209.
Pope, B. J., Wagner, P. D., and Weeds, A. G. (1977), *J. Mol. Biol. 109,* 470-473.
Reisler, E., Burke, M., and Harrington, W. F. (1974), *Biochemistry 13,* 2014-2022.
Sarkar, S. (1972), *Cold Spring Harbor Symp. Quant. Biol. 37,* 14-17.
Schaub, M. C., and Perry, S. V. (1971), *Biochem. J. 123,* 367-377.
Schaub, M. C., Watterson, J. G., and Waser, P. G. (1975), *Hoppe-Seyler's Z. Physiol. Chem. 356,* 325-339.
Seidel, J. C., and Gergely, J. (1971), *Biochem. Biophys. Res. Commun. 44,* 826-830.
Sekine, T., and Yamaguchi, M. (1963), *J. Biochem. (Tokyo) 54,* 196-198.
Sekine, T., Barnett, L. M., and Kielley, W. W. (1962), *J. Biol. Chem. 237,* 2769-2772.
Sobieszek, A., and Bremel, R. D. (1975), *Eur. J. Biochem. 55,* 49-60.
Starr, R., and Offer, G. (1973), *J. Mol. Biol. 81,* 17-31.
Strauch, L. (1965), *Z. Klin. Chem. Klin. Biochem. 3,* 165-167.
Taylor, E. W., Lymn, R. W., and Moll, G. (1970), *Biochemistry 9,* 2984-2991.
Thomas, D. D., Seidel, J. C., Hyde, J. S., and Gergely, J. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 1729-1733.
Trentham, D. R., Bardsley, R. G., Eccleston, J. F., and Weeds, A. G. (1972), *Biochem. J. 126,* 635-644.
Wagner, P. D., and Yount, R. G. (1975a), *Biochemistry 14,* 1900-1907.
Wagner, P. D., and Yount, R. G. (1975b), *Biochemistry 14,* 5156-5162.
Watterson, J. G., and Schaub, M. C. (1973), *Hoppe-Seyler's Z. Physiol. Chem. 354,* 1619-1625.
Watterson, J. G., Schaub, M. C., Locher, R., Di Pierri, S., and Kutzer, M. (1975), *Eur. J. Biochem. 56,* 79-90.
Weber, A., and Murray, J. M. (1973), *Physiol. Rev. 53,* 612-673.
Weeds, A. G., and Lowey, S. (1971), *J. Mol. Biol. 61,* 701-725.
Weeds, A. G., and Pope, B. (1977), *J. Mol. Biol. 111,* 129-157.
Weeds, A. G., and Taylor, R. S. (1975), *Nature (London) 257,* 54-56.
Weeds, A. G., Hall, R., and Spurway, N. C. (1975), *FEBS Lett. 49,* 320-324.
Werber, M. M., Szent-Györgyi, A. G., and Fasman, G. D. (1972), *Biochemistry 11,* 2872-2883.
Yount, R. G., Ojala, D., and Babcock, D. (1971), *Biochemistry 10,* 2490-2496.

Modulator Protein as a Component of the Myosin Light Chain Kinase from Chicken Gizzard†

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ABSTRACT: The Ca^{2+}-dependent regulation of smooth muscle actomyosin involves a myosin light chain kinase (ATP:myosin light chain phosphotransferase). It has been shown (Dabrowska, R., Aromatorio, D., Sherry, J. M. F., and Hartshorne, D. J. 1977, *Biochem. Biophys. Res. Commun. 78,* 1263) that the kinase is composed of two proteins of approximate molecular weights 105 000 and 17 000. In this communication it is demonstrated that the 17 000 component is the modulator protein. This conclusion is based on: (1) the identical behavior of the 17 000 kinase component and modulator protein in assays of actomyosin Mg^{2+}-ATPase activity, phosphorylation of myosin, and phosphodiesterase activity, and, (2) the similarity of the 17 000 kinase component and the modulator protein with respect to amino acid composition, absorption spectrum, and electrophoresis in urea-polyacrylamide gels. It is shown also that the modulator protein from smooth muscle and troponin C are distinct proteins.

