Heat Treatment Could Affect the Biochemical Properties of Caldesmon*

Shaobin Zhuang, Katsuhide Mabuchi, and C.-L. Albert Wang‡

From the Muscle Research Group, Boston Biomedical Research Institute, Boston, Massachusetts 02114

Smooth muscle caldesmon (CaD) exhibits apparent heat stability. A widely used purification procedure of CaD involves extensive heat treatment (Bretscher, A. (1984) J. Biol. Chem. 259, 12873-12880). CaD thus purified co-sediments with actin, inhibits actomyosin ATPase activity, and interacts with Ca2+/calmodulin, similarly to the unheated protein. On the other hand, heat-treated CaD binds to actin filaments in a tether-like fashion, whereas lengthwise binding dominates in vivo (Mabuchi, K., Lin, J. J.-C., and Wang, C.-L. A. (1993) J. Muscle Res. Cell Motil. 14, 54-64), suggesting that differences do exist between heat-purified CaD and the native protein. We have isolated, without heat treatment, full-length recombinant chicken gizzard CaD overexpressed in insect cells (High-Five™) using a baculovirus expression system. We found that such unheated CaD interacts with calmodulin 10 times stronger than does the heated CaD; its inhibitory action on actomyosin ATPase is reversed by a much lesser amount of calmodulin. Moreover, electron microscopic examination indicated that actin binding at the N-terminal region is more frequent in the unheated CaD, resulting in more lengthwise binding. These findings point to the fact that CaD is not entirely heat-stable; the C-terminal CaM-binding regions and the N-terminal actin-binding region are possibly affected by heat treatment.

Caldesmon (CaD)§ is an actin-binding protein found in smooth muscle and many non-muscle cells (for reviews, see Marston and Redwood (1991) and Matsumura and Yamashiro (1993)). It also interacts with myosin, tropomyosin, and calmodulin (CaM). In vitro CaD inhibits the actomyosin ATPase activity, and this inhibition is reversible by Ca2+/CaM. An inhibitory role of CaD in vivo was also implicated by the observation that an 18-residue CaM-binding CaD peptide (GS17C), which also binds actin in a manner similar to the intact protein, is able to induce force in a permeabilized smooth muscle cell, presumably by nudging off the endogenous CaD from its inhibitory position (Katsuyama et al., 1992). It was proposed that CaD regulates smooth muscle contraction by a troponin-like mechanism (Marston et al., 1994), but direct evidence for such a hypothesis has not been established.

CaD, when free in solution, is extremely sensitive to proteolysis. Because of its apparent heat stability, a widely used purification procedure of CaD involves extensive heat treatment (Bretscher, 1984; Lynch and Bretscher, 1986). Upon boiling for 10–15 min most of the proteins in the tissue homogenate, including proteases, precipitate, and are thereby easily removed by centrifugation, resulting in a protein preparation that is relatively stable in the absence of Ca2+. Since the secondary structure of CaD undergoes reversible helix-coil transition upon heating (Graceffia and Jancsó, 1993; Wang et al., 1991), it is generally accepted that thermally unfolded CaD would return to its “native” conformation upon cooling. CaD thus purified retains its capacity to co-sediment with actin, to interact with Ca2+/CaM, as well as the ability to inhibit the actomyosin ATPase activity. On the other hand, heat-purified CaD binds to reconstituted actin filaments in a tether-like fashion, whereas lengthwise binding appears to dominate in the native thin filament (Lehman et al., 1989; Mabuchi et al., 1993), suggesting subtle differences between heat-purified CaD and the native protein.

In this work we have isolated full-length recombinant chicken gizzard CaD from insect (High-Five™) cells without heat treatment. We were able to do this rather easily, probably because of the much lower proteolytic activities present in these cells. We found that such unheated CaD binds CaM with an affinity that is at least an order of magnitude higher than that of CaD purified with heat treatment. More interestingly, electron microscopy indicates stronger actin binding at the N-terminal region, in comparison to the heated CaD. This finding indicates possible existence of an additional, heat-labile, actin-binding site in the N-terminal region of CaD, and also raises the possibility that the difference previously observed in the binding modes between the native and the reconstituted systems can be explained, at least partly, by alterations of CaD by heating.

