Observations on Actomyosin.

by

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If a 0,5 *M* KCl-solution of myosin and a solution of actin are mixed actomyosin is formed. If the solution is sufficiently strong it can be pulled into threads.* If it is diluted with water it precipitates and can be washed salt-free giving a fine and fairly stable suspension.

Actomyosin is much less soluble than myosin or actin is. While myosin dissolves in water actomyosin only swells up. While 0,2 *M* KCl dissolves myosin 0,6 *M* KCl will be needed to dissolve actomyosin. But on the whole the collodial reactions of actomyosin are a reflection of the reactions of myosin.

Free actin is not precipitated by alkali salts, myosin is. Actomyosin will be precipitated similarly to myosin. In Tab. I. the precipitation of myosin and actomyosin by KCl are compared and it will be seen that the precipitation of both, at small salt concentrations, go parallel.

ATP has a solvatising action on myosin and will, in absence of salts, dissolve actomyosin also.

While myosin and actomyosin react thus to salts and ATP in a similar way; they will respond in a different way to the joint action of salt and ATP. While in the case of myosin the sole effect of ATP was to weaken the precipitating action of salts, in the case of actomyosin small amounts of ATP will greatly intensify the action of the salt. If the salt, in itself, had a precipitating action, ATP will make this precipitation much

* Technic of threads see vol. 1 of these studies.

Table I.

M . KCl	0,2	0,1	0,05	0,025	0,0125	0,006	0,003	0,0015	0,0008	0
Acto-myosin	++	++	+++	+++	++	++	+	+	0	0
	15	14	11	12	16	20	28	44	65	65
Myosin	0	0	+	++	++	++	+	+	0	0

Upper line: molar concentration of KC . Middle line: volume of the actomyosin precipitate in an arbitrary unit. Crosses mean precipitation. 0,1% solution of myosin and 0,1% suspension of a 25% actomyosin.

Table II.

The action of varied KCl and ATP concentration on an actomyosin suspension

M KCl	mg ATP per ml						
	0,4	0,2	0,1	0,05	0,025	0,0125	0
0,4	!!!	!!!	!!!	!!!	!!!	!!!	!!
0,3	!!!	!!!	!!	!!	!	0	!
0,2	!!	!	xx	xx	xx	x	++
0,05	xxx	xxx	xxx	xxx	xx	xx	+++
0,0125	!	!	0	0	x	x	+
0,003	!!	!!	!	!	!	x	+
0,0008	!!!	!!!	!!!	!!	!	0	0

0, 1 % suspension of 25% actomyosin.

+ = precipitation, x = superprecipitation, ! means clearing up of the suspension. !!! means complete dissolution. O means no change. In this experiment first the KCl was added to the actomyosin suspension, the resul noted and then the ATP introduced.

stronger. If the salt (owing to its higher concentration) solvatised actomyosin in, ATP will make the dissolution complete. (Tab. II.).

Not only will the precipitation, caused by KCl in presence of ATP, be much intenser. The whole character of the precipitate will be changed: it will become granular and settle quickly to a small volume. This change is due not only to the intenser precipitation but at the same time to the strong shrinking of the particles. I will call this effect „super-precipitation" to distinguish it from the simple precipitation given by salts alone.

The shrinking of actomyosin can better be observed on actomyosin threads which give under the same conditions a violent contraction which is, in fact, but an extreme degree of shrinking. Superprecipitation and contraction are identical phenomena caused by the shortening of the actomyosin micels, as proved by the anisodimensional contraction of oriented myosin threads (M. GERENDÁS[1]). We can thus sum up our results by saying that if salts act on an actomyosin particle, they will simply discharge and precipitate it. If they act on the ATP complex of actomyosin they will cause not only discharging and precipitation of the particles but also their shortening. This is what has been called superprecipitation and what is observed in threads as contraction.

We must distinguish between the action of small and high concentrations of ATP. Small concentrations will have the effect described. Big doses will have a solvatising action only. Comparing the solvatising action of this excess of ATP at different salt concentrations (Tab. II.) we will find that the stronger the KCl precipitation and thus the stronger the KCl—ATP superprecipitation, the more ATP will be needed to cause dissolution.