The regulation by Ca^{2+} of the actin-myosin interaction in smooth muscle is thought to be due to the concerted action of a protein kinase and a phosphatase (Gorecka et al., 1976; Sobieszek, 1977; Chacko et al., 1977). In the presence of Ca^{2+} the kinase phosphorylates the 20 000-dalton light chain of myosin (1 mol of phosphate/light chain) and thereby allows the activation by actin of the Mg^{2+}-ATPase activity. As long as Ca^{2+} is present cyclic actin-myosin interactions proceed with the concurrent hydrolysis of ATP and development of tension or shortening. In the absence of Ca^{2+}, i.e., when the muscle relaxes, the protein kinase is not active and the phosphatase removes the phosphate groups from the myosin molecule. This prevents the actin-activation of ATPase activity and the contractile apparatus is turned "off". Thus the regulatory proteins of smooth muscle are the kinase and the phosphatase and are quite different from the troponin-tropomyosin system in skeletal muscle. It should be pointed out that, although the phosphorylation theory has received wide support, it is not unanimously accepted. Head et al. (1977) have suggested that a troponin-like mechanism is involved in the regulation of smooth muscle activity, and Mikawa et al. (1977) have isolated an activator which does not phosphorylate myosin.

In an attempt to establish the correlation between the activation of ATPase activity and the phosphorylation of myosin we recently isolated the myosin light chain kinase. Our rea-

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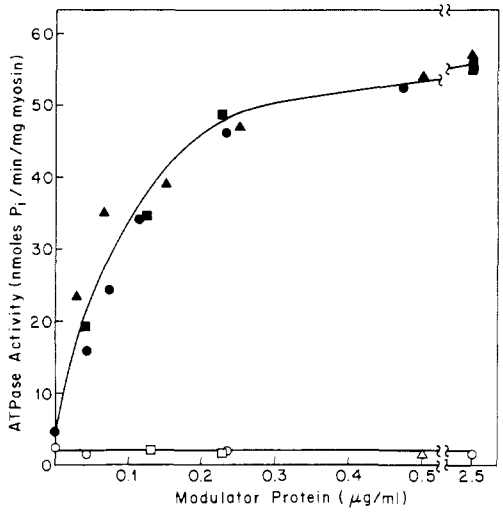

FIGURE 1: The effect of 17K and modulator proteins on the actin-activated ATPase activity of gizzard myosin at a constant level of 105K. Assay conditions: 10 mM $MgCl_2$, 50 mM KCl, 2.5 mM ATP, 20 mM Tris-HCl (pH 7.6), 25 °C, gizzard myosin 0.46 mg/mL, skeletal muscle actin 0.26 mg/mL, skeletal muscle tropomyosin 0.075 mg/mL, gizzard 105K 6.4 μg/mL. Final assay volume 2 mL. The amount of 17K and modulator proteins was varied as shown. 17K (●, ○), adrenal medulla modulator (▲, △), brain modulator (■, □). Open symbols indicate the inclusion of 1 mM EGTA.

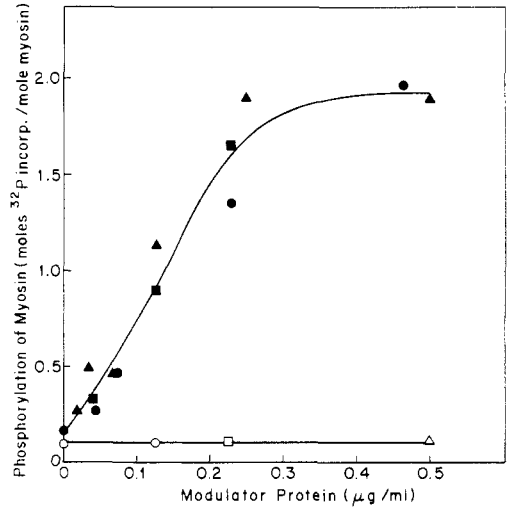

FIGURE 2: The effect of 17K and modulator proteins on the phosphorylation of gizzard myosin at a constant level of 105K. Assay conditions as in Figure 1, except that the final assay volume was 1 mL. 17K (●, ○); adrenal medulla modulator (▲, △), brain modulator (■, □). Open symbols indicate the inclusion of 1 mM EGTA.

soning was that any correlation between the two events should become obvious with increasing purity of the kinase. The results that were obtained were consistent with the dominant role of phosphorylation in the regulatory mechanism. It was discovered (Dabrowska et al., 1977) that the protein kinase was composed of two proteins, neither of which alone possessed any activity. The approximate molecular weights for each were estimated to be 105 000 and 17 000; these will be referred to as 105K[1] and 17K, respectively. During the characterization of each it was realized that the smaller component resembled in many respects the modulator protein which regulates the activity of cyclic nucleotide phosphodiesterase and adenylate cyclase. The data that are presented in this paper indicate that the activator of the myosin light chain kinase, i.e., 17K, is identical with the modulator protein.

