

New Type of Protein-Protein Interaction Demonstrated by Actomyosin

V.V. Matveev's report for Albert Szent-Györgyi Symposium „The Living State”
Sümege, Hungary, May 23, 2002

First slide. Title of the report.

Dear Colleagues, first of all, I would like to say that I am much grateful to the Organizing Committee of the Conference for the honor to present here some unusual observations of mine on properties of actomyosin.

Next slide

Slide 2. The goal of my communication.

The goal of my communication is to show that actomyosins with different calcium sensitivity interact with each other. The desensitized actomyosin interacts with the natural actomyosin and modifies its calcium sensitive system. This modification consists both in a decrease of the natural actomyosin calcium sensitivity and in its complete disappearance. However, this disappearance is reversible. The character of interaction of the desensitized actomyosin with natural actomyosin is unusual and is worth studying in detail.

I studied actomyosin of rabbit skeletal muscle. The actomyosin was obtained by standard procedure. To evaluate the results that will be presented in my communication, it is to remember that all of them were obtained in actomyosin gels in solutions with a low ionic strength.

Next slide

Slide 3. Dependence of actomyosin particle size on ionic strength (scheme).

It is known that the colloid state of actomyosin in solution is determined by the ionic strength of the solution. This slide demonstrates dependence of sizes of actomyosin particles in solution on the ionic strength.

At the ionic strength 0.5 and higher, the actomyosin particles represent actin threads decorated with myosin. With reduction of the ionic strength, these particles started aggregating, and the size of the particles increased to reach the size visible by eye. All experiments I will refer to below were performed only at the ionic strength about 0.1. The buffer system seen on the slide was used. Thus, in our experiments, we were dealing with the actomyosin colloid particles, whose sizes fluctuated within large limits.

In due time, I came across the task of obtaining actomyosin preparations with different calcium sensitivity. I decided to achieve this goal by mixing natural actomyosin, with its high calcium sensitivity, with desensitized actomyosin deprived of calcium sensitivity. I believed that calcium sensitivity of such two-component suspensions would be easily regulated by changing ratio between the natural and desensitized actomyosins. Indeed, it looked that the

higher content of desensitized actomyosin in the mixture, the lower calcium sensitivity of the two-component preparation.

Next slide.

Slide 4. Removal of Ca^{2+} -sensitive complex by washing.

Preparation of desensitized actomyosin was done by washing natural actomyosin at 4-6°C in pure water containing a minimal amount of Tris-buffer, pH 8.5-9.0 (Schaub, M.C. and Perry, S.V. 1969, Biochem. J. 115: 993).

I obtained desensitized actomyosin from natural one by washing out natural actomyosin from the calcium-sensitive complex.

Next Slide.

Slide 5. Preparation of two-component suspensions of actomyosin.

Then I prepared the two-component suspensions that represented mixtures of natural actomyosin with desensitized one.

The first experiment on measurement of calcium sensitivity of two-component suspensions has given unexpected result.

Next Slide.

Slide 6. Ca^{2+} -sensitivity of two-component suspensions.

Abscissa shows the content of desensitized actomyosin in the two-component suspension. The desensitized actomyosin content changes within the limits from 0 to 100% ("0" means the one-component suspension containing natural actomyosin only, whereas "100" means desensitized actomyosin only). The calcium sensitivity equal to "1" means the complete absence of the calcium sensitivity.

I expected that the value of calcium sensitivity would reflect mechanic ratio of the gel particles that do have and do not have the calcium sensitivity. In this case the dependence should have changed linearly, as shown by the red line. However, to my surprise, the experimental curve differed essentially from the expected one.

It had the quite clear nonlinear character (shown by the black line), which is possible only in the case of interaction of these two actomyosins. It is certainly seen that this interaction is of cooperative character. It has turned out that as little as 40% of desensitized actomyosin in the mixture is sufficient to knock out the calcium sensitive system of natural actomyosin, whose percentage in the mixture still is rather high, 60%. What happens here is an amazing event. To understand its reason, let us see the next slide.

 Slide 7. Mechanical contact is needed to modify Ca^{2+} -sensitivity of natural actomyosin.

* Particles of *n*- and *d*AM came into collision.

* Two-layer system modeling the interaction of the particles

It is quite clear that interaction of the natural and desensitized actomyosin can occur only under the condition of their mechanical contact, as shown on the left side of the slide. Such interaction of particles can be modeled by obtaining two-layer systems from the natural and desensitized actomyosin gels. This experimental approach allows forming two-layer systems with different ratios of the natural and desensitized actomyosin.

Next Slide.

 Slide 8. Formation of two-layer gels.

The two-layer systems were obtained by a consecutive sedimentation first of desensitized actomyosin, then of natural actomyosin, by changing the weight ratio between the upper and lower layer.

In experiments with two-component suspensions, it might be suggested that the natural and desensitized actomyosin do not interact with each other until they enter reaction of superprecipitation, that is (i.e.) they interact only in the presence of ATP. To check this suggestion, the experiments with two-layer gels were performed.

Next slide.

 Slide 9. Threshold manner of the natural actomyosin-desensitized actomyosin interaction in the two-layer system

On the left side of the Slide, it is seen that the dependence of the calcium sensitivity of the upper layer on natural actomyosin is similar to that for the two-component suspensions. Like in the case of suspensions, when the percentage of desensitized actomyosin in the system reaches 40%, the calcium sensitivity of the natural actomyosin in the upper layer disappears completely.

Hence, the studied actomyosin preparations interact with each other even without ATP, that is already in the two-layer system.

