ON FACTORS AFFECTING THE REVERSIBLE SUPERPRECIPITATION OF ACTOMYOSIN

by

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In view of the work of SZENT-GYÖRGYI[1] and his co-workers it is highly probable that the reactions immediately responsible for muscular contraction involve colloidal changes brought about by adenosine triphosphate (ATP) in the actin-myosin system.

Polymerized actin, F-actin, combines with myosin to form actomyosin. Actomyosin is capable of exhibiting very striking reactions in the presence of ATP, the type of these reactions depending on the salt concentration. SZENT-GYÖRGYI assumes that the molecular mechanism is essentially the same, no matter what the salt concentration. At K^+ concentrations of the order of 0.5 *M*, ATP produces a drop in viscosity of actomyosin solutions. At K^+ concentrations of about 0.05–0.15 *M* on addition of ATP a coarse precipitate is formed called superprecipitate by SZENT-GYÖRGYI. Under certain carefully controlled conditions, such as the order of mixing the ingredients, or amount of shaking or mixing, ATP induces formation of a contracted actomyosin plug.

VARGA[2] in SZENT-GYÖRGYI's laboratory has worked out a theory for muscle contraction, the basis of which is a postulated reversible transformation of actomyosin from one molecular state corresponding to relaxation, into another corresponding to contraction. If the reaction of actomyosin with ATP is essentially this postulated reaction, it would seem of interest to study the possible reversibility of it. In this paper we report experiments that were carried out with optical methods with a view to following the superprecipitation reaction. The effects of p_H and temperature were studied.

EXPERIMENTAL

Actin was obtained according to FEUER, MOLNAR, PETTKO, AND STRAUB[3] from rabbit muscle.

Myosin was prepared according to SZENT-GYÖRGYI, with a modification described by us in another paper[4]. The ATP, used as a sodium salt in our experiments, was a product of Nutritional Biochemicals Corporation.

Superprecipitation was followed by measuring photoelectrically the intensity of light** transmitted through the actomyosin solution. Since superprecipitation produces a gross turbidity, *i.e.*, light scattering occurs, it was desirable to obtain some rough information about the angular distribution of the transmitted light***. A glass cell with optical windows (light path 5 mm) contained

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** An incandescent lamp was used as lightsource. The protein solutions used did not have any significant absorption in the visible part of the spectrum.

*** Because of the non-homogenity of the actomyosin solutions a theoretical interpretation of light scattering data is not possible.

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the sample. A collimated light beam passed through the cell, and the transmitted light fell on a photocell. By means of a diaphragm the angle within which light could reach the photocell was regulated. The apperture is expressed as the angle subtended by the diameter of the opening of the diaphragm at the center of the glass cell. The diameter of the direct beam was equal to that of the smallest opening used, 2° as defined above. Therefore we subtracted this amount from every actual opening. The smallest aperture was thus designated by 0°, indicating that no light outside the direction of the primary beam could reach the photocell. With this convention the other openings used were 2°, 14° and 40°. The transmission of light is expressed as per cent of the amount transmitted by a H_2O blank. The cells were well shaken before a reading was taken, in order to ensure a uniform suspension of the superprecipitate.

If not stated otherwise, the total K^+ concentration was 0.13 *M* in all experiments. Actin and myosin were mixed in such a ratio that there would be neither free actin nor free myosin[4].

RESULTS

Fig. 1 shows a typical curve of the light transmission of an actomyosin superprecipitate. It shows also the curves for actin, myosin, and actomyosin without ATP.

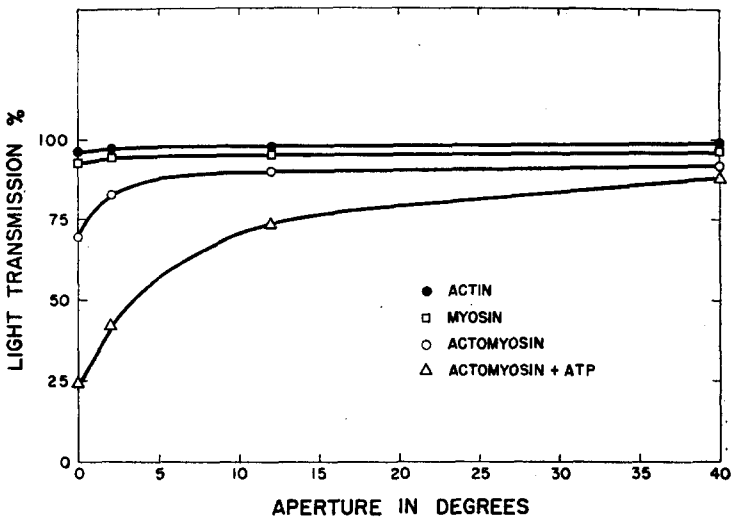

Fig. 1. Variation of light transmission with aperture in diaphragm
● Actin, protein 0.25 mg/ml
□ Myosin, protein 0.56 mg/ml
o Actin, protein 0.25 mg/ml + Myosin, protein 0.56 mg/ml
△ Actin, protein 0.25 mg/ml + Myosin, protein 0.56 mg/ml + ATP 100 μg/ml
Readings were taken 5 minutes after addition of ATP.
In each case 0.05 *M* histidine buffer, K^+ conc. 0.13 *M*, p_H 7.5, 25° C.

