently complex the carboxylic residues which normally coordinate Ca^{2+} or Mg^{2+} on the DTNB light chain. The same should be true for TnC and parvalbumin. We may expect binding of Na^+ or K^+ to both proteins and their consequent structural perturbation. Experimental investigation of TnC and parvalbumin has verified these expectations.

Circular dichroism studies of TnC produce similar results as those seen in the DTNB light chain. Far-UV scans (Figure 4) show that high salt concentrations perturb the secondary structure of TnC in the same manner as calcium ions (transitions between curves A and B for salt and curves A and C or D for Ca^{2+} effect). The magnitude of the salt effect (at 0.5 M NaCl) is about one-half of that of calcium. The effect of Mg^{2+} on the α-helical content of TnC is smaller than the Ca^{2+} effect and approximates the transition induced by 0.5 M NaCl. The calcium-induced increase in the α-helical content of TnC amounts to ~20% when measured in a 10 mM NaCl solvent (from 39 to 58% α helix; curves A and C in Figure 4). Like with the DTNB light chain, high salt concentrations do not appreciably affect the CD spectra of Ca^{2+}-saturated TnC (curves C and D in Figure 4). However, in the absence of divalent cations, increasing the salt concentration results in a gradual increase in the CD signal and in the α-helical content of TnC.

Local perturbations in TnC may be monitored through changes in the intrinsic fluorescence of tyrosine groups or by following the fluorescence of a dansylaziridine chromophore attached to the protein. The binding of divalent cations to TnC perturbs the environment of Tyr-109 (close to the Ca^{2+}—Mg^{2+} strong binding site) and leads to a large fluorescence enhancement (Leavis & Lehrer, 1978). The equivalent proton-induced change in fluorescence does not parallel the ellipticity increase at 222 nm. Thus, fluorescence is only sensitive

Table II: Fluorescence Properties of TnC_{DANZ}

KCl (M)	divalent cations (3 mM)	λ_{max} (emission)	f/f_0[a]
0.01		525	1.00
0.01	Ca^{2+}	512	2.75
0.60		518	1.67
0.01	Mg^{2+}		1.00
0.05			1.09
0.05	Mg^{2+}		1.01
0.10			1.18
0.10	Mg^{2+}		1.00
0.60			1.65
0.60	Mg^{2+}		1.03

[a] f_0 refers to the fluorescence intensity (in arbitrary units) of TnC_{DANZ} in the presence of 10 mM KCl. Protein concentrations and other conditions are given under Materials and Methods.

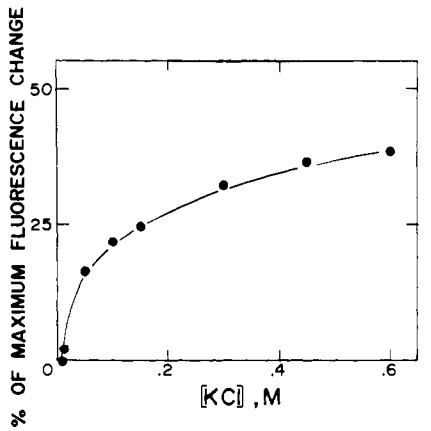

FIGURE 5: Fluorescence titration of TnC_{DANZ} (0.7 mol of DANZ per mol of TnC) as a function of the salt concentration. 100% increase in fluorescence corresponds to the effect of 3 mM Ca^{2+} in the presence of 10 mM KCl. All titrations were carried out on 0.1 mg/mL solutions of TnC_{DANZ} in 10 mM potassium phosphate and 2 mM EDTA at pH 7.0.

to conformational changes in the regions of the Ca^{2+} binding sites and not in the protein's backbone (Leavis & Lehrer, 1978). A salt-induced increase in the tyrosine fluorescence of TnC follows the same pattern. At 0.6 M NaCl it approaches only 30% of the Ca^{2+}-caused fluorescence enhancement (Table I). Moreover, the fluorescence change saturates at lower salt concentrations than the CD change. This may be attributed to the difference between overall and local structural changes in TnC.