The modulator protein was discovered in rat brain by Cheung (1970) and Kakiuchi et al. (1970) and subsequently identified in a wide variety of tissues (Cheung et al., 1975b; Waisman et al., 1975; Egrie and Siegel, 1975; Kuo and Coffee, 1976b; Stevens et al., 1976; Dedman et al., 1977). It was recognized initially that the modulator protein played a role in the Ca^{2+}-dependent regulation of phosphodiesterase activity. More recently a second function was discovered, namely, in the regulation of adenylate cyclase activity (Brostrom et al., 1975; Cheung et al., 1975a). The modulator protein is known to bind Ca^{2+} and in this and other respects resembles troponin C (Stevens et al., 1976; Watterson et al., 1976; Amphlett et al., 1976). Because of its wide distribution and high concentration in many tissues (approximately 1% of the soluble protein of brain [Watterson et al., 1976]), it has been suggested by many investigators (Waisman et al., 1975; Egrie and Siegel, 1975; Hait and Weiss, 1976; Watterson et al., 1976) to have a functional role in enzymatic pathways other than phosphodiesterase and adenylate cyclase activities.

[1] Abbreviations used are: 105K, 105 000 kinase component; 17K, 17 000 kinase component; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

Our results identify an additional function for the modulator protein and show that in smooth muscle it is an integral part of the Ca^{2+}-sensitive mechanism that regulates the contractile apparatus.

Experimental Procedure

The procedures used to isolate the various proteins were as follows: chicken gizzard myosin, Hartshorne et al. (1977); rabbit skeletal muscle actin and tropomyosin, Driska and Hartshorne (1975); rabbit skeletal muscle troponin I and troponin C, Greaser and Gergely (1971); 105K and 17K, Dabrowska et al. (1977); activator-deficient phosphodiesterase and bovine brain modulator protein, Watterson et al. (1976); adrenal medulla modulator protein, Kuo and Coffee (1976a).

The Mg^{2+}-activated ATPase activity and the phosphorylation of myosin were assayed as described previously (Aksoy et al., 1976). The phosphodiesterase assay was essentially as described by Lin et al. (1974a) except that 2.5 mM $MgCl_2$ was substituted for $MnCl_2$. Amino acid composition was determined using a Durrum D-500 analyzer following a 22-h hydrolysis. Trimethyllysine was eluted as a distinct peak before lysine, and quantitated using a color constant of 0.89× the color constant of lysine. Electrophoresis in 7.5% polyacrylamide gels and 7 M urea was as described by Perrie and Perry (1970). Tyrosine emission fluorescence (excitation at 278 nm) was measured using a Farrand spectrofluorometer, MkI, equipped with corrected excitation and emission modules.

Results

(a) *Functional Characterization.* The effect on ATPase activity of 17K in comparison with modulators from brain and adrenal medulla is shown in Figure 1. At a constant level of 105K increasing amounts of either 17K or modulator protein caused an activation of the Mg^{2+}-ATPase activity of actomyosin. This occurred only in the presence of Ca^{2+}. It is clear that 17K and the modulators from brain and adrenal medulla were equally effective in activating the ATPase activity. Under identical assay conditions troponin C, at concentrations up to 30 μg/mL, did not activate the ATPase activity.

In Figure 2 a similar experiment is described except that the phosphorylation of myosin is the measured parameter. Both

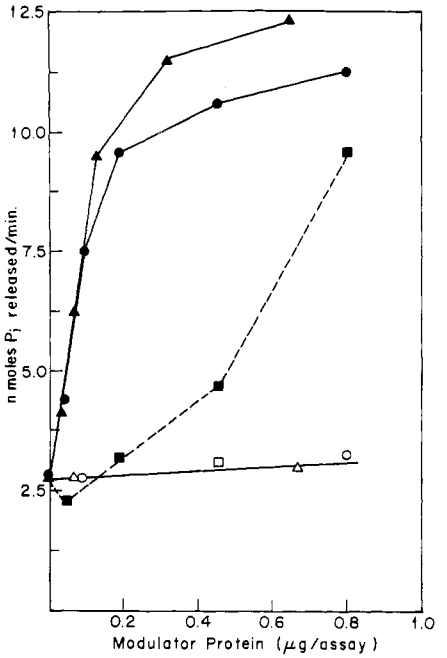

FIGURE 3: The effect of 17K and modulator proteins on phosphodiesterase activity. Assay method given in text. Phosphodiesterase level was constant, with varying amounts of 17K (●, ○) and adrenal medulla modulator (▲, △). The effect of 17K on a mixture of phosphodiesterase and 105K (5 μg/0.5 mL assay volume) is also shown (■, □). Open symbols indicate the inclusion of 1 mM EGTA.

