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Evidence of a New Type of Protein-Protein Interaction: Desensitized Actomyosin Blocks Ca^{2+}-Sensitivity of the Natural One. A Possible Model for an Intracellular Signalling System Related to Actin Filaments

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Abstract: Actin filaments are certainly believed to function as an intracellular signalling system; however, this is not confirmed by direct evidence. We used a two-layer actomyosin gel with a concentration gradient of the troponin-tropomyosin complex (TT-complex, Ca^{2+}-sensitive system) between the two layers. To prepare one layer of the system, natural actomyosin (*n*AM) rich in TT-complex was used. To prepare the second layer, we used desensitized actomyosin (*d*AM) without the complex. All experimental studies were made in medium with a low ionic strength. Two phenomena were observed: (1) *d*AM blocks Ca^{2+}-sensitivity of *n*AM when the *d*AM weight portion in the system (as well as in mixed *n*AM+*d*AM suspension) reaches 40% and more; further increase of the *d*AM portion does not affect the Ca^{2+}-sensitivity; (2) it was electrophoretically shown that a rapid diffusion of the TT-complex from *n*AM gel into the *d*AM gel took place. The apparent diffusion coefficient for the TT-complex in *d*AM gel is about $(1\text{-}4)\cdot10^{-4}$ cm^2/sec, i.e. three orders higher than the same values for protein diffusion in water.

THE CYTOSKELETON and, particularly, actin filaments are believed to function as an intracellular signalling system (by signal we mean any cellular event which has a regulatory significance). Edelman (1) was the first to suggest participation of actin filaments in the transfer of signals from the plasma membrane to the cytoplasm and nucleus.

To elucidate the mechanism of transfer of a signal related to elements of the cytoskeleton, experiments with protein model systems reproducing some important features of the cytoplasm can be useful. Such a convenient model system can be actomyosin. Natural actomyosin (*n*AM) contains a regulatory troponin-tropomyosin complex (TT-complex), whose content may be easily changed by washing out actomyosin with a solution of a low ionic

strength and alkaline pH. After removal of the TT-complex, the actomyosin superprecipitation by addition of MgATP becomes Ca^{2+}-independent, i.e., actomyosin becomes desensitized (*d*AM). Addition of the TT-complex to *d*AM results in recovery of Ca^{2+}-sensitivity (2). Therefore, after removal of the TT-complex from actomyosin, its binding sites still are able to bind again the regulatory complex. Thus, the use of actomyosin preparations with different contents of TT-complex can produce gels with a concentration gradient of the actin-binding proteins.

It should be emphasized that the TT-complex is strongly bound to actin filaments at physiological ionic strength and pH and serves as a Ca^{2+} trigger of muscle contraction (3,4). Hence, the function and distribution of this regulatory complex can be evaluated not only by analysis of protein content in various areas of the gel, but also by measurement of its functional parameter, Ca^{2+}-sensitivity of the reaction of actomyosin superprecipitation.

The goal of the present work was to study the interaction of actomyosin gels with different contents of TT-complex to detect possible redistribution of the TT-complex as a result of its diffusion, translocation, transport, etc.

Materials and Methods

Isolation of natural actomyosin (*n*AM) from rabbit skeletal muscles was performed at 4-6°C, as described by Haga *et al.* (5), with minor modifications. The time of the extraction was 15-20 h. The actomyosin gel (about 30 mg/ml of protein) with 50 mM KC1 was diluted by the same volume of cold glycerol and stored at -20°C (6). Prior to the experiments, the glycerinated actomyosin was washed free of glycerol with a solution containing 90 mM KCl and 14 mM Na-K-phosphate buffer, pH 6.7. After two washings by tenfold volumes of this phosphate buffer (PB), *n*AM was dissolved in 0.5 M KC1 and centrifuged for 20 min at 10,000 g. The supernatant was diluted with 9 volumes of cold deionized water, and the collected precipitate was washed twice with PB and then resuspended in PB.

