Observations on myosin and actomyosin.

by

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1. *Comparative Study.*

I compared the behaviour of the striated muscle of different animal species during extraction. I used the muscle of the rabbit, pigeon (breast muscle), frog (Rana esc.), fish (amiurus nebulosus), cattle, hen (leg and breast) and the heart of the rabbit. The muscles of the freshly killed animal were quickly cooled, minced on a cooled Latapie mincer, suspended in 0,6 *M* KCl, 3 ml being taken per g of muscle. The suspension was stirred at 0° C and then centrifuged. My problem was to see how much myosin is extracted and how active it is when the time of extraction is varied. The results are summed up in the two curves.

Fig. 1a. and 1b. show the relation between activity and the time of extraction. As can be seen the extract of rabbit muscle shows little activity during the first 15 min. and begins to rise after this time. This rise is continuous and after 3 hours the rabbit (as well as the pigeon) muscle has given a 120% active myosin. The activity of frog muscle extract rises steep in the beginning to remain unchanged afterwards. Very remarkable is the behaviour of the fish which gives in 20 min. a 120% active myosin. Other fishes (esox mucius) behave in like manner. The actin thus releases the stucture very easily. This might explain the easy digestibility of fish meat.

Fig. 2a and b. shows the relation between the time of extraction and myosin content of the extract. These fig. show that the rabbit is an especially favorable object. The values obtained in 20 min. in the other animals seem to lie close to

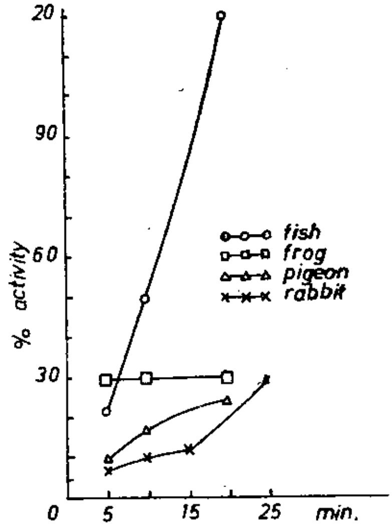

Fig. 1. a.

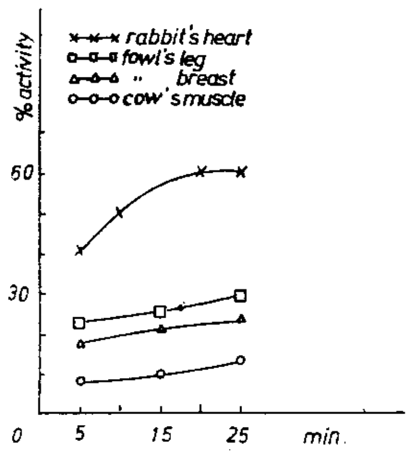

Fig. 1. b.

the limit, for, in the pigeon even after 3 hours, no more than 9 mg per ml was extracted.

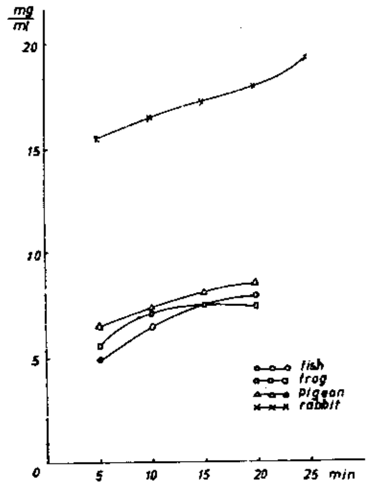

Fig. 2. a.

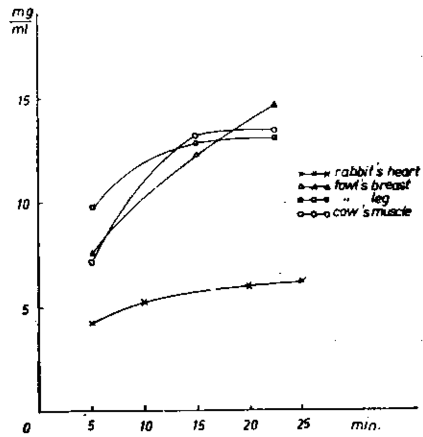

Fig. 2. b.