The right side of the Slide shows that the dependence of the calcium sensitivity of the upper layer is described by hyperbolic function. This function is known to have asymptotes to both axes. This figure shows asymptote to the X-axis, which is certainly interpreted as a threshold desensitized actomyosin content in the system. Starting with this content, desensitized actomyosin begins affecting the calcium sensitivity of the upper layer. If the desensitized actomyosin content is below the threshold one, its interaction with natural actomyosin in the upper layer does not begin in spite of that these two preparations are in a contact with each other. The threshold desensitized actomyosin value for the two-layer system is determined to be 18%, whereas for suspensions, twice lower.

The essential, cardinal question at description of interaction of the two gels is the question as to why natural actomyosin, when contacting desensitized actomyosin, loses its calcium sensitivity?

Let me repeat the question again: which events result in cessation of activity of the natural actomyosin calcium sensitive system?

Why does the troponin-tropomyosin regulatory complex stop functioning?

One, although presumably not the only, answer to this question is as follows: the regulatory complex in the upper layer does not function just because it is absent there. The most available, although far from simple, way to prove this is the electrophoretical analysis.

Next slide.

Slide 10. Formation of two-tube systems for electrophoretical analysis of tropomyosin and troponin T distribution.

For this purpose, gels of the actomyosin preparations were placed into plastic tubes that subsequently were connected end-to-end.

After incubation for 16 hr, the contents of the tubes were submitted to the electrophoretical analysis. The obtained results are presented on the right of the figure. It is seen that the contents of tropomyosin and troponin T in natural actomyosin decrease. The degree of this decrease depends on the portion of desensitized actomyosin in the two-tube systems. The content of these proteins in desensitized actomyosin rose, but I failed to describe it quantitatively.

To determine the diffusion rate, the contents of tropomyosin and troponin T in different segments of the tube with desensitized actomyosin were measured depending on the time after the beginning of diffusion. The calculated values of the apparent diffusion coefficient for the regulatory complex in desensitized actomyosin gel is shown on this slide (about $(1-4) \cdot 10^{-4}$ cm²/sec), that is three orders higher than the same values for protein diffusion in water. However, this result is so unusual that it requires a separate and detailed study.

Next slide.

Slide 11. Maintenance of the actin band optical density in the linear part of the dependence is the main problem of the electrophoretical study.

The main difficulty of the quantitative determination of proteins by electrophoretical method is the necessity of maintaining the optical density of the actin band in the linear area of dependence of optical density of the band on the content of protein in it. In practical work, to meet this condition is difficult, while every difficulty is a source of errors. Therefore, data on diffusion are of a preliminary character.

Next slide.

Slide 12. Structural changes in actomyosin gel as a result of interaction of natural actomyosin with desensitized actomyosin. Structural changes were tested by the dye, neutral red.

I have already mentioned that 40% of desensitized actomyosin is already sufficient for the complete inactivation of the natural actomyosin calcium sensitive system both in the two-component suspensions, and in the two-layer systems.

The question arises: is there any other way to show that natural actomyosin does, indeed, interact with desensitized actomyosin? For this purpose, I studied effect of desensitized actomyosin on the number of binding sites for the dye, neutral red, in the two-component actomyosin gel.

In these experiments, each determination of the calcium sensitivity and of limit of the dye absorption was performed in the same preparation. The left and the right figures show results of independent experiments.

The X-axis shows the weight portion of desensitized actomyosin in the two-component suspension, while left Y-axis, the number of binding sites of the dye molecules.

It is evident that this parameter changes depending on the amount of desensitized actomyosin in the two-component suspension. This is possible only in the presence of structural changes in actomyosin.

These data are another evidence in favor of that the studied preparations do not coexist in the suspension, but are involved in an interaction, whose nature is unknown.

Next Slide.

Slide 13. The Ca^{2+} -sensitivity of the suspension mixture containing 60% of natural actomyosin and 40% of desensitized actomyosin plotted as a function of n-propanol or ethanol concentration after 15-20 h incubation of the protein mixture with the alcohols.

Then the next question arises whether the loss of the calcium sensitivity is reversible in the two-component suspension composed of 60% natural actomyosin and 40% desensitized actomyosin? Since the protein complexes are stabilized by hydrophobic forces, I decided to try effects of alcohols, as at their action on proteins, the major interactions are hydrophobic ones.

The initial calcium sensitivity of this two-component suspension is practically absent. However, with increase of alcohol concentration, the calcium sensitivity of the preparations starts rising, reaches maximum, and then is rapidly inhibited.

These data indicate that the loss of the calcium sensitivity caused by desensitized actomyosin is reversible. The calcium sensitivity is restored by a modification of hydrophobic interactions by changes of alcohol concentrations. The value "0" on the X-axis means the control preparation of the mixture without alcohols.

Next slide.

Slide 14. CONCLUSIONS.

- * The studied protein complexes interact in the gel state as colloidal particles.
 - * For this reason the interaction begins at a surface of interacting gel particles and extends over the entire gel volume.
 - * The interaction of actomyosin gels reversibly disturbs normal operation of the calcium sensitivity system and can change the troponin-tropomyosin complex distribution in the whole gel volume.
 - * The revealed peculiarities of the interaction allow claiming a new type of protein-protein interaction.
-

Next slide (Acknowledgements)

I am much grateful to all these people for help in performance of this investigation.

Thank you for your attention.

Vladimir Matveev
Email: vm@vm1616.spb.edu
Home page: <http://actomyosin.narod.ru>