The fluorescent probe dansylaziridine (DANZ) is particularly attractive in studying the conformational changes in TnC. It is incorporated into the TnC tryptic fragment, T3, attaching to Met-25 near a Ca^{2+}-specific site (Johnson et al., 1978). When Ca^{2+} or Mg^{2+} binds to the Ca^{2+}–Mg^{2+} sites (in the presence of 90 mM KCl), a small decrease in TnC_{DANZ} fluorescence is noted. Ca^{2+} binding to Ca^{2+}-specific sites produces a twofold fluorescence enhancement (Johnson et al., 1978). Consequently, the DANZ chromophore is well suited for probing structural perturbations around the Ca^{2+}-specific sites. Examination of the salt effect on the fluorescence of TnC_{DANZ} reveals spectral changes consistent with perturbation of the Ca^{2+}-specific sites. Increasing the salt concentration from 10 mM to 0.6 M KCl leads to a 7-nm blue shift in the wavelength of the fluorescence maximum emission (Table II). This is nearly 50% of the blue shift observed upon Ca^{2+} addition. Increasing the concentration of KCl results in an increase in TnC_{DANZ} fluorescence (Figure 5), suggesting that monovalent cations affect the weak Ca^{2+} binding sites. Interestingly, the salt-induced enhancement of fluorescence is

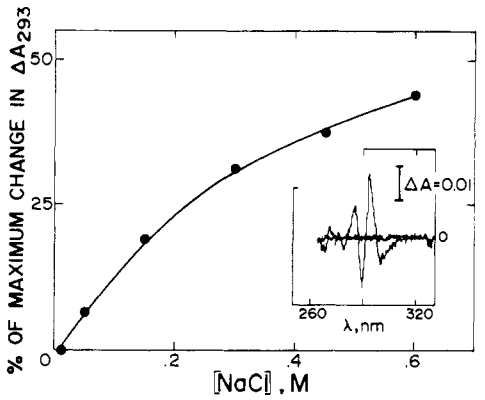

FIGURE 6: Difference absorption titration of Whiting parvalbumin at 293 nm as a function of salt concentration. 100% increase in ΔA_{293} (0.084 OD) corresponds to the effect of 3 mM Ca^{2+} in the presence of 10 mM NaCl. Protein concentration was 0.4 mg/mL. Titration buffer contained 50 mM Tris-HCl and 2 mM EDTA at pH 7.5.

canceled by addition of Mg^{2+} to TnC_{DANZ} (Table II). This implies that Mg^{2+} saturation of the Ca^{2+}–Mg^{2+} sites affects the vicinity of the Ca^{2+}-specific sites and their interaction with monovalent cations.

The original observation of the binding of sodium cations to Ca^{2+}-binding proteins had been made on carp parvalbumin by employing magnetic resonance techniques (Grandjean et al., 1977). Recently, this finding has been challenged on the basis of NMR measurements at a single salt concentration (Parello et al., 1979). We have been prompted to examine the interaction of parvalbumin with monovalent cations in order to resolve this contradiction. We have also wished to ascertain whether the salt effect observed in TnC and in the DTNB light chains could be detected in a structurally related protein. Whiting parvalbumin has been chosen for these studies because of the presence of tryptophan and tyrosine, which are absent in other parvalbumins (Joassin, 1974). The tryptophan residue, Trp-102, is located close to a calcium binding site. Difference spectra studies on Whiting parvalbumin show relatively large salt-dependent changes (Figure 6). They have been titrated with salt by monitoring tryptophan perturbation at 293 nm (Figure 6). As already observed with TnC and the DTNB light chain, the magnitude of this change is a function of the salt concentration.

At 0.6 M NaCl our results show ~45% of the Ca^{2+} effect. These findings support the original observations of Grandjean et al. (1977) on the binding of Na^+ to parvalbumin.

Discussion

Calcium-binding regulatory proteins undergo large conformational changes upon the binding of Ca^{2+}. These changes have been most extensively investigated in troponin-C. They are believed to be important and may be functional under physiological conditions (Leavis et al., 1978). In this contribution we report studies which indicate that monovalent cations induce striking changes in DTNB light chain, TnC, and Whiting parvabumin. We observe by digestion studies and CD, fluorescence, and difference spectra that salt-induced perturbations in these proteins are qualitatively similar to those caused by calcium binding. The binding of sodium or potassium cations to TnC, DTNB light chain, and parvalbumin is probably analogous to the previously described binding of H^+ to TnC (Lehrer & Leavis, 1974; Leavis & Lehrer, 1978). Quantitatively, the effects of Na^+ and K^+ may not be identical, but in view of their similarity they are referred throughout as "salt" effects.

The magnitude of the salt effect (at 0.6 M NaCl) on the α-helical content of both TnC and DTNB light chains is close to 50% of the calcium-induced change. Local perturbations around tyrosine or tryptophan residues, monitored by intrinsic fluorescence measurements, are smaller. High salt concentrations induce ~30% of the Ca^{2+}-affected change.