MATERIALS AND METHODS

Expression and Purification of Recombinant CaD—500 ml of High-Five™ cells (Invitrogen) at a density of 4 × 10⁶ cells/ml were grown in suspension in a 1-liter spinner (CYTOSTIR, Knotes) in Ex-cell 405 medium (JRH Bioscience) at 28 °C for 24 h with stirring at 60 rpm. The cells were infected with pVLCaD viral stock (a gift from Dr. J. Bryan) at a multiplicity of infection of 5. The infected cells were harvested at maximal expression (48 h after infection). Cells were pelleted by centrifugation, resuspended in 50 ml of buffer A (100 mM KCl, 20 mM Tris-HCl, pH 7.5, 50 mM EDTA, 0.25 mM PMSF, 5 μM leupeptin), frozen and thawed twice, and centrifuged at 180,000 × g for 40 min. The supernatant was, after addition of CaCl₂ to a final concentration of 2 mM, loaded onto a CaM-Sepharose 4B column. The column was washed with buffer B (100 mM KCl, 20 mM Tris-HCl, pH 7.5, 1 mM DTT, 1 mM CaCl₂, 0.25 mM PMSF, 5 μM leupeptin), followed by elution with 5 mM EDTA in buffer B. Fractions containing CaD, as determined by SDS-PAGE, were pooled and loaded onto a fast protein liquid chromatography mono Q column (Pharmacia Biotech Inc.). Proteins were eluted with a 0.1–0.6 M NaCl gradient in buffer C (0.1 mM EDTA, 20 mM Tris-HCl, pH 7.0, 1 mM DTT, 0.25 mM PMSF, 5 μM leupeptin). The

*This work was supported by National Institutes of Health Grant P01-AR41637. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom the correspondence should be addressed: Boston Biomedical Research Institute, 20 Stanford St., Boston, MA 02114. Tel.: 617-742-2010 (ext. 376); Fax: 617-523-6649; E-mail: Wang@bbri.harvard.edu.

§ The abbreviations used are: CaD, caldesmon; CaM, calmodulin; DTT, dithiothreitol; MLCK, smooth muscle myosin light chain kinase; PMSF, phenylmethlysulfonyl fluoride; PAGE, polyacrylamide gel electrophoresis; PIPES, piperazine-N,N’-bis(2-ethanesulfonic acid).
purity of CaD (eluted at ~0.2 M NaCl) was examined by SDS-PAGE. The elution yield was ~20 mg/liter of cells. CaD was also purified after heat treatment; the procedure was the same as described above, except for a 2-min boiling of the cell suspension in buffer A after freeze and thaw. Heating had little effect on the yield. For the concentration determination of purified CaD, an extinction coefficient of ε_{280 nm} = 3.3 was used (Graceffa et al., 1988).

Other Proteins—Recombinant chicken brain CaM was purified from *Escherichia coli* (BL21/DE3) cells by phenyl-Sepharose column chromatography (Dedman and Kaetzel, 1983). Skeletal actin was prepared from rabbit skeletal muscle according to Spudich and Watt (1971). Smooth muscle CaD (Lynch and Bretschner, 1986; Wang, 1988), myosin (Ikebe et al., 1978), MLCK (Walsh et al., 1983), and tropomyosin (Graceffa, 1987) were purified from chicken gizzard. Purified smooth muscle myosin was phosphorylated by MLCK as described previously (Zhang et al., 1995).

**Fluorescence Titrations with CaM—Binding of CaM to both heated and unheated recombinant CaD was studied by adding aliquots of the CaM stock solution (190 μM) to 0.6 ml of a solution containing 1–3 μM CaD in 50 mM KCl, 1 mM MgCl₂, 20 mM Pipes, pH 6.9, and monitoring the tryptophan emission (λ_{em} = 295 nm; λ_{exc} = 280 nm) of CaD on a Perkin-Elmer MPF-4 fluorimeter. The titration data were fitted to a binding equation as described previously (Zhang et al., 1995).