Similarly to precipitation also superprecipitation is reversible. Any agent that brings about a dissolution and dissociation of actomyosin reverts it to its uncontracted form. It has been shown earlier (3) that contracted actomyosin threads can be brought to relaxation by alkaline salt solutions or by the combined action of salts and ATP (4). These relaxed threads are capable of contracting again.

Superprecipitated or contracted myosin can be dissolved by 0,05 % ATP in absence of salts. It is also dissolved by

higher salt concentrations or salt plus ATP. Substances that will dissolve it will also restore to it its DRF, restore thus also the original shape and dimensions of its particles.

ADP.* Threads prepared form synthetic actomyosin contract energetically in presence of salts and ATP. They are somewhat more labile than threads prepared from the impure natural actomyosin (our earlier myosin B). It was repeatedly observed that a freshly pulled thread gave only a sluggish contraction and became very active spontaneously in a few hours. The initial sluggish contraction could be speeded up by soaking the thread in an aqueous muscle extract.

There is one very sharp difference between the reaction of threads prepared from pure actomyosin and from myosin B. If the myosin B thread is suspended in a 0,05 *M* KCl containing 0,001 *M* $MgCl_2$, and 0,1% ADP is added, the thread will contract in the same way as if ATP had been added. If the pure actomyosin thread is treated likewise no contraction occures. If, however, in addition to ADP also $^1/_{10}$ parts of an aqueous muscle extract** are added, the thread will contract.

The aqueous extract can be inactivated by 20 minutes boiling. The contraction of the thread will be faster if the extract is mixed with the ADP solution before this latter is added to the thread. If the extract is allowed to stand with the ADP for a few minutes and the mixture then boiled for twenty minutes, it will cause rapid contraction if added to the thread. This makes it evident that the aqueous muscle extract contains a thermolabile substance which alters the ADP in such a way that it is capable of causing contraction. Evidently this catalyst is identical with K. LAKI's watersoluble factor and I. BANGA's isomerase. In all probability it restores to the nucleotide the active pyrophosphate configuration.

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* The ADP was prepared by I. BANGA from ATP by having one phosphate split off by myosin.

** The aqueous extract was prepared in the following way: The muscle was minced, suspended in water, 3 ml being taken for every g of muscle. The suspension was stored over night at 0° during which time the ATP present was split. The clear filtrate contains no ATP and added to threads, gives, in itself, no contraction.

This experiment was repeated with a purified isomerase solution of I. BANGA. It was found that the protein solution reactivated the ADP only in presence of Mg (0,001 *M*). If isomerase and ADP were allowed to stand in absence of Mg, boiled and then added to the muscle, no contraction ensued.

We can thus say that the difference between myosin B and pure actomyosin is due the presence of isomerase in myosin B As shown by I. BANGA, myosin B can be freed from isomerase by repeated washing. Threads, prepared from such myosin B. behave in respect to ADP as the synthetic actomyosin. As shown by I. BANGA, such an isomerase-free actomyosin does not split ADP either. There is thus, at this point, a close analogy between splitting and contraction.

Salts. It has been shown before that there is a close analogy between the precipitation of actomyosin by KCl and the superprecipitation elicited by KCl and ATP. This analogy between salt-precipitation and superprecipitation breaks down in the case of Ca and Mg. Both ions have an equally strong precipitating action on actomyosin and could both be expected to act as K and give contraction in presence of ATP and enhance the K-ATP contraction. But contrary to this expectation Ca will give with ATP no contraction at all and Mg will do so only in presence of higher ATP concentrations which makes it probable that the contraction, in this case, is also due to the joint action of the Mg and K, the latter being present as cation of the ATP. Ca inhibits the K-Mg-ATP contraction also. There is thus an antagonism between K and Ca on the one side and Ca and Mg on the other side which makes it probable that Ca and Mg exert their influence by some other mechanism than K does. The antagonism of K and Ca in living muscle has been known for a long time. The predominance of K causes tetany, Ca suppresses it. Also the antagonism of Ca and Mg has been known from pharmacology (*Melzer* narcosis).