Preparation of desensitized actomyosin (*d*AM) was done by washing *n*AM at 4-6°C in pure water containing a minimal amount of Tris-buffer, pH 8.5-9.0 (2). 15-20 ml aliquots of the stored glycerolized actomyosin preparation were placed into every 100-ml centrifuge test tube, and water was added up to the volume of 70 ml. After the second or third washing, with centrifugation for 20 min at 10,000 g, the actomyosin gel swelled (as a result of desalination) to such a degree that it could no longer be precipitated. After this step, prior to every centrifugation, 1-2 drops of 3 M KC1 were added to every tube so that the actomyosin gel volume was about 50% of the suspension volume after centrifugation. After each centrifugation, the supernatant was replaced by fresh pure water. This procedure was repeated 10 times. The above procedure was confirmed electrophoretically to result in the nearly complete removal of the TT-complex and in the complete elimination of the Ca^{2+}-sensitivity. The sensitivity was effectively restored by addition of the TT-complex isolated as described by Spudich and Watt (7) to such *d*AM preparations. After washing, *d*AM was centrifuged in 0.5 M KC1 and precipitated by dilution with 10 volumes of water. The precipitate was washed twice with PB and then resuspended in the third portion of the buffer.

Superprecipitation of *n*AM, *d*AM, and their mixtures was carried out at 20°C in PB containing 0.5 mM Ca^{2+} or 0.5 mM EGTA (ethylene glycol-bis(β-aminoethyl ether)

N,N,N',N'-tetraacetic acid) and 0.25 mM MgATP at a protein concentration of 0.1 mg/ml. The reaction of superprecipitation was recorded by the method of 90°-angle light scattering at 450 nm, using a Specol spectrometer (Carl Zeiss, Jena, Germany) with a built-in magnetic mixer for continuous stirring of the samples in the course of superprecipitation. For the superprecipitation, 30-ml cells were used; the volume of samples was 20 ml.

After addition of MgATP to the actomyosin suspension, the light scattering began to fall, reaching the minimum steady-state level after 1-2 min (for *n*AM) or after 5-7 min (for *d*AM*)*. For *n*AM preparations, the decreasing value of the light scattering varied depending on the presence of Ca^{2+} or EGTA in the solution. This sensitivity to Ca^{2+} (S_{ca}) was expressed quantitatively as a light scattering decrease (LSD): LSD $=I_0-I$, in the presence both of 0.5 mM Ca^{2+} (LSD_{ca}) and of 0.5 mM EGTA (LSD_{egta}), where I_0 is the initial and I, the final level of the light scattering (in arbitrary units). So $S_{ca} = LSD_{ca}/LSD_{egta}$. As a rule, three measurements of S_{ca} were done for each actomyosin sample.

The S_{ca} values for *d*AM preparations were close to 1, which indicates that the superprecipitation of these preparations did not depend on Ca^{2+} and, accordingly, that no significant amount of the regulatory TT-complex remained in *d*AM. The S_{ca} values for *n*AM preparations were more than 1. This method of measurement of Ca^{2+}-sensitivity is described in detail in our earlier work (6).

Electrophoresis in polyacrylamide gels in the presence of sodium dodecyi sulphate (disc-SDS-electrophoresis) was performed by the method of Sheludko (8), with some modifications for block-electrophoresis. The gel components were from Reanal (Hungary). Their composition in the separation gel, unless otherwise specified, was the following: 0.43 M Tris-HCl (tris(oxymethyl)aminomethane), pH 8.9, 12% acrylamide (AA), and 0.3% N,N'-methylene-bis-acrylamide (MBA), in the concentration gel: 0.05 M Tris-HCl, pH 6.8, 2.5% AA, 0.6% MBA, 20% sucrose, and 2% sodium dodecyl sulphate. The electrode solutions contained 0.6 M glycine and 0.033 M Tris-buffer, pH 8.3; sodium dodecyl sulphate was added to the upper electrode solution up to a concentration of 0.1%. Actomyosin samples were solubilized for 5 min in a boiling water bath in a solution containing 50% glycerol, 5% sodium dodecyl sulphate, 50 mM dithiothreitol (Serva, Germany), and 25 mM EDTA (ethyienediaminetetraacetic acid), pH 7.0. The gels were fixed by a mixture of ethanol-CH_3COOH-H_2O (1:3:6) and then stained with 0.125% Coomassie brilliant blue G-250 (Serva, Germany) dissolved in the fixation mixture. After the dye excess had been removed from the gels with 7% acetic acid, densities of protein bands were measured three times, using an Elscript-3 densitometer (Hirschmann, Germany). The relative contents of TT-complex proteins were expressed as percentage to actin.

Protein concentration was determined by the micro-biuret method (9), using bovine serum albumin as a standard.

