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THE PROPERTY AND CONTRACTION PROCESS OF ISOLATED MYOFIBRILS*

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In our laboratory, many endeavourments have been devoted to obtaining the clue to the understanding of the normal mechanism of muscular contraction[1]. For the past decade our experiments have been carried out with various contractile materials such as living muscle fibres, dried fibres, degenerated fibres with chemical treatments and extracted threads, and their respective visco- and thermo-elastic properties and physico-chemical activities in contraction have been extensively studied. In pursuit of these investigations we were led to consider that, in order to approach our purpose, it is of the highest importance that the sarcolemma, sarcoplasm and myofibrils should be separated from one another and their individual natures should be researched.

The first desirable requirement was to remove the myofibril intact from the live fibre. A direct procedure was attempted. The first isolation was performed in a bath of isotonic KCl solution or a saline solution made so that it contained the constituents similar to those of the sarcoplasm[2]. The myofibril isolated in these solutions inevitably came into contracture, though it almost returned to its original length if stretched out after a while. In various experiments, this fibril was proved to have the characters closely resembling those of the intact fibre. However, it was less sensible to electric shocks, heat, actions of contracture producing drugs such as nicotine, quinine, caffeine, *etc.*, suggesting that the structure of its protein molecules might be somewhat disorganized.

The next goal of our investigations was to separate the myofibril possessing the properties and contractilities of the native myofibrils in the living muscle fibre. In varying attempts, finally, the fibril isolated with a method stated in this report fulfilled the requirement. The present study is concerned with further investigations of the properties of this myofibril, which contributed to the more thorough understanding of the mechanism of contraction.

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Preparation of the myofibril

A single muscle fibre was isolated in RINGER's solution from the adductor magnus, gastrocunemius, sartorius, *etc.* of the toad; then placed on a dried glass plate and the solution adhering to it was completely removed. After that, the fibre was immersed in a bath of refined whale oil. Then, under microscopic observation (*ca.* 50–100× magnification was most suitable) the sarcolemma was carefully stripped from the fibre by a sharply pointed knife. After stripping the sarcolemma off completely, the bared fibre was gently separated with fine needles into small bundles of myofibrils. No contracture was produced during the manipulation, provided the solution adhered to the fibre was completely removed before placing into oil.

Results

1. Visco-elastic properties of the myofibril

The following experiment was designed to facilitate an accurate comparison of the visco-elastic properties between the intact fibre and the separated myofibril. A part of a single fibre was stripped of the sarcolemma to expose a region of myofibrils and the other part was left intact the respective rates of extension of both parts were estimated when this whole fibre was stretched out. A typical example of these experiments is given in Table 1. It proves that the rates of

Table 1.

Part of myofibrils Diameter 29μ		Part of intact fibre Diameter 32μ		
Length (μ)	% of extension (e)	Length (μ)	% of extension (E)	e/E
300	0	300	0	1.00
327	9	320	7	1.29
342	14	336	12	1.17
364	21	351	17	1.24
382	27	375	25	1.08
401	34	382	27	1.26
425	42	404	35	1.17
558	86	415	38	2.26

both parts are closely resembling within the range of 0 to 40% extension; *i.e.* the elastic modulus of the myofibril is quite analogous to that of the living fibre within this range of extension.

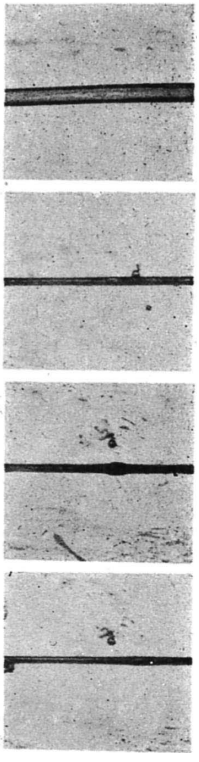
2. Contractions of the isolated myofibril produced by various solutions

The amount of solutions to be added to myofibrils must be very small. For this purpose, a thin piece of wood with a finely sharpened tip was utilized. A small drop of the solution was placed on its tip, and the excess solution was then wiped off with a small piece of gauze to allow only an adequate amount to leave adhering to it. By this method very small amounts of solutions were applied to a point of the myofibril in all experiments.

a) Contraction by water

When water was added to the isolated myofibril, it contracted intensely. The curve of this contraction plotted against time was identical to that shown in water rigor of the living fibre.