**ATPase Assay**—The Mg-ATPase activities of the actomyosin complex were measured as described previously (Zhang et al., 1995). Briefly, phosphorylated chicken gizzard myosin (~2 μM), rabbit skeletal F-actin (12 μM), chicken gizzard tropomyosin (1.7 μM) were mixed in a solution containing the following reagents: 60 mM NaCl, 20 mM Tris-HCl (pH 7.5), 0.4 mM DTT, 2 mM ATP, 4 mM MgCl₂, and 0.2 mM CaCl₂. The reaction was allowed to proceed for 40 min at 25 °C before termination with AcMNPV-based vectors is SF-9 cell line. Full-length recombinant chicken brain CaD was previously overexpressed in SF-9 cells and purified by conventional procedures that includes a heating step; the insect cell-expressed CaD exhibited identical biochemical properties as CaD purified from gizzard (Wang et al., 1994). In this report we took advantage of the High-Five™ cell line (BTI-TN-5B1-4) that has recently become commercially available. Compared to SF-9 cells, High Five™ cells have shorter doubling time and offer high expression levels for many recombinant proteins (Phillips et al., 1994).

The insect cell-expressed CaD, purified without heat treatment by a two-step procedure (see "Materials and Methods"), was reasonably pure on SDS-gel electrophoresis (Fig. 1), and analytical ultracentrifugation showed a single Gaussian peak with a sedimentation coefficient s_{20\,\text{w}} = 2.9 S (data not shown), corresponding to a monomeric molecular mass (M_{app}) of ~88,000. Overexpressed CaD inside the insect cells appeared to be in aggregated forms, which could be broken up by treatment with high concentrations (e.g. 50 mM) of DTT in the extraction buffer and 1 mM DTT in the subsequent buffers.

**Binding of CaD to CaM—CaD** was originally identified as a CaM-binding protein (Sobue et al., 1981). Subsequent studies on the CaD-CaM interactions by various methods yielded an affinity at around μM range (Malencik et al., 1988; Pritchard and Marston, 1989; Shirinsky et al., 1988; Smith et al., 1987). In particular, since binding is accompanied by a conformational change in CaD that increases its tryptophan fluorescence intensity, by monitoring the tryptophan fluorescence change, the binding constant was determined to be 1.8 × 10^{9} M^{-1} (Shirinsky et al., 1988). A C-terminal synthetic peptide, GS17C, also binds CaM with a similar affinity (Zhan et al., 1991). Such a weaker affinity relative to other CaM targets (e.g. MLCK, K_{app} = 10^{9} M^{-1}) (see Blumenthal et al., 1985) has raised concerns...
whether or not there is enough CaM in the cell to allow CaM-CaD interactions to be physiologically relevant (Marston and Redwood, 1991). These concerns have led some laboratories to yet inconclusive searches for other Ca\(^{2+}\)-binding proteins that would exhibit higher affinities for CaD (Pritchard and Marston, 1988). On the other hand, despite its lower association constant, CaM’s regulatory action may still be accomplished by a relatively rapid on-rate of binding to CaD, as demonstrated by kinetic studies (Kasturi et al., 1993).

We have examined the interactions between CaM and recombinant CaD by monitoring the tryptophan fluorescence of CaD. Both the heated and unheated CaD were titrated with CaM, and the fluorescence changes were analyzed by curve fitting to a binding equation (Morris and Lehrer, 1984). The binding constant for the heated CaD was (2.3 \pm 0.3) \times 10^6 \text{ M}^{-1}, in agreement with previous studies of heated gizzard CaD (Shirinsky et al., 1988); other parameters include the apparent stoichiometry \( n = 0.76 \pm 0.08 \), and the overall fluorescence enhancement \( (F/F_0)_{\text{max}} = 3.05 \pm 0.04 \) (Fig. 2). The binding constant for the unheated CaD, on the other hand, was found to be \( (2.0 \pm 0.9) \times 10^5 \text{ M}^{-1} (n = 0.69 \pm 0.10); (F/F_0)_{\text{max}} = 2.18 \pm 0.03 \); see Fig. 2). Thus CaD purified without heat treatment interacts with CaM one order of magnitude more strongly than that prepared by boiling. The simplest explanation is that CaM-binding of CaD is weakened by heating; such an effect may result from a heat-induced, apparently irreversible, conformational change in the C-terminal portion of the CaD molecule. This would also suggest that the CaM-CaD interaction has a better chance to play a physiologically significant role.