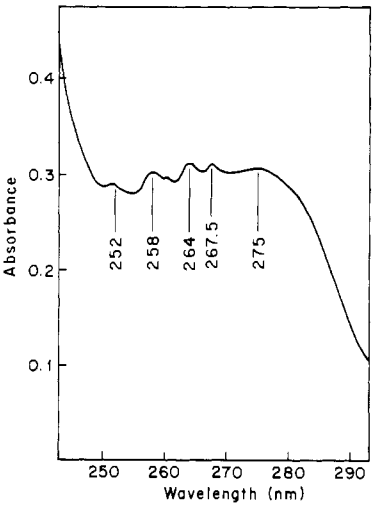

FIGURE 4: Absorption spectrum of 17K. The concentration of 17K was 1.62 mg/mL, in 10 mM Tris-HCl (pH 7.6).

of the modulators and 17K were equally effective in promoting phosphorylation. The maximum extent of phosphorylation, i.e., 2 mol of phosphate incorporated/mol of myosin, was obtained with 0.35 μg of modulator. This occurred at a molar ratio of 1:3:49 for the modulator, 105K, and myosin, respectively. In the absence of Ca^{2+}, myosin was not phosphorylated.

From a comparison of Figures 1 and 2, it is evident that a correlation exists between the phosphorylation of myosin and the activation of actomyosin Mg^{2+}-ATP activity. Both events were dependent upon the presence of Ca^{2+} and both required the presence of two kinase components, i.e., 105K and 17K. It is also apparent that 17K may be exchanged with either modulator protein without any alteration of kinase response.

The activation of brain phosphodiesterase by 17K and the modulator protein of adrenal medulla is shown in Figure 3. Both proteins were effective in activating the phosphodiesterase activity. At relatively high levels of activator the adrenal medulla modulator gave a slightly higher activity, although the difference is not significant. In the absence of Ca^{2+}, neither protein activated the phosphodiesterase activity.

Addition of 105K to the phosphodiesterase assay system resulted in an inhibition of phosphodiesterase activity (Figure 3). The most reasonable explanation for this effect is that 17K formed a complex with both the phosphodiesterase and 105K. A competition for the activator would thus be established. The association of the two kinase components was also demonstrated by chromatography on Sepharose 6B. In the presence of Ca^{2+} and at an ionic strength of approximately 0.04, the 17K and 105K components were eluted as a complex. In the absence of Ca^{2+}, or at a higher ionic strength ($I \simeq 0.8$), the two proteins were dissociated. (The details of these experiments will be published later.)

In "hybrid" assay systems where the phosphodiesterase and 105K were interchanged, it was found that in the presence of modulator protein the phosphodiesterase did not activate actomyosin ATPase activity and 105K did not exhibit phosphodiesterase activity.

It can be concluded from the above results that, at least under the functional boundaries imposed by the assay techniques, the 17 000 smooth muscle protein (i.e., 17K) is indistinguishable from the modulator protein.

(b) *Physical Characterization.* The amino acid composition of 17K is given in Table I. By comparison with the modulator proteins of adrenal medulla, brain, and heart, it is obviously very similar. Tryptophan was not present in 17K, as indicated by the absence of its distinctive fluorescence emission spectrum. A significant feature also was the presence of trimethyllysine which has been discovered previously in the modulator protein (Amphlett et al., 1976).

The absorption spectrum of 17K is shown in Figure 4. The spectrum is characteristic of the phenylalanine absorption maxima and is similar to that reported for modulator proteins by other investigators (Liu and Cheung, 1976; Stevens et al., 1976; Watterson et al., 1976).

The above physical properties of 17K are remarkably similar to those of troponin C and in order to distinguish between the two proteins an additional procedure was applied, namely, electrophoresis in urea-polyacrylamide gels. The results are shown in Figure 5. The modulator protein from adrenal medulla and brain had identical electrophoretic mobility to 17K. However, troponin C had a higher mobility and was clearly distinguishable from the modulator proteins. This is best demonstrated by gel f in Figure 5, which shows the resolution of a mixture of troponin C and 17K. A similar pattern was observed by Amphlett et al. (1976) for troponin C and bovine brain modulator protein.