It will be seen that superprecipitation causes a marked change in the course of the curves. There is a decrease in light transmission at narrow angles, indicating a light scattering by the superprecipitate. For this reason we have decided to use the light transmission data with the smallest opening as a measure of superprecipitation, since the method is most sensitive with this aperture*.

Fig. 2 shows results of experiments in which the effects of temperature and p_H were studied. It will be noted that at 0° there is no increase in turbidity on addition of ATP at any p_H within the range p_H 5.5–9.5. The actomyosin solutions above p_H 7

* Decrease of light transmission with this opening corresponded always to a visible increase of flocculence, increase of light transmission to a clearing up of the solution.

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clear up upon addition of ATP as indicated by the increased light transmission. At higher temperatures ATP induces superprecipitation and this is optimal at about p_H 6.5–7.5. At p_H 9.5 there is either an increased light transmission or no apparent effect.

We would add that, as shown in Fig. 2, in the absence of ATP the turbidity of an actomyosin solution does not significantly change either with the temperature or with p_H.

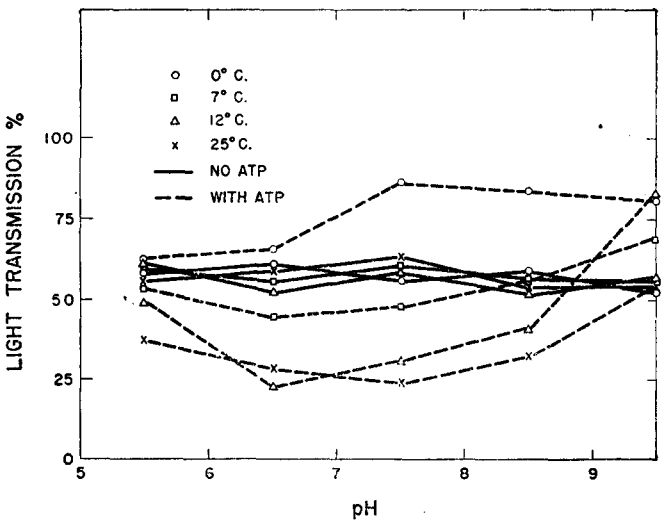

Fig. 2. Effect of p_H and temperature on light transmission of actomyosin and actomyosin + ATP. Light transmission with o° opening in diaphragm (for explanation see text). Reaction mixture: Actin, protein 0.25 mg/ml + Myosin, protein 0.56 mg/ml. 0.05 *M* histidine buffer, K^+ conc. 0.13 *M*. The actomyosin solution was kept at the appropriate temperature for 15 minutes. A reading was taken and after 5 minutes in the constant temperature bath ATP was added (final concentration 100 μg/ml) and a reading taken after another 5-minute interval.

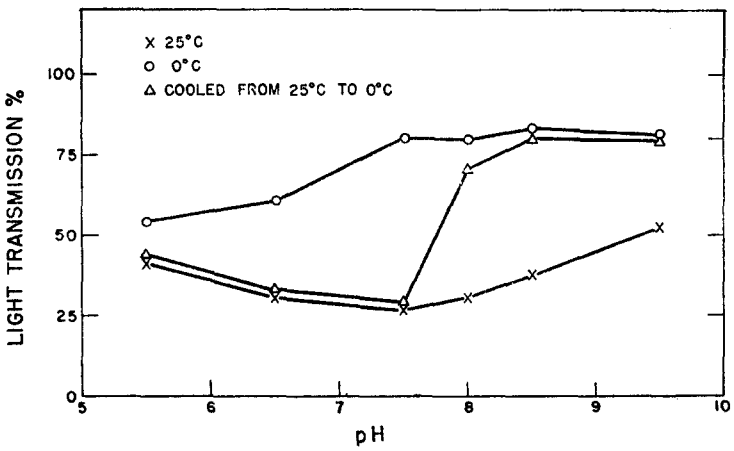

Fig. 3. Temperature reversibility of the Actomyosin-ATP reaction.
Light transmission with o° aperture (for explanation see text). Reaction mixture was the same as in Fig. 2. 100 μg of ATP were added per ml at 25° C, and readings taken after 5 minutes (×—×). The same amount of ATP was added again, and the reaction mixture was put in a o° C bath. Readings were taken after 15 minutes (△—△). 100 μg of ATP were added per ml at o° C, and readings taken after 5 minutes (o—o). As shown by Fig. 2, light transmission of actomyosin solutions did not change either with temperature or p_H in the absence of ATP.