Formation of two-layer gels. To form an actomyosin gel with non-uniform distribution of the TT-complex, a two-layer system was prepared in 10-ml centrifuge tubes (Figure 1), using the following steps. First, 30 mg of the *d*AM in suspension were placed in each tube and precipitated by centrifugation for 20 min at 10,000 g (with the final protein concentration of 30-40 mg/ml in the precipitate). Then the supernatant was removed; different amounts of the *n*AM suspension were added very carefully, and the centrifugation was repeated. The second centrifugation produced a two-layer system (Figure 1) with the overlying super-

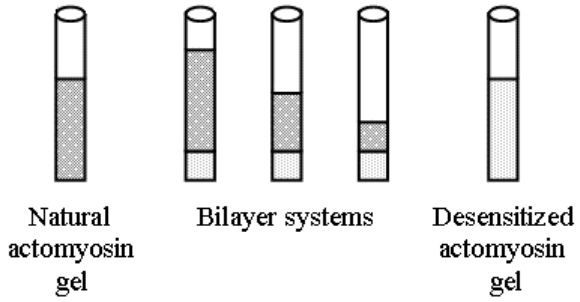

FIGURE 1. A diagrammatic sketch of the two-layer actomyosin systems used, each with a different ratio of natural (*n*AM) and desensitized (*d*AM) actomyosin. The sizes of the layers are overestimated to better demonstrate the arrangement of the experiments.

natant that remained in the tubes during the entire experiment. In order to test deformability of the interlayer surface during the second layer formation, the surface of the first layer was marked with the dye neutral red or with colloidal carbon. No deformation was detected after the second centrifugation. So the possibility of a mixture of different actomyosin preparations can be ruled out.

In separate experiments, it was found that the *n*AM layer (in the two-layer system with the *n*AM/*d*AM ratio 60:40, respectively) lost its Ca^{2+}-sensitivity as early as after 1-3 min. But to insure the steady-state conditions, we used an overnight incubation both for the two-layer systems and for two-component (*n*- and *d*AM) suspensions. However, the apparent diffusion coefficient was determined during much shorter time periods.

After formation of the two-layer systems, the tubes were chilled for 15-20 h at 4-6°C before the electrophoresis and superprecipitation. After incubation, minimal, about 2 mg, samples of actomyosin were carefully taken exactly from the surface of the upper layer of the two-layer system for measurements of the Ca^{2+}-sensitivity and electrophoretic analysis. The stock *n*AM and *d*AM preparations used to form the two-layer system served as control.

To determine the apparent diffusion coefficient (ADC) for the TT-complex (tropomyosin was used as a marker of the complex), the *n*AM-*d*AM interaction was performed in thin polyvinyl chloride tubes that were chosen so that the outside diameter of one tube corresponded to the inside diameter of the other. This made it possible to form a very tight, mechanically strong connection. Tubes with the internal diameters of 3 and 6 mm were used; they formed 2-3-cm-long gels; one end of each tube was sealed.

The thin tubes were filled with *n*AM, the thick ones, with *d*AM. The filling of the tubes with actomyosin gel was carried out by the same centrifugation procedure, as in the preparation of the two-layer systems (10,000 g, 20 min). After formation of the precipitate, the tubes were cut at the level of the precipitate/supernatant line and connected to each other very carefully. The protein contamination from the surface of the joined tubes was thoroughly removed.

Several joined tubes were then incubated at 4-6°C. These two-tube systems were quickly frozen on ice at -20°C after different incubation time (from 3 min to 1 h). Then the thin and thick tubes were separated into two sections, left and right, respectively, 1 mm from the point of gel contact. The segment isolated after these first two cuts was discarded.