It should be pointed out that several earlier studies also utilized CaD prepared without heating (Bretscher, 1984; Ngai and Walsh, 1984; Sobue et al., 1985), although quantitative measurements of the affinity for CaM have been lacking. The only detailed report using unheated CaD (from sheep aorta) yielded a binding affinity of \( 0.8 \times 10^6 \text{ M}^{-1} \) (Smith et al., 1987); but since the reported purity of this preparation by a rather lengthy procedure was only 70–80\% (Smith and Marston, 1985), it can not be ruled out that the observed weaker CaD-CaM interaction was due to proteolysis during purification. It is known that CaD is extremely susceptible to proteolysis; in fact one of the major gains of the heat treatment was to rapidly remove the proteases, so that a better yield could be achieved. Apparently High-Five\textsuperscript{TM} cells contain much less proteases than gizzard smooth muscle cells, thus allowing us to purify CaD without heating.

**FIG. 2. Effect of heat treatment on the affinity of CaD for CaM.** Unheated (closed symbols) and heated (open symbols) CaD (1.4 \text{ \mu M}) was titrated with a stock solution of CaM (190 \text{ \mu M}) in the presence of 1 mM CaCl\(_2\). The tryptophan fluorescence (\( \lambda_{\text{ex}} = 295 \text{ nm}; \lambda_{\text{em}} = 320 \text{ nm} \)) was monitored. The curves are best fits to a single-sited binding equation.

**FIG. 3. Effect of heat treatment on (A) the ability of CaD to inhibit actomyosin ATPase activity and (B) the efficiency of CaM to reverse such inhibition.** For assay conditions, see "Materials and Methods." In A, 0.5–2.0 \text{ \mu M} unheated recombinant CaD (closed circles), heated recombinant CaD (open circles), and heated gizzard native CaD (open triangles) were used; in B, 2 \text{ \mu M} heated (open circles) or unheated recombinant CaD (closed circles) was used to inhibit the ATPase activity, and 4–24 \text{ \mu M} CaM was used to restore the activity. Smooth curves were drawn only to show the trend. 100\% ATPase activity corresponds to 35 nmol of P/mg of myosin/min.

**Effect of CaD on the Actomyosin ATPase Activity—**Unheated recombinant CaD inhibited the actin-activated ATPase activity of phosphorylated smooth muscle myosin more effectively than both the heat-treated recombinant CaD and chicken gizzard CaD (Fig. 3A). For the same amount of added CaD, the unheated protein consistently resulted in a greater inhibition than the heated proteins. The inhibition caused by either heated or unheated CaD was reversed by Ca\(^{2+}\)/CaM, but the efficiency of such reversal appeared to be different between the two cases. A mere 1-fold excess of total CaM resulted in about 70\% recovery of the inhibited ATPase activity caused by heated CaD, whereas a 4-fold excess of CaM was needed to achieve the same level of deinhibition when the heated CaD was used (Fig. 3B), as shown by much of the earlier reports. That CaM is able to reverse the inhibition induced by unheated CaD at a lower concentration is consistent with its observed higher affinity toward the unheated CaD.

It has become apparent that the molecular mechanism involved in the inhibitory effect of CaD on the actomyosin interaction and its reversal by Ca\(^{2+}\)/CaM is more complicated than
the previously suggested “flip-flop” model (Sobue et al., 1982). There are experimental data indicating that even an excess amount of CaD fails to completely inhibit the smooth muscle actomyosin ATPase activity (Nomura and Sobue, 1987; Sobue et al., 1985), and that the pattern of CaM-induced deinhibition is not always parallel to the binding of CaM to CaD (Pritchard and Marston, 1989). While a more satisfactory mechanism for the deinhibition is still to emerge, the true picture may have been obscured by the varying quality of the purified CaD itself. From this study it is clear that, compared to the conventionally prepared CaD, unheated CaD is more effective in both inhibiting the actomyosin ATPase activity and being de-inhibited by CaM, indicating that either heating itself or the cooling process following the heat treatment may cause alterations in the functional properties of CaD.