It is known that in the presence of Ca^{2+} the complex of troponin I and troponin C (Perry et al., 1972) or brain modulator protein (Amphlett et al., 1976) is not dissociated under the conditions used for urea-polyacrylamide electrophoresis. We obtained similar results for mixtures of troponin I and 17K and troponin I and the adrenal medulla modulator (each mixture at equal weight ratios). In 7 M urea each complex was stable, and a band corresponding to the free modulator was not observed. However, the complex of 105K and 17K or 105K and adrenal medulla modulator was not as stable and dissociated under the conditions used for electrophoresis.

A Ca^{2+}-dependent conformation change of 17K is illus-

TABLE I: Amino Acid Compositions[a] of the 17 000 Kinase Component, Various Modulator Proteins, and Troponin C.

Amino acid	17K	Modulators from			Troponin C[e]
		Adrenal medulla[b]	Bovine brain[c]	Bovine heart[d]	
Lys	7	8	8	9	9
His	1	1	1	1	1
Me_3Lys	1		1[h]		
Arg	6	5	7	6	7
Asp	23	22	24	25	22
Thr[f]	12	11	12	12	6
Ser[g]	5	5	5	3	7
Glu	28	25	29	30	31
Pro	2	2	2	2	1
Gly	12	12	12	12	13
Ala	11	11	12	12	13
½-Cystine		1	0	0	1
Val	7	9	8	9	7
Met	9	8	10	9	10
Ile	8	7	8	8	10
Leu	9	8	10	10	9
Tyr	2	2	2	2	2
Phe	8	7	8	9	10
Trp		0	0	0	0

[a] Given as moles of amino acid/mole of protein. Molecular weights assumed are: 17K, 17 000; adrenal medulla modulator, 16 000; bovine brain modulator, 18 000; bovine heart modulator, 17 500; troponin C, 18 000 (see following references). [b] From Kuo and Coffee (1976a). [c] From Watterson et al. (1976). [d] From Stevens et al. (1976). [e] From Collins et al. (1973) and Collins (1974). [f] Corrected assuming a 6% loss during 22 h of hydrolysis. [g] Corrected assuming a 10% loss during 22 h of hydrolysis. [h] Listed by Watterson et al. (1976) as compound X.

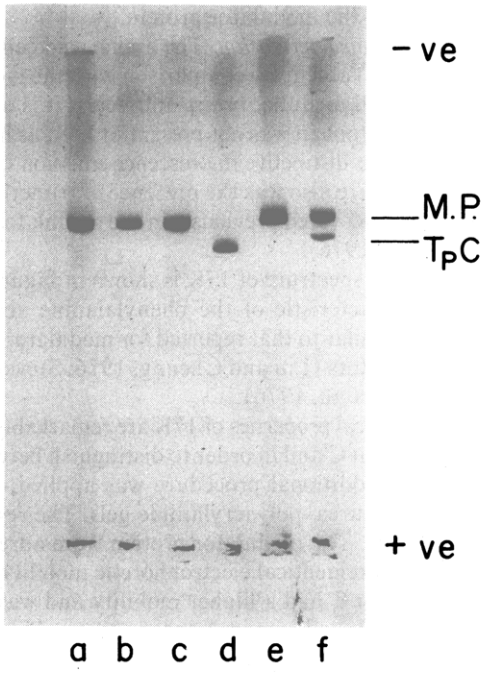

FIGURE 5: Urea-polyacrylamide gel electrophoresis of 17K, modulator proteins, and troponin C. Polyacrylamide, 7.5%, 7 M urea, other conditions as in method cited in text. M.P. is modulator protein; TpC is troponin C. (Gel a) Five micrograms 17K; (gel b) 5 μg of adrenal medulla modulator protein; (gel c) 5 μg of brain modulator protein; (gel d) 5 μg of troponin C; (gel e) mixture of 17K, adrenal medulla, and brain modulator proteins, 3 μg each; (gel f) mixture of troponin C and 17K, 5 μg each.

trated by the tyrosine fluorescence data given in Figure 6. These determinations were done in the presence of 1 mM $MgCl_2$. At low concentrations of Ca^{2+} ($<1 \times 10^{-6}$ M), the tyrosine emission was quenched. The quenching was removed as the Ca^{2+} concentration was increased. The midpoint of the titration curve was at approximately 3×10^{-6} M Ca^{2+}. A similar titration curve was observed for troponin C (Figure 6), and this suggests that the Ca^{2+} binding constants for those sites affecting tyrosine fluorescence are similar.

The physical properties given above for 17K are indistinguishable from those exhibited by the various modulator proteins. It can be concluded, therefore, that in both functional and physical properties 17K is identical with the modulator protein.