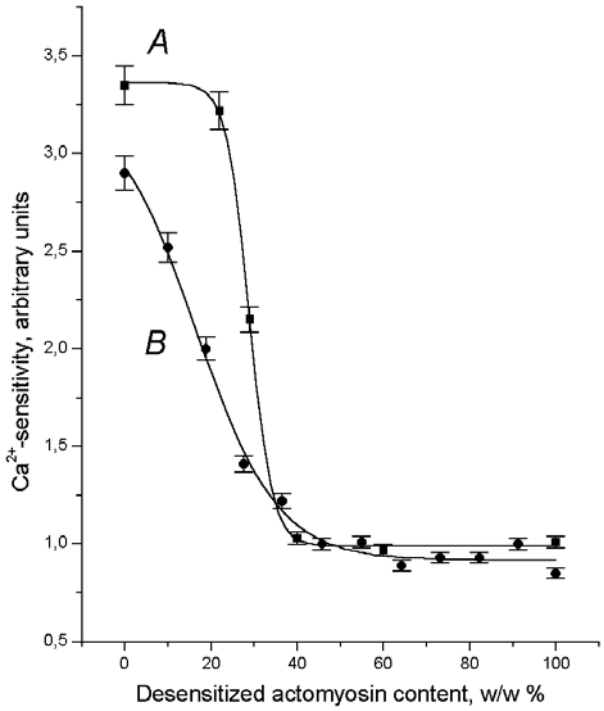

FIGURE 2. The Ca^{2+}-sensitivity of the natural actomyosin *(n*AM*), A*, arranged as the upper part of the two-layer system (See Figure 1), and the Ca^{2+}-sensitivity of the two-component suspension mixtures (1 mg of protein per ml) of *n*AM with desensitized actomyosin *(d*AM*), B,* following the 15-20 h incubation (at 4-6°C) plotted as a function of the weight portion of the *d*AM. The value "0" on the X-axis means *n*AM preparation alone. The value "100" on the X-axis means *d*AM preparation alone. The value "1" on the Y-axis means the absence of the Ca^{2+}-sensitivity. The data points are the means ± SEM from triplicate assays.

The separated tubes were cut into 5 mm slices (resulting in 4-6 segments). After each cut, the cutting tool was cleaned of protein contamination. Protein from each segment was solubilized and subjected to electrophoresis. This allowed for the detection of the time-dependent tropomyosin decrease in the *n*AM gel and the tropomyosin increase in *dAM.* The TT-complex diffusion was studied at a *n*AM/*d*AM weight ratio 1:4. The ADC was calculated as described by Chang (10).

Statistics. Results of the experiments were expressed as means ± SEM. The differences were considered statistically significant at $P < 0.05$, using Student's *t*-test.

Results

Figure 2 (curve *A*) illustrates typical results of the experiments with the two-layer systems. The control *n*AM preparation definitely demonstrated a high sensitivity to Ca^{2+} (curve *A*, the left end, 0% of *d*AM), while the other control preparation, *d*AM, was completely desensitized (curve *A*, the right end, 100% of *d*AM). In the two-layer system, an increase in the proportion of the *d*AM weight within narrow limits from 20 to 40% (20% range) resulted in the complete loss of Ca^{2+}-sensitivity in *n*AM (the top layer).