Example: actomyosin thread prepared from 12,5% actomyosin. The thread was soaked for a few minutes in the salt solution and then 0,125 % ATP was added in the form of its neutral K salt. The numbers give the % linear contraction reached in one minute, as measured by the length of the thread.

KCl 0,05*M*	38
KCl 0,05*M* and $MgCl_2$ 0,001 *M*.	52

KCl 0,05*M* and $CaCl_2$ 0,01 *M* 2
KCl 0,05*M*, $MgCl_2$ 0,001 *M* and $CaCl_2$ 0,01 *M* 18

The bending of actomyosin threads as a model of contraction. It has been shown that, under action of salts and ATP, actomyosin threads shrink rapidly i.e. become shorter and thinner: they contract. If, however, there is an assymmetry in the system which makes that the two long sides of the thread do not contract equally, then the thread, before

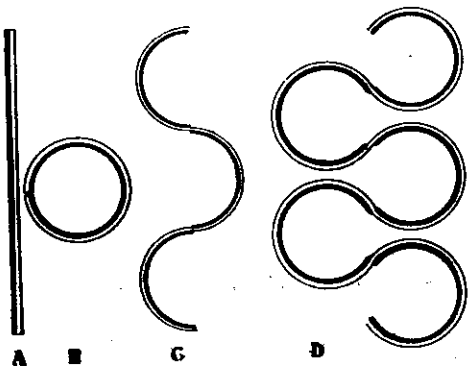

Fig. 1.

contracting, will bend or curl up. This is shown in fig. 1A and B where the more reactive side is marked with black. Such an assymmetry in the reaction can be produced by introducing the ATP on the one side of the thread. An assymmetry may also be found in the substance of the thread. Threads, if not pulled under special precautions, have some such assymmetry in their composition and tend to bend before they contract. One very often observes the curling up as shown in fig. 1B. It is immaterial what the source of the assymmetry is, the result will be the same: the bending of the thread. This is not a new phenomenon, it is an undesirable experience of every-day life: if two boards are stuck together and shrink or swell unequally then humidity will bend them.

Such an assymmetrically built myosin thread may serve as a model of the actomyosin micel. If a myosin and actin micel stick together side by side an assymmetrical structure will be obtained, as symbolised in fig. 1A. But myosin and actin

are both hydrophil colloids and rather similar in their constitution and reactions. A high degree of assymmetry will be introduced by ATP which reacts only with the myosin moiety of actomyosin forming with it an ATP-myosin* complex which is most sensitive to ions. The one sided shrinking of the actomyosin micel will have to cause its bending. This bending will make the micel effectively shorter and so the whole mass of the actomyosin gel will shrink and the thread, made herefrom, shorten. The maximum contraction will be reached when the micels have curled up as in fig 1B. In this case the thread has to contract to $^1/_3$ of its original length (more exactly $l = \frac{l_0}{\pi} + d$ d being the diameter of the thread). The maximum contraction of actomyosin threads was found to be 66%.

It is also evident that in threads in which the actomyosin micels have been arranged coaxially to the thread the curling of the micels must give anisodimensional contraction: the thread must become shorter and thicker and at the same time its double refraction must be lost.

Figure 1B makes it evident that, if a contracted actomyosin is brought to dissociation, we obtain the original uncontracted actin and myosin which, if put together, will give uncontracted actomyosin, as is actually the case in the experiment.

The geometrical limitations given above do not hold if the actin forms a continuous structure, as is the case in muscle. Combination with myosin and ATP will give shortening also here but in this case the limit will not be $1/\pi$. The contraction will theoretically have no limits. In fig. 1C is pictured a shortening of $^1/_3$, in fig. 1D a shortening of $^3/_4$.

References.

1. *M. Gerendás.* These studies, **1**, 47, 1941—42.
2. *A. Szent-Györgyi.* Ibid. p. 67.
3. „ „ „ „ „ 22.
4. „ „ „ „ **2**, 25, 1942.

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* Should it be found that myosin is phosphorylated then the only change will be in regard of this model that we will have to write P-myosin in stead of ATP-myosin.