Discussion

The most popular theory which accounts for the regulation of actin–myosin interactions and, hence, contractile activity in smooth muscle is based on the phosphorylation and dephosphorylation of the 20 000-dalton myosin light chains. Stated simply, myosin in the phosphorylated state can interact with actin, hydrolyze ATP, and thus can generate tension; when the phosphate groups are removed by a phosphatase, the Mg^{2+}-ATPase activity of myosin is no longer activated by actin, with the net result that relaxation follows. It has been known for many years that the contraction–relaxation in smooth muscle as in skeletal muscle, is regulated by the intracellular Ca^{2+} concentration, and thus at the contractile protein level it is essential that either the myosin light chain kinase or the phosphatase is subject to regulation by Ca^{2+}. Most investigators (Aksoy et al., 1976; Sobieszek, 1977; Chacko et al., 1977) accept that the Ca^{2+}-sensitive site is associated with the kinase system. Recently we have shown (Dabrowska et al., 1977) that the kinase is composed of two components, both of which are essential for activity. These have molecular weights of 105 000 and 17 000. From the results presented above we have identified the smaller component as identical with the modulator protein, which has previously been implicated in other Ca^{2+}-dependent mechanisms. The binding of Ca^{2+} to the modulator protein has been demonstrated (Lin et al., 1974b; Kuo and Coffee, 1976a; Watterson et al., 1976; Wolff et al., 1977) and Ca^{2+}-dependent conformation changes have also been reported (Liu and Cheung, 1976; Kuo and Coffee, 1976b; Wolff et al., 1977; Klee, 1977). It is thus reasonable to assume that in smooth muscle function the modu-

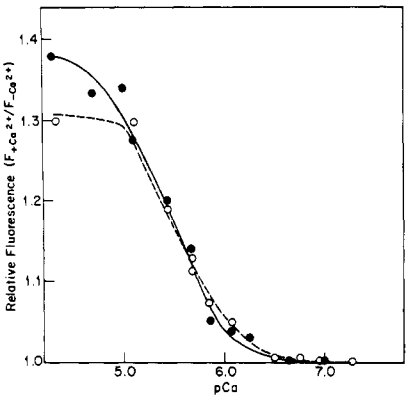

FIGURE 6: Tyrosine fluorescence of 17K and troponin C as a function of Ca^{2+} concentration. Solvent conditions were 0.1 M KCl, 1 mM $MgCl_2$, 10 mM phosphate (pH 7.0). Ca-EGTA and EGTA were varied. The Ca-EGTA binding constant under these conditions was taken as 1.1×10^6 M^{-1}. The tyrosine emission was monitored at 305 nm. The relative fluorescence is given as the fluorescence at any Ca^{2+} concentration divided by the fluorescence in the absence of Ca^{2+}, i.e., at Ca^{2+} concentrations $< 1 \times 10^{-7}$ M. Protein concentration for troponin C and 17K was less than 0.1 mg/mL. 17K (●); troponin C (○).

lator acts as the detector of intracellular Ca^{2+} transients and, as the Ca^{2+}-modulator complex, initiates contraction. In skeletal muscle this is the function of troponin C, and in many respects the two Ca^{2+} receptors are remarkably similar (Watterson et al., 1976; Stevens et al., 1976; Amphlett et al., 1976; Dedman et al., 1977). However, it is clear from our results that the two proteins are distinct. On an equal weight basis, troponin C was not as effective as the modulator protein in activating 105K in assays of ATPase activity. A similar conclusion was reached previously by Stevens et al. (1976) who reported that troponin C failed to activate phosphodiesterase activity. However, Dedman et al. (1977) found recently that troponin C at relatively high concentrations could activate phosphodiesterase activity, and this raises the possibility that troponin C and modulator protein might both be functioning in smooth muscle. In our opinion this is unlikely. Most available evidence suggests that troponin is not present in smooth muscle; for example, troponin subunits are not found in Ca^{2+}-sensitive actomyosin (Driska and Hartshorne, 1975; Sobieszek and Bremel, 1975) or thin filaments (Driska and Hartshorne, 1975; Sobieszek and Small, 1976) from chicken gizzard. The amount of troponin C that could effectively substitute for the modulator protein (assuming a 600-fold excess as reported by Dedman et al., 1977) also imposes an unrealistic situation.