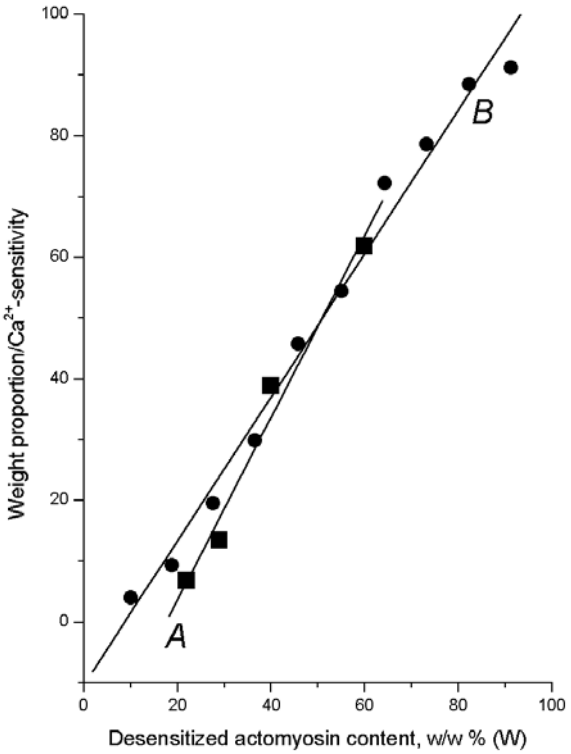

FIGURE 3. The linear representation of the data from Figure 2 for natural (*n*AM) + desensitized actomyosin (*d*AM) preparations in a form of two-layer systems, *A*, and as two-component suspension, *B*. The experimental results (except for the data for individual *n*AM and *d*AM preparations, in which the studied interaction does not occur) are well fitted by the linear model: $W/S = a + b \cdot W$, where **W** is the weight portion (in %) of *d*AM; **S** is the Ca^{2+}-sensitivity (in arbitrary units) of *n*AM (as a part of the two-layer system) or of the *n*AM + *d*AM suspension mixtures. The parameters of the equation for the suspensions are: $W/S = -10.4(\pm 2.7) + 1.18(\pm 0.05) \cdot W$, correlation coefficient = 0.994 (P-value < 0.01); for the two-layer systems: $W/S = -26.4(\pm 6.6) + 1.50(\pm 0.16) \cdot W$, correlation coefficient = 0.989 (P-value < 0.05). The statistically significant linearity of the relationships indicates that the data both for the two-layer systems and for the *n*AM + *d*AM suspension mixtures demonstrate a hyperbolic relation between **S** and **W** values. If so, it is possible to calculate the X-axis asymptote for each curve in Figure 2 as a value for the threshold *n*AM/*d*AM ratio in accordance with the relation: Threshold = *a/b,* where *a* and *b* are parameters of the linear model mentioned above (the absolute value of *a* is calculated). The following values for the thresholds were obtained: 9±2% for the suspensions, and 18±5% for the two-layer systems (means ± SEM). These thresholds are the levels of *d*AM (in %, w/w) when the redistribution of TT-complex begins in the systems.

As seen from the shape of the curve *A*, the degree of the TT-complex redistribution depends on the weight ratio of the layers, therefore, diffusion of the minor components is triggered by the weight ratio, rather than by the existence of the gradient alone. This two-layer system as a whole initiates the protein diffusion at a definite *n*AM/*d*AM threshold (critical) ratio.

This "threshold" hypothesis is confirmed by the data shown in Figure 3. As seen from Figure 2, the curve describing the dependence of Ca^{2+}-sensitivity on the weight portion of *d*AM (without the points for 0 and 100% *d*AM because these points do not reflect interactions in two-layer systems) looks like a hyperbola. After transformation of the data of

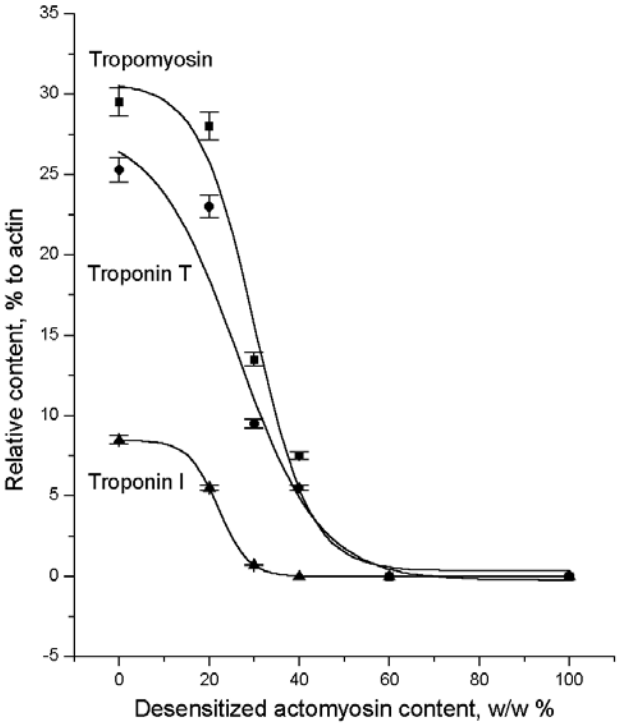

FIGURE 4. The tropomyosin, troponin T, and troponin I contents in the natural actomyosin (*n*AM) arranged as the top layer of the two-layer systems (see Figurel), plotted as a function of the weight portion of the desensitized actomyosin (*d*AM) in the lower layer after 15-20 h of incubation (at 4-6°C). These data indicate that the TT-complex diffuses from the *n*AM to the *d*AM layer in the two-layer systems. The value "0" on the X-axis means *n*AM preparation alone. The value "100" on the X-axis means *d*AM preparation alone. The data points are the means ± SEM from triplicate assays.

Figure 2 into the coordinates of Figure 3, this dependence becomes linear. The linear character of the relationships demonstrated by Figure 3 is statistically significant for the two-layer system at the 95% confidence level (correlation coefficient = 0.989, $P < 0.05$) and for the two-component suspension, at the 99% confidence level (correlation coefficient = 0.994, $P < 0.01$). This linearity proves the hyperbolic character of the relationships seen in Figure 2 for *interacting* actomyosin preparations. Therefore, we can regard the X-axis asymptote (see the legend for Figure 3) for each curve as a corresponding *n*AM/*d*AM threshold ratio. The threshold value for *d*AM content to initiate blockage of Ca^{2+}-sensitivity of *n*AM was calculated to be 18 ± 5% for the two-layer system and 9 ± 2%, for the two-component suspension.