2. *KCl curve and activity.*

In these experiments I compared the viscosity of myosins of different activity — thus different actin content — at varied

KCl concentration with and without the addition of ATP. The 10% and 90% active myosin were prepared directly from muscle. The 50 and 150 % myosin were prepared by mixing actin and myosin. My solutions contained 1,6—1,8 mg myosin per ml. The pH was stabilised with veronal acetate buffer of pH 7 (see BALENOVIĆ and STRAUB[1]). The solution contained 0,001 M $MgCl_2$. To 4 ml of fluid 0,05 ml of a 1,2 % ATP solution

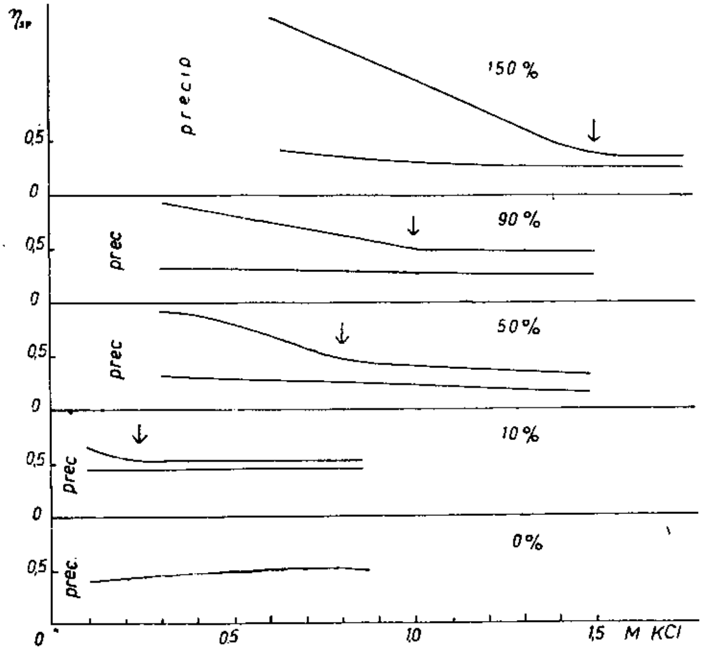

Fig. 3.

was added. Foaming was prevented by the addition of one drop of caprylic alcohol. The viscosity was determined in the capillary viscosimeter. The time of outflow of the salt solution varied between 70 and 120 sec. First the viscosity was determined without and then with ATP. Temp. 0°.

My results are summed up in Fig. 3. The blank zone on the left means precipitation. In every case the lower line is the ATP curve.

In the case of 0% active myosin ATP had no effect and the two curves were identical. F. B. STRAUB[2] found that ATP at a lower pH greatly affects the viscosity of myosin A. At that time, when those experiments were made, however, we were not yet able to prepare entirely inactive myosin. Myosin A was not quite inactive; it had only a low actin content. Now, repeating these experiments with completely inactive myosin, ATP is found to have no effect at all.

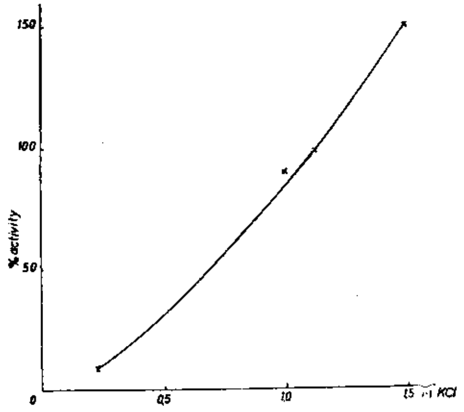

Fig. 4.