It is known that Ca^{2+}, via its interaction with the modulator protein, can regulate cyclic nucleotide metabolism. Recently it was reported that the modulator protein also activates erythrocyte Ca^{2+}-Mg^{2+} ATPase activity (Gopinath and Vincenzi, 1977; Jarrett and Penniston, 1977). Our studies add another function for the modulator protein, that of regulating the activity of smooth muscle actomyosin. It is intriguing that each quite distinct pathway is activated at the same Ca^{2+} concentration, and the interrelationship of each must be established by further studies. It is likely that functions as yet unknown will also be subject to regulation by the modulator protein. Similarly it is possible that mechanisms discovered in one cell type might be functional in other cell types. The latter possibility is relevant to our results since it is known that a wide variety of nonmuscle eucaryotic cells contain actin and myosin (Pollard and Weihing, 1974). It would be very instructive to know whether or not the regulation of actin–myosin interactions by the modulator protein is a general phenomenon, or, one restricted to muscle tissue. It is intriguing that a modulator binding protein isolated from brain by Wang and Desai (1977) has a molecular weight (95 000) and several properties similar to 105K.

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References

Aksoy, M. O., Williams, D., Sharkey, E. M., and Harthshorne, D. J. (1976), *Biochem. Biophys. Res. Commun. 69,* 35.

Amphlett, G. W., Vanaman, T. C., and Perry, S. V. (1976), *FEBS Lett. 72,* 163.

Brostrom, C. O., Huang, Y. C., Breckenridge, b. McL., and Wolff, D. J. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 64.

Chacko, S., Conti, M. A., and Adelstein, R. S. (1977), *Proc. Natl. Acad. Sci. U.S.A. 74,* 129.

Cheung, W. Y. (1970), *Biochem. Biophys. Res. Commun. 38,* 533.

Cheung, W. Y., Bradham, L. S., Lynch, T. J., Lin, Y. M., and Tallant, E. A. (1975a), *Biochem. Biophys. Res. Commun. 66,* 1055.

Cheung, W. Y., Lin, Y. M., Liu, Y. P., and Smoake, J. A. (1975b), in Cyclic Nucleotides in Diseases, Weiss, B., Ed., Baltimore, Md., University Park Press, p 321.

Collins, J. H. (1974), *Biochem. Biophys. Res. Commun. 58,* 30.

Collins, J. H., Potter, J. D., Horn, M. J., Wilshire, G., and Jackman, N. (1973), *FEBS Lett. 36,* 268.

Dabrowska, R., Aromatorio, D., Sherry, J. M. F., and Hartshorne, D. J. (1977), *Biochem. Biophys. Res. Commun. 78,* 1263.

Dedman, J. R., Potter, J. D., and Means, A. R. (1977), *J. Biol. Chem. 252,* 2437.

Driska, S., and Harthshorne, D. J. (1975), *Arch. Biochem. Biophys. 167,* 203.

Egrie, J. C., and Siegel, F. L. (1975), *Biochem. Biophys. Res. Commun. 67,* 662.

Gopinath, R. M., and Vincenzi, F. F. (1977), *Biochem. Biophys. Res. Commun. 77,* 1203.

Gorecka, A., Aksoy, M. O., and Harthshorne, D. J. (1976), *Biochem. Biophys. Res. Commun. 71,* 325.

Greaser, M. L., and Gergely, J. (1971), *J. Biol. Chem. 246,* 4226.

Hait, W. N., and Weiss, B. (1976), *Nature (London) 259,* 321.

Hartshorne, D. J., Gorecka, A., and Aksoy, M. O. (1977), in Excitation-Contraction Coupling in Smooth Muscle, Casteels, R., Godfraind, T., and Ruegg, J. C., Ed., Amsterdam, Elsevier, p 377.

Head, J. F., Weeks, R. A., and Perry, S. V. (1977), *Biochem. J. 161,* 465.

Jarrett, H. W., and Penniston, J. T. (1977), *Biochem. Biophys. Res. Commun. 77,* 1210.