Since *n*AM (the top layer) lost its Ca^{2+}-sensitivity, the question arises as to whether the change in the content of the TT-complex actually takes place in this layer of the two-layer system under the conditions of our experiment. To answer this question, several two-layer systems with different *n*AM/*d*AM ratios were formed (Figure 1), and the content of the regulatory proteins in the top layer of the systems was determined (after 10-15 h of incubation) by the method of electrophoresis. Figure 4 shows that the content of all the studied components of the regulatory complex fall sharply when the weight proportion of *d*AM exceeded a certain threshold.

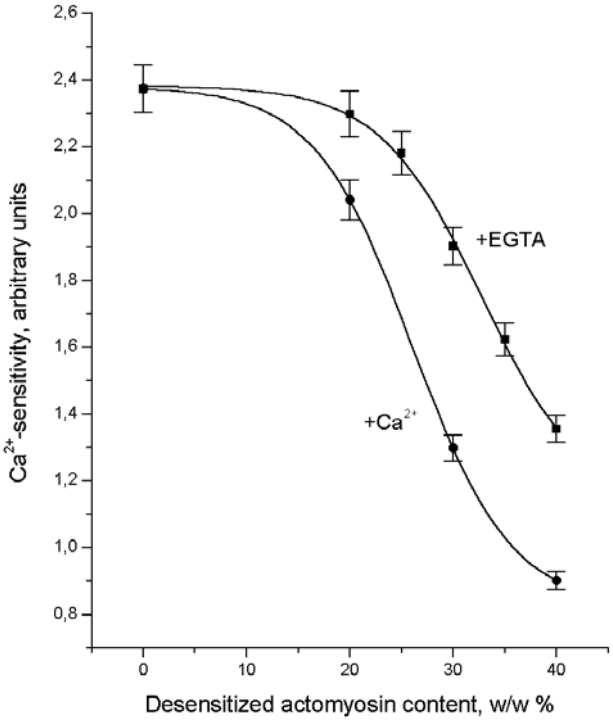

FIGURE 5. The Ca^{2+}-sensitivity of the two-component suspension mixtures (1 mg of protein per ml) of natural actomyosin (*n*AM) with desensitized actomyosin (*d*AM) plotted as a function of the weight portion of the *d*AM in the mixture after one-hour incubation (at 4-6°C) in the presence of 0.5 mM EGTA or 0.5 mM Ca^{2+}. When Ca^{2+}-sensitivity of the preparations with EGTA was measured, excess of Ca^{2+} was injected about 1-2 sec prior to the MgATP injection. The value "0" on the X-axis means 100% *n*AM content in the suspension. The data points are the means ± SEM from triplicate assays.

With respect to the Ca^{2+}-sensitivity changes, similar results were also obtained for mixed *n*AM and *d*AM suspensions containing different proportions of *d*AM (Figure 2, curve *B*) (e.g., with a final protein concentration of 1 mg/ml, each ml in the 40% *d*AM mixture would contain 0.4 mg of *d*AM and 0.6 mg of *n*AM). Like in the case when the *d*AM weight proportion in the two-layer systems reached the 40% level, the two-component mixture completely lost Ca^{2+}-sensitivity at the same level of *d*AM, although it consisted of 60% of *n*AM containing a significant amount of the native TT-complex. The cooperative character of the *B* curve (Figure 2) might indicate an interaction between the two types of actomyosin preparations, otherwise the relationship between the Ca^{2+}-sensitivity and *d*AM content would have had a linear character.

As the TT-complex is the receptor of Ca^{2+}-, the Ca^{2+}-dependence of the *n*AM-*d*AM interaction was studied. These sets of experiments were performed as were other experiments with two-component suspensions but with the following differences: incubation time was 1 h; incubation medium contained 0.5 mM EGTA or 0.5 mM Ca^{2+}. When Ca^{2+}-sensitivity of the preparations with EGTA was measured, excess Ca^{2+} was injected about 1-2 sec prior to the MgATP injection. Data plotted in Figure 5 show that free Ca^{2+} enhances the inactiva-

tion of the Ca^{2+}-sensitive system (by *d*AM*)* but does not affect the regression slope of the Ca^{2+}-sensitivity *versus d*AM*:* the close-to-linear parts of the curves are almost parallel.