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In the p_H range 8.0–9.5 the ATP effect is reversible with respect to temperature, as shown by Fig. 3. Below p_H 8 the precipitate once formed does not dissolve if brought to 0° C. At p_H 8.5, *e.g.*, the precipitate formed at 25° C dissolves if cooled down to 0° C. Brought to higher temperatures the precipitate appears again. The presence of ATP is essential for the reversibility. If the ATP is split by the ATP-ase action of the actomyosin before cooling, no increase in light transmission ensues. To ensure the presence of a sufficient amount of ATP in the reversibility experiments extra ATP was added (100 μg/ml) before bringing the superprecipitate to 0° C. A second addition of ATP at p_H 8 did not produce any change in turbidity at 25° C; therefore, we were satisfied that reversibility was produced by the change in temperature and not by the repeated adding of ATP. If the sample was put in the 0° C bath immediately after the formation of the superprecipitate, no addition of ATP was necessary for reversibility.

It was found that with myosin B, *i.e.*, actomyosin extracted as such, or mixtures of actin and myosin that were allowed to stand for more than 4 hours, no reversibility can be obtained.

DISCUSSION

It is known from the studies of SZENT-GYÖRGYI and others that temperature affects the superprecipitation reaction of actomyosin. Quantitative studies have not been carried out and the effect of p_H has not been taken into consideration. We have now shown that p_H plays an important role in the formation of the superprecipitate in a threefold sense: First, at 0° C, where there is no superprecipitation at all ATP produces an increase in light transmission, and this effect increases with p_H up to p_H 7.5. This may be due either to a dissociation of actomyosin or to formation of a hydrated ATP-actomyosin complex. Second, at higher temperatures there is a p_H optimum for the superprecipitation. This seems to have roughly the same value as that recently found to be optimal for the contraction of a superprecipitate[5]. Third, the temperature-dependent reversibility of the superprecipitation reaction depends on p_H in that reversibility occurs only at higher p_H values. It is difficult to decide whether the effect of temperature on superprecipitation is due to a change in solubility or to a shift in the equilibrium position of a chemical reaction the product of which would be insoluble. The first possibility would indicate that the actomyosin-ATP complex has a positive heat of solution since the solubility decreases with increasing temperature. In view of the fact that the highly charged ATP-actomyosin complex would have quite a strong tendency for hydration, this picture does not seem unreasonable. The second possibility would be an endothermic transformation between two forms of actomyosin. This on the other hand is in agreement with VARGA's results on muscle slices and fibers. The theoretical significance of reversible reactions involving the actomyosin system has been pointed out in the introduction. Our experiments seem to prove the existence of such a reaction *in vitro*, though it seems to occur outside the physiological p_H range. This, however, may not affect the relevance of our results since other factors may shift this range *in vivo*.

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SUMMARY

1. The reaction between actomyosin and ATP was studied by measuring light transmission within various angles.

2. The effects of p_H and temperature on superprecipitation as measured by light transmission is described.

3. The temperature effect is reversible within the p_H range 8.0–9.5.

4. The significance of superprecipitation and its reversibility is discussed with respect to muscular contraction.

RÉSUMÉ

1. Nous avons étudié la réaction de l'actomyosine avec l'ATP en mesurant l'intensité de la lumière transmise à travers la solution d'actomyosine à l'intérieur de divers angles.

2. Nous avons décrit les effets du p_H et de la température sur la "superprécipitation" tels que nous les avons mesurés par la transmission de la lumière.

3. L'effet de la température est réversible entre les limites de p_H 8.0–9.5.

4. Nous avons discuté la signification de la "superprécipitation" et de sa réversibilité par rapport à la contraction musculaire.

ZUSAMMENFASSUNG

1. Die Reaktion zwischen Actomyosin und ATP wurde durch Messung der Lichtdurchlässigkeit innerhalb verschiedener Winkel untersucht.

2. Der Einfluss von p_H und Temperatur auf die "Superprecipitation", der sich aus unseren Lichtdurchlässigkeits-Messungen ergibt, wurde beschrieben.

3. Der Temperatureffekt ist innerhalb des p_H-Bereiches 8.0–9.5 reversibel.

4. Die Bedeutung der "Superprecipitation" und deren Umkehrbarkeit wurde in Bezug auf die Muskelkontraktion erörtert.

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