**Electron Microscopic Examination of Unheated CaD Molecules**—To visualize the molecular shape of unheated CaD, insect cell-expressed CaD, and F-actin were first mixed in high salt (0.25 M ammonium acetate) and examined by electron microscopy. We found that a globular structure appeared at the C terminus (arrows at the end opposite to the bound antibody; Fig. 4A) of many unheated CaD, including those isolated from chicken.
gizzard (Fig. 4C, arrows), but rarely seen for the heated proteins (Fig. 4B). As there was no detectable difference between heated and unheated CaD in their SDS-gel electrophoretograms, it is unlikely that such a globular structure represents another bound protein. Moreover, since the globular structure was not detected when examined by the spray method (data not shown), which is known to generate strong shearing force, it is quite possible that the C-terminal region of unheated CaD is indeed globular but flexible enough to be deformed by heating or shearing force. The change in the molecular shape of the C-terminal region of CaD may be related to the observed difference in the affinity toward CaM.

Also noted was that many unheated CaD remained unbound and only a few molecules were closely associated with the actin filament (Fig. 4A). There appeared to be more CaD/anti-CaD complexes associated with actin filaments when heated CaD was used (Fig. 4B). This apparently higher degree of binding, however, may have resulted from the fact that heated recombinant CaD easily formed oligomers (asterisks). Because of the aggregation problem, these samples were deemed not suitable for the analysis of actin-binding by electron microscopy, and CaD purified from chicken gizzard with heating was used in-

---

**Fig. 5. Rotary shadowed electron micrographs of CaD incubated with reconstituted actin filaments.** Insect cell-expressed unheated CaD (A) or conventionally purified (heated) chicken gizzard CaD (B) was first labeled with anti-CaD and then mixed with actin filaments in a solution containing 0.1 M ammonium acetate and 30% glycerol for visualization of complexes. It was assumed that the presence of anti-CaD on or very near the actin filaments (arrowheads, which were placed without masking any antibodies) indicates the presence of CaD molecules bound to actin filaments at their N termini (see text). The C termini of a few free CaD molecules were marked by arrows. Magnification, × 75,000.

---

2 P. Graceffa, unpublished results.
stead (see below).

Electron Microscopic Examination of Heating Effect on CaD Binding to F-actin—There also appeared some differences between heated and unheated CaD in actin binding. Such differences mainly occurred in the N-terminal region of CaD. We found that the N-terminal region of unheated CaD exhibited stronger actin binding than that of the heated CaD. To quantitatively describe the difference in actin binding, we have carried out a statistical analysis of the location of the anti-N terminus antibody in the electron micrograph. As the heated, insect-expressed CaD was unsuitable for this analysis owing to oligomer formation (see above and Fig. 4), conventionally purified chicken gizzard CaD was used instead as a comparison. This is not unreasonable in view of the fact that biochemical studies indicated no differences between the two heat-treated CaD preparations (Wang et al., 1994). In doing so, we assumed that antibodies on or very near the actin filaments (Fig. 5, A and B, arrowheads) represent CaD bound at its N terminus, while those slightly away from the filaments, but within a distance corresponding to the length of a CaD molecule (∼70 nm), represent CaD bound at its C terminus. Examination of a total of ∼1400 (unheated recombinant CaD) and ∼2000 (heated gizzard CaD) antibody/CaD molecules indicated that ∼60% of unheated CaD, but only ∼30% of heat purified CaD, bind at its N terminus, as exemplified in Fig. 5, A and B.

To avoid subjective judgment in describing the distribution of anti-CaD, we have measured the distances between the far edge of antibody molecules and the nearest actin filaments in Fig. 5 (magnification of × 150,000) and plotted these distances on histograms (Fig. 6). The results clearly demonstrate that there are more antibodies on or very near the actin filament in the unheated sample (Fig. 6B) than the heated one (Fig. 6A). The abrupt drop of antibody counts at 10 mm (corresponding to ∼70 nm) in both CaD preparations is reasonable for the distribution of the bound CaD considering its dimension. Since the epitope of this antibody is in the N-terminal region of CaD, the higher degree of close association between the antibody and actin filament indicates that unheated CaD is likely to bind actin in a more lengthwise manner than heated CaD.