Our measurements showed that the tropomyosin (in fact, the TT-complex) ADC (apparent diffusion coefficient) varied within the limits of $4.2\text{-}4.4 \cdot 10^{-4}$ cm^2/sec in the presence of Ca^{2+} (0.5 mM) and within the limits of $2.7\text{-}4.2 \cdot 10^{-4}$ cm^2/sec in the presence of EDTA (0.5 mM). Thus, it is evident that divalent cations (Ca^{2+} and Mg^{2+}) do not significantly affect the rate of the tropomyosin/TT-complex diffusion under the conditions of our experiments.

Discussion

An understanding of the nature of the Ca^{2+}-sensitivity of actomyosin superprecipitation is essential to interpreting the results obtained in this work. The TT-complex has been shown to be the final component of the excitation-contraction coupling system (11). This complex also determines Ca^{2+}-sensitivity of the natural actomyosin superprecipitation (2-4). Its removal results in the loss of Ca^{2+}-sensitivity in actomyosin, but addition of the TT-complex to a reconstituted actomyosin that contains no other proteins but actin and myosin produces Ca^{2+}-sensitivity of its superprecipitation.

In this connection, of interest are our data using the two-layer gel (Figure 1). We found a new type of interaction of the contractile proteins both in two-layer systems and in suspension mixtures. The *n*AM preparations studied had a high Ca^{2+}-sensitivity (3.3 arbitrary units) before interaction with the *d*AM layer (Figure 2). However, after the *n*AM gel contacts with the *d*AM gel, the Ca^{2+}-sensitivity of *n*AM (the top layer) begins to depend on the *d*AM/*n*AM weight ratio in the two-layer system; this dependence has a *threshold* and *cooperative* pattern: *n*AM completely loses Ca^{2+}-sensitivity within the narrow limits of this ratio changes, from 22/78 to 40/60. This suggests that *n*AM, the top layer of the two-layer gel, loses the regulatory TT-complex.

The amount of the Ca^{2+}-sensitive complex in the top layer is actually reduced with an increase in the amount of *d*AM in the system (Figure 4). At the *d*AM/*n*AM ratio = 4/6, the tropomyosin amount, for example, in the top layer decreases more than 3 times. The amount of other regulatory proteins also falls markedly. The above-mentioned ratio, 4/6, is the limit, after which the actomyosin preparations lose completely their Ca^{2+}-sensitivity.

The change in the Ca^{2+}-sensitivity of *n*AM in the two-layer system has a definite cooperative character that indicates that two actomyosin preparations somehow interact with each other and this interaction spreads far from the line of contact of the two gels.

It is clear that the *n*AM-*d*AM interaction begins in a zone of contact of the two gels, but then this process involves the entire system. Thus, the molecular events in the protein system studied appear spatially interconnected.

The mechanism of such changes requires the ability of the minor components of actomyosin gel to move inside the gel. This conclusion is based on the data presented in Figure 4. These data indicate that in the bulk of the two-layer gel, after the start of contact was made, the spatial redistribution (diffusion) of the TT-complex molecules begins to be visible as evidenced by the minor components leaving the top layer of the system (*n*AM) to the point where *n*AM loses its Ca^{2+}-sensitivity. It is highly probable that the structural basis for the diffusion of the TT-complex is F-actin, because tropomyosin and the complex strongly bind

to it (4), and the sigmoid character of the interaction is caused by the cooperative properties of actin filaments and proteins associated with them (12,13).

The *n*AM-*d*AM interaction also occurs in the two-component suspensions (Figure 2, curve *B*). It is seen that with a decrease in the Ca^{2+}-sensitive *n*AM fraction, the decrease in the Ca^{2+}-sensitivity of the whole mixture *is not linear;* this relationship might indicate a more complex, unexpected interaction. In fact, as soon as the *n*AM content falls below 60%, the Ca^{2+}-sensitivity of the mixture disappears completely.

The interaction of *n*AM with *d*AM in the presence of free Ca^{2+} causes a greater decrease in the Ca^{2+}-sensitivity (Figure 5). This is definitely reasonable, considering multiple Ca^{2+} effects on the properties and structure of contractile and regulatory proteins and on their complexes (4).

It is well known that Ca^{2+}, in addition to its effect on the structure and properties of the TT-complex (14), enhances the cooperativity and changes some other properties of actin filaments (13,15) as well as reduces by twice or more the actin affinity to the TT-complex (16). The latter effect is of interest, as the weakening of the F-actin-TT-complex interaction may facilitate diffusion of the complex. In the presence of EGTA, actin filaments are more rigid than in the presence of Ca^{2+} (17); but in the presence of Ca^{2+}, the regulated actin (F-actin + TT-complex) and F-actin alone become very flexible (18,19). From our point of view, the flexibility of actin filaments is one of the factors modulating the rate of the decrease or loss of the Ca^{2+}-sensitivity after the beginning of the *n*AM-*d*AM interaction: the flexible filament facilitates the diffusion process and thus accelerates redistribution of the TT-complex.