Kakiuchi, S., Yamazaki, R., and Nakajima, H. (1970), *Proc. Jpn. Acad. 46,* 587.
Klee, C. B. (1977), *Biochemistry 16,* 1017.
Kuo, I. C. Y., and Coffee, C. J. (1976a), *J. Biol. Chem. 251,* 1603.
Kuo, I. C. Y., and Coffee, C. J. (1976b), *J. Biol. Chem. 251,* 6315.
Lin, Y. M., Liu, Y. P., and Cheung, W. Y. (1974a), *Methods Enzymol. 38,* 262.
Lin, Y. M., Liu, Y. P., and Cheung, W. Y. (1974b), *J. Biol. Chem. 249,* 4943.
Liu, Y. P., and Cheung, W. Y. (1976), *J. Biol. Chem. 251,* 4193.
Mikawa, T., Toyo-Oka, T., Nonomura, Y., and Ebashi, S. (1977), *J. Biochem. (Tokyo) 81,* 273.
Perrie, W. T., and Perry, S. V. (1970), *Biochem. J. 119,* 31.
Perry, S. V., Cole, H. A., Head, J. F., and Wilson, F. J. (1972), *Cold Spring Harbor Symp. Quant. Biol. 37,* 251.
Pollard, T. D., and Weihing, R. R. (1974), *CRC Crit. Rev. Biochem. 2,* 1.
Sobieszek, A. (1977), *Eur. J. Biochem. 73,* 477.
Sobieszek, A., and Bremel, R. D. (1975), *Eur. J. Biochem. 55,* 49.
Sobieszek, A., and Small, J. V. (1976), *J. Mol. Biol. 102,* 75.
Stevens, F. C., Walsh, M., Ho, H. C., Teo, T. S., and Wang, J. H. (1976), *J. Biol. Chem. 251,* 4495.
Waisman, D., Stevens, F. C., and Wang, J. h. (1975), *Biochem. Biophys. Res. Commun. 65,* 975.
Wang, J. H., and Desai, R. (1977), *J. Biol. Chem. 252,* 4175.
Watterson, D. M., Harrelson, Jr., W. G., Keller, P. M., Sharief, F., and Vanaman, T. C. (1976), *J. Biol. Chem. 251,* 4501.
Wolff, D. J., Poirier, P. G., Brostrom, C. O., and Brostrom, M. A. (1977), *J. Biol. Chem. 252,* 4108.

Arginine Decarboxylase from *Escherichia coli* B: Mechanism of Dissociation from the Decamer to the Dimer†

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ABSTRACT: The mechanism by which arginine decarboxylase dissociates from a decamer to a dimer has been examined by allowing a sulfhydryl group, available in the dimer but not the decamer, to react with 5,5′-dithiobis(2-nitrobenzoic acid). Initial rates of dissociation were obtained by following the resulting increase in absorbance at 412 nm in a stopped-flow spectrophotometer. The rate of dissociation increases linearly with the protein concentration and reaches a maximum as a function of the concentrations of 5,5′-dithiobis(2-nitrobenzoic acid), Na^+, and $1/[H^+]$. Experiments in which the rate of dissociation was measured while one reagent was varied at fixed levels of a second indicate that dissociation requires three events: binding of one Na^+ ion, dissociation of one proton, and the irreversible dissociation of subunits, in that order. The results also show that the decamer dissociates in stages rather than all at once. The activation energy for the overall process is 16 kcal/mol.

Interactions between the subunits of enzymes have been investigated almost exclusively from a thermodynamic point of view, since instruments such as the analytical ultracentrifuge provide information only about the equilibria that exist between associated and dissociated states. An entirely different type of information, the underlying mechanism of the dissociation process, would be obtained if kinetic techniques could be adapted to the study of dissociating systems. This is not ordinarily possible, since enzymes which dissociate undergo only a molecular weight change, not a chemical change; there is usually no way to assay the newly formed species. In cases where dissociation makes available a previously unreactive group, however, it should be possible to develop such assays and to study dissociation kinetically.

The inducible arginine decarboxylase of *Escherichia coli* B appears to be particularly favorable for such a study; dissociation from the decamer to the dimer unmasks one sulfhydryl group per subunit (Boeker et al., 1969). Since there are no other available sulfhydryls, it is possible to assay for dissociation by titrating this group with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB)[1] (Boeker, 1977).

As determined in the analytical ultracentrifuge (Boeker and Snell, 1968), arginine decarboxylase occurs as a decamer (mol wt 820 000) at pH values below 6.5 or salt concentrations above 0.08 M and dissociates to dimers (mol wt 160 000) as the pH increases and the salt concentration decreases. Dissociation appears to be complete above pH 7 and below 0.04 M salt. The ultimate subunits (mol wt 82 000) are identical by several criteria (Boeker et al., 1969). Electron micrographs show that the decamer consists of five dimers arranged in a pentamer. Unless there is more than one type of interdimer bond in this structure, these bonds must be heterologous, or head to tail (Boeker et al., 1969). The intradimer bonds, on the other hand, must be isologous, or head to head, since the decamers themselves show no tendency to associate. The subunit structure of *E. coli* lysine decarboxylase is very similar (Sabo et al., 1974).

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[1] The abbreviation used is: DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

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