On the other hand, when compared with the heated sample (Fig. 6A), the unheated CaD preparation (Fig. 6B) exhibited a greater number of antibody molecules much farther away from the filaments, which obviously are the free CaD molecules, thus indicating a smaller fraction of bound CaD. An estimate of the stoichiometry of bound CaD to actin by counting the average number of anti-CaD molecules on a unit length of actin filament yielded 1 CaD per ∼14 actin subunits for the heated CaD and 1 CaD per ∼20 actin subunits for the unheated CaD. Such a difference in the stoichiometry may be explained by the lengthwise binding of the unheated CaD which results in fewer available binding sites along the actin filament. Further studies are needed to verify this point.

There have been three groups showing independently that purified CaD forms tether-like structure when it binds to actin filaments (Katayama and Ikebe, 1995; Mabuchi et al., 1993; Moody et al., 1990). However, while the two earlier reports acknowledged that tethering might be artificial, the more recent one (Katayama and Ikebe, 1995) argued that tethered binding is the only way CaD associates with actin filaments. It is known that freshly prepared thin filaments do not bundle, whereas aged samples do. Since bundling activity could result from the ability of tethered CaD to cross-link actin filaments, it is conceivable that some CaD molecules in the native thin filaments that have been handled improperly or stored too long become tethered form. Similar phenomenon may account for the observations made with the reconstituted filaments using heated CaD.

We have previously shown that both ends of heat-purified CaD could tether to actin filaments in vitro, although the majority of binding occurred at the C-terminal region (Mabuchi et al., 1993). In contrast, native thin filaments isolated from chicken gizzard did not show any sign of tethering of CaD (Mabuchi et al., 1993), indicating that the N-terminal region of CaD in situ is not projecting away from actin filaments. Therefore, it was proposed that the N-terminal portion of intact CaD may actually interact with actin filaments strongly enough to confer a lengthwise binding, instead of tethered binding as seen in the reconstituted system. This difference raises the possibility that the structure of the N-terminal region of CaD may be altered during purification, such as heat treatment. Our finding of the unheated CaD indeed supports this hypothesis.

CONCLUSION

We have shown that it is possible to purify CaD more readily from insect cells using the baculovirus expression system by a two-step procedure without heat treatment. Compared to the similarly prepared CaD from smooth muscle tissues, the recombinant protein offers much less aggregation, lower proteolysis, and therefore, a much higher yield. More significantly,
such unheated CaD exhibits more lengthwise binding to actin filaments than heat-treated CaD, suggesting that, although the majority of the structural characteristics of CaD is heat-resistant, there are certain subtle, heat-labile elements, such as an additional actin-binding site in the N-terminal region of CaD. Also affected by heating is the tertiary folding in the C-terminal domain, which confers a stronger interaction with CaM, thus allowing CaM to function as a physiological regulator for CaD's inhibitory effect. These results suggest that full-length recombinant CaD purified without heat treatment alleviates complications owing either to improper renaturation after heating or to proteolysis when purified from tissues and not using heating, thus offering a better and more consistent quality of preparations. Furthermore, the difference previously observed in the binding modes between the native and the reconstituted systems can also be explained without invoking another protein component.

Acknowledgment—We thank Dr. Philip Graceffa for helpful discussions, Dr. Walter F. Stafford for ultracentrifugation analysis, Dr. Jim J.-C. Lin for the monoclonal anti-CaD antibodies, and Drs. Joseph Bryan and Samuel Chacko for sharing their pVLCaD viral stock and insect cells.