It is interesting to compare the TT-complex apparent diffusion coefficient (ADC) obtained in this work for the condensed actomyosin gel (at 4-6°C) with that for diffusion of other substances in water (at 25°C) (10): $1.10 \cdot 10^{-5}$, ethanol; $1.18 \cdot 10^{-5}$, urea; $4.6 \cdot 10^{-6}$, sucrose; $6.9 \cdot 10^{-7}$, hemoglobin; $13.3 \cdot 10^{-7}$ cm^2/sec, myoglobin. It should be noted again that in our experiments, tropomyosin was used as a protein marker for the TT-complex. It might well be that the ADC obtained for "tropomyosin" is in fact the coefficient for TT-complex.

Thus, the TT-complex ADC measured at a lower temperature and in much more viscous, almost solid, medium than water has an ADC value that is one order of magnitude higher than that for small molecules of ethanol and urea and three orders of magnitude higher than that of hemoglobin and myoglobin. The diffusion coefficients for other proteins (lysozyme, ovalbumin, bovine serum albumin), and tobacco virus, as well as for DNA, also appeared to be three orders of magnitude lower (20) than that for TT-complex. Hence, if the TT-complex diffused in the water inside the actomyosin gel, its diffusion coefficient would be much lower. In other words, the mechanism of the fast diffusion does not allow TT-complex to dissociate from actomyosin.

On the other hand, protein diffusion in the cytoplasm (condensed protein system) is much slower than in aqueous solutions. Thus, the ADCs for proteins in cytoplasm are: $1.0 \cdot 10^{-8}$ cm^2/sec, bovine serum albumin (human fibroblasts, at 22°C) (21); $5.7 \cdot 10^{-8}$ cm^2/sec, aldolase (Swiss 3T3 cells, at 36°C) (22); $2\text{-}3 \cdot 10^{-9}$ cm2/sec, G-actin (embryonic chicken gizzard cells, at 36°C) (23); $1.2 \cdot 10^{-7}$ cm^2/sec, myoglobin (muscle fibers, at 22°C) (24). As a rule, the protein mobility in the cytoplasm is much slower than in aqueous solutions. This indicates that the mobility inside the cell is limited by interaction of diffusible proteins with the cytoplasmic matrix (25).

It is interesting to discuss which properties of F-actin can promote the "sliding" diffusion of regulatory proteins bound to it: (1) tropomyosin bound to actin has an ability for lateral movement (26-30); (2) F-actin itself can facilitate diffusion of the TT-complex by spontaneous bends (18,19), a "twist" of monomeric units in coordination with each other (31), and rotational movements of actin globules (32).

Our findings seem to indicate that the actomyosin gel can be a convenient model for studies on processes of intracellular signalling systems related to actin filaments. This model (a protein gel based on F-actin) is most likely to provide promising opportunities for studies of conditions required for protein diffusion in condensed protein systems like the cytoplasm. The phenomenon of the fast diffusion of TT-complex recalls findings of a higher mobility of cations bound to polyanions in comparison with the their lower motility in water (for review see 33). These data indicate that the association does not necessarily lead to a pronounced reduction in mobility.

So the data obtained can be summarized in the following words. Natural actomyosin enriched in the troponin-tropomyosin complex interacts specifically (by a mechanism still unknown) with desensitized actomyosin free of the regulatory complex. This results in (i) loss of Ca^{2+}-sensitivity in natural actomyosin placed both as a layer of the two-layer system and as a component of its mixture with desensitized actomyosin, and (ii) redistribution of the TT-complex in the actomyosin gel. The unknown mechanisms of these effects may play an important role in the living cell.

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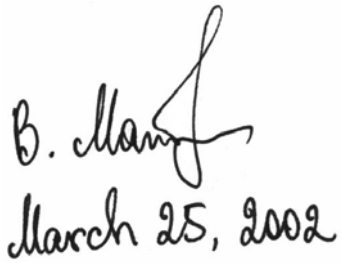
Author's remark made after publication of this article

In our conditions, diffusion of tropomyosin-troponin complex began in 6 hour's time after creation of the two-tube systems and came to an end after 12 hours. So, as a whole the diffusion takes up about 6 hours.



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