A better insight into the salt effect on the Ca^{2+} binding sites of the investigated proteins is obtained through a combination of intrinsic and extrinsic fluorescence studies on TnC. Enhancement of intrinsic tyrosine fluorescence by monovalent cations can be equated with the perturbation of Tyr-109 near the strong Ca^{2+}–Mg^{2+} binding site. This is based on the analogy to the Ca^{2+} effect on TnC fluorescence (Leavis & Lehrer, 1978; Leavis et al., 1978). The effect of salt is not limited to the Ca^{2+}–Mg^{2+} binding sites. Dansylaziridine fluorescence of labeled TnC shows that the Ca^{2+}-specific sites are also affected. The fact that Mg^{2+} binding to the high-affinity sites can completely cancel the salt-induced effect on the danzylaziridine probe provides strong evidence of site–site interactions within TnC. This type of interaction is indicated by NMR studies, which detect that Ca^{2+} saturation of the Ca^{2+}–Mg^{2+} sites produces changes in the structure of Ca^{2+}-specific sites (Levine et al., 1977; Seamon et al., 1977). Measurements of Ca^{2+} binding to proteolytic fragments of TnC are also consistent with site–site interactions. Once the first mole of Ca^{2+} is bound, the other sites have a reduced affinity for Ca^{2+} (Leavis et al., 1978). In our experiments, the binding of Mg^{2+} to the high-affinity sites apparently reduces the affinity of the weak binding sites for the monovalent cations. This suggests a more subtle form of site–site interaction than implied by the quenching of the TnC_{DANZ} fluorescence by Mg^{2+} binding to the Ca^{2+}–Mg^{2+} sites (Johnson et al., 1978). We believe that the Mg^{2+}-caused quenching of fluorescence reflects the cancellation of the salt effect on the Ca^{2+}-specific sites.

At present, no data are available about the nature and the extent of structural changes that DTNB light chains complexed to myosin undergo upon the binding of calcium. Paramagnetic resonance spectra of Mn^{2+} bound to the isolated DTNB light chain and glycerinated muscle are virtually identical (Bagshaw & Reed, 1977). This suggests that there are no changes occurring in the metal binding site upon the isolation of the light chain. Our own results indicate that there is a correlation between the α-chymotryptic susceptibility of the isolated and complexed DTNB light chain (S. Oda and E. Reisler, unpublished experiments). Thus, it appears that the DTNB light chain complexed to myosin can undergo conformational changes similar to those of the isolated chain. It is questionable whether such changes actually occur in the myosin filament upon the binding of Ca^{2+}. Although the hydrodynamic properties of thick filaments seem to change with the binding of Ca^{2+} (Morimoto & Harrington, 1974), fluorescence depolarization studies (Mendelson & Cheung, 1976), cross-linking experiments (Sutoh & Harrington, 1977), and electron microscopy (Elliott & Offer, 1978) fail to confirm any specific Ca^{2+} effect. Some studies present evidence for the involvement of DTNB light chains in actomyosin ATPase (Lehman, 1977; Malhotra et al., 1979). However, it appears that Ca^{2+} displaces bound Mg^{2+} too slowly to act as the myosin-linked switch in the activation of contraction (Bagshaw & Reed, 1977).

The strongest evidence for Ca^{2+}-mediated structural changes in the thick filament has been obtained from chymotryptic digestion studies of myosin (Weeds & Pope, 1977). A switch in the digestion products of polymeric myosin (low salt), from subfragment 1 to heavy meromysin, occurs upon the binding of Ca^{2+}. Our data suggest that there is a correlation between

this switch in products in low salt and the proteolytic production of heavy meromyosin in high salt. As shown in this work, monovalent cations and Ca^{2+} (or Mg^{2+}) induce similar structural changes in the isolated DTNB light chain. It is deduced that both salt (at high concentrations) and Ca^{2+} apparently protect the DTNB light chain and consequently prevent proteolytic cleavage at the subfragment 1 site. This site is apparently masked by the DTNB light chains. Digestions of monomeric myosin in very low salt concentrations (in pyrophosphate buffer) support this conclusion (S. Oda and E. Reisler, unpublished experiments). Such digestions yield subfragment 1 in the absence of divalent cations and heavy meromyosin in the presence of Ca^{2+}. In this case, the switch in the products must be related to the protection of the DTNB light chain rather than to changes in myosin structure or organization.

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