REFERENCES
Blumenthal, D. K., Takio, K., Edelman, A. M., Charbonneau, H., Titani, K., Walsh, K. A., and Krebs, E. G. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3187–3191
Bretscher, A. (1984) J. Biol. Chem. 259, 12873–12880
Dedman, J. R., and Kaetzel, M. A. (1983) Methods Enzymol. 102, 1–8
Fiske, C. H., and Subbarow, Y. (1925) J. Biol. Chem. 66, 375–400
Graceffa, P. (1987) FEBS Lett. 218, 139–142
Graceffa, P., and Jancsò, A. (1993) Arch. Biochem. Biophys. 307, 21–28
Graceffa, P., Wang, C.-L. A., and Stafford, W. F. (1988) J. Biol. Chem. 263, 14196–14202
Ikebe, M., Aiha, T., Onishi, H., and Watanabe, S. (1978) J. Biochem. (Tokyo) 83, 1643–1655
Kasturi, R., Vuulk, C., and Johnson, J. D. (1993) J. Biol. Chem. 268, 7958–7964
Katayama, K., and Ikebe, M. (1995) Biophys. J. 68, 2419–2428
Katsuyama, H., Wang, C.-L. A., and Morgan, K. G. (1992) J. Biol. Chem. 267, 14555–14558
Lehman, W., Craig, R., Lui, J., and Moody, C. (1989) J. Muscle Res. Cell Motil. 10, 101–112
Lin, J. J.-C., Davis-Nanthakumar, E. J., Jin, J.-P., Lourim, D., Novy, R. E., and Lin, J. L.-C. (1991) Cell Motil. Cytoskeleton 20, 95–108
Lynch, W., and Bretscher, A. (1986) Methods Enzymol. 134, 37–42
Mabuchi, K. (1991) J. Struct. Biol. 107, 22–28
Mabuchi, K., Lin, J. J.-C., and Wang, C.-L. A. (1993) J. Muscle Res. Cell Motil. 14, 54–64
Maiencik, D. A., Ausio, J., Byles, C. E., Modrell, B., and Anderson, S. R. (1989) Biochemistry 28, 8227–8233
Marston, S. B., and Redwood, C. S. (1991) Biochem. J. 270, 1–16
Marston, S. B., Fraser, I. D. C., and Huber, P. A. J. (1994) J. Biol. Chem. 269, 32104–32109
Matsumura, F., and Yamashiro, S. (1993) Curr. Opin. Cell Biol. 5, 79–76
Moody, C., Lehman, W., and Craig, R. (1990) J. Muscle Res. Cell Motil. 11, 176–185
Morris, E. P., and Lehrer, S. S. (1984) Biochemistry 23, 2214–2220
Ngai, P. K., and Walsh, M. P. (1984) J. Biol. Chem. 259, 13656–13659
Nomura, M., and Sobue, K. (1987) Biochem. Biophys. Res. Commun. 144, 936–943
Philips, J. E., Cooper, S. T., Potter, E. E., and Church, F. C. (1994) J. Biol. Chem. 269, 16696–16709
Pritchard, K., and Marston, S. B. (1988) in Sarcomeric and Non-Sarcomeric Muscles: Basic and Applied Research Prospects for the 90's (Carraro, U., ed) pp. 649–654, Unipress Padova, Padova, Italy
Pritchard, K., and Marston, S. B. (1989) Biochem. J. 257, 839–843
Shirinsky, V. P., Bushueva, T. L., and Frolova, S. I. (1988) Biochem. J. 255, 203–208
Smith, C. W. J., and Marston, S. B. (1985) FEBS Lett. 184, 115–119
Smith, C. W. J., Pritchard, K., and Marston, S. B. (1987) J. Biol. Chem. 262, 116–122
Sobue, K., Muramoto, Y., Fujita, M., and Kakiuchi, S. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 5652–5655
Sobue, K., Morigumo, K., Inui, M., Kanda, K., and Kakiuchi, S. (1982) Biomed. Res. 3, 188–196
Sobue, K., Takahashi, K., and Wakabayashi, I. (1985) Biochem. Biophys. Res. Commun. 132, 645–651
Spudich, J. A., and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871
Walsh, M. P., Hinkins, S., Dabrowska, R., and Hartshorne, D. J. (1983) Methods Enzymol. 99, 279–288
Wang, C.-L. A. (1988) Biochem. Biophys. Res. Commun. 156, 1033–1038
Wang, C.-L. A., Chalovich, J. M., Graceffa, P., Lu, R. C., Mabuchi, K., and Stafford, W. F. (1991) J. Biol. Chem. 266, 13958–13963
Zhan, Q., Wang, E., and Wang, C.-L. A. (1991) J. Biol. Chem. 266, 21810–21814
Zhuang, S., Wang, E., and Wang, C.-L. A. (1995) J. Biol. Chem. 270, 19964–19968