The other curves show that at a high KCl concentration the two curves lie close to each other and are parallel. A high

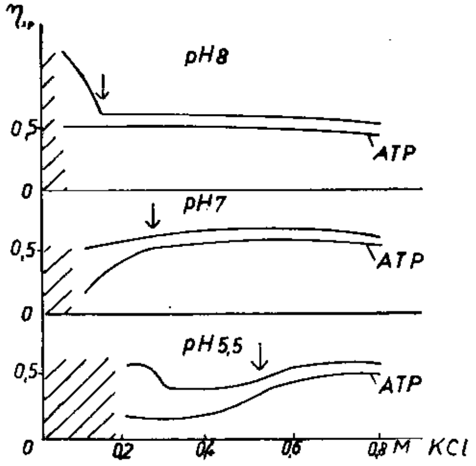

Fig. 5.

salt concentration has thus the same effect as ATP, i. e. it is capable of dissociating the actomyosin.

The curves show that according to the activity (actin content) of the actomyosin, the parting of the two curves lies at different KCl concentrations i. e. actomyosins of different activity are dissociated by the salt alonce at different concentrations. This means that actomyosins of different % composition behave as individuals. The less actin they contain the less KCl is needed for their dissociation.

If the points, at which the two curves part, are plotted against the activity, Fig. 4. is obtained which is, in fact, the curve of complete hydration and dissociation of actomyosins of different actin content.

In the case of 10% active actomyosin I also studied the effect of pH (see curve Fig. 5.) I found that higher pH shifts the point of parting to the left, lower pH to the right. At pH 8 this point lies at 0,15 *M* KCl, at pH 5,5 at 0,6 *M* KCl. In both cases the pH was stabilised by a veronal acetate buffer.

As a practical consequence we may deduce that the measurement of activity of actomyosins of low actin content can be made with advantage at low KCl concentration and at lower pH, which, so to say, magnifies the effect of ATP on viscosity.

The theoretical consequence of these curves is still more interesting. We have seen that the 10% active myosin behaves, in absence of ATP, as an individual and not as myosin plus actomyosin. This 10% active actomyosin contains only 1,6% actin to 98,4% myosin. It can be concluded herefrom that actin is capable of coating itself with several layers of myosin. Myosin in itself has a tendency towards coaxial association and the actin seems to act as the nucleus of these associated particles. The force, by which actin holds this great mass of myosin can be but small because 0,25 *M* KCl suffices to cause dissociation. As has been shown elsewhere (F. B. STRAUB[3]) maximum activity is 170%. Also fermentatively this complex is the most active (BANGA[4]). It corresponds to the natural actomyosin of muscle and contains actin and myosin in the proportion of 1:3. Very probably this is the actomyosin in which the actin micels are coated with one layer of myosin. This one layer is held very firmly and a very high KCl concentration is needed for dissociation.

The smaller the % activity, the thicker this layer of myosin held by the actin, but the smaller the force by which myosin is held and, correspondingly, the smaller the KCl concentration which is capable of effecting dissociation.

If the salt concentration of the actomyosin solutions of different activity is gradually reduced at a certain point the solution precipitates. The more active the actomyosin, the more salt is needed to keep it in solution. This precipitation is reversible. If the salt concentration is increased again the precipitate dissolves. One very interesting fact, which emerges from these experiments of Fig. 3, is that if the precipitate is dissolved by increasing the salt concentration in presence of ATP the actomyosin not only dissolves but also dissociates at the same time into actin and myosin. Actomyosin, dissolved thus in presence of salt and ATP means dissociated actin and myosin.

If the KCl concentrations, at which the actomyosin dissociates without ATP, are plotted against the activity of the actomyosin, a straight line is obtained (Fig. 5).

References.

1. *K. Balenović* and *F. B. Straub.* These studies **1**, 43, 1941–42.
2. *F. B. Straub.* Ibid **2**, 6, 1942.
3. *F. B. Straub.* Ibid **1**, 43, 1941–42.
4. *I. Banga.* Oral comm.