However, when a very small amount of water was applied to a site of the myofibril placed in an oil bath, it contracted instantly and relaxed subsequently. Fig. 1 illustrates pictures of contractions of myofibrils on adding a small drop of water *ca* 10 μ. in diameter. This twitchlike contraction could be repeated by repeating addition of water as often as at least 90 to 100 times. The larger the drop of water, the stronger became the contraction and the weaker the relaxation. The time of contraction as well as relaxation was parallel to the temperature, *i.e.* the higher temperature, the faster the time (Table 2).



b) Contractions by NaCl, KCl, $MgCl_2$, $CaCl_2$, $AlCl_3$, $CaCl_2$, K_2HPO_4, and acids and alkalis

On adding small amounts of solutions of 0.001M. to 0.15 M. of KCl and NaCl, the myofibril contracted and relaxed similarly as in addition of water (Table 3), through it was very poorly contracted by KCl solution over 0.15 M..

When a small piece of KCl crystal was applied, the myofibril contracted intensely once, but no longer repeated contraction in repetition of appliance, suggesting that its constractility must have been to some extent injured by the effect of KCl. Likewise, the myofibril contracted on adding 0.001M. to 0.3 M. K_2HPO_4 solutions, though it basely contracted to solutions, *e.g.* such as 0.6 M. (only 5 to 10%). On adding solutions of $MgCl_2$ and $CaCl_2$, the myofibril strongly contracted, but relaxed less completely as compared with relaxations observed

Table 2.

Temperature (°C)	Phase of contraction (sec.)	Phase of plateau (sec.)	Phase of relaxation (sec.)
5	12	2	11
	3	0	4
	15	3	14
	5	1	4
	3	0	3
7	4	0	3
	13	2	15
	3	0	3
	8	2	7
	11	2	10
10	4	0	3
	5	1	6
	2	0	2
	2	0	2
	2	0	3
20	1	0	1
	1	0	1
	3	0.5	3
	2	0	1
	1	0	1
25	1	0	2
	1	0	1
	3	0	2
	1	0	1

when KCl and NaCl solutions were added. Idential contraction of the myofibril was also produced by adding $AlCl_3$ and $CaCl_2$ solutions.

To acid and alkali solutions of varying pH the myofibril contracted and relaxed similarily as when water or NaCl solution was applied. Table 4 gives the grades of contractions in relation to the pH of solutions. As indicated in this table, the contraction of the myofibril to acid or alkali solution of higher concentrations was not so remarkable.

c) Contraction by ATP

When solutions or small crystal pieces of K, Na and Mg. salt of ATP were applied, the myofibril quickly contracted and relaxed. It was a twitch-like contraction just as seen on adding a small amount of water.

The grade of this contraction was concerned not with the amount of ATP

solutions. If a large amount of ATP solution was applied, contracture was induced from the beginning. If ATP was added, to the myofibril which had beed sufficiently contractured by water, further weak contraction (10 % or so) was produced, but no relaxation followed even after 20 to 30 minutes.

d) Contraction due to Quinine, Nicotine, Veratorine and Acetylcholine

Also by applying solution of varying concentration of these drugs similar concentrations of the myofibril was produced, the process of which was in many respects identical to that in contractions caused by water or other solutions. However, no small conductive contracting waves, which are usually seen when these drugs act upon the living fibre, could be produced on the myofibril.

3. *Contraction produced by electric current*

An unpolarizable capillary electrode as follows was specially designed for the electrical stimulation of the myofibril. A glass capillary tube was filled with KCl agar. A small amount of agar was made protruding out of the orfice of KCI the tip of the tube, and then dried so that it might be adequately with so much solution that permitted electric current to flow but to cause the myofibril to contract. If the agar was excessively wet with KCl solution, it would induce same effect as produced when KCl solution was added, which might overshadow the effect of electric current.

Table 3.

KCl (mol)	% of contraction
0.30	##
0.15	##
0.04	###
0.02	###
0.01	###
0.001	###
NaCl (mol)	
0.30	###
0.15	###
0.04	###
0.01	###
0.001	###
$MgCl_2$ (mol)	
0.30	###
0.15	###
0.04	###
0.01	###
0.001	###
$CaCl_2$ (mol)	
0.30	###
0.15	###
0.04	###
0.01	###
0.001	###
$AlCl_3$ (mol)	
0.30	###
0.15	###
0.04	###
0.01	###
0.001	###
K_2HPO_4 (mol)	
0.60	+
0.30	##
0.15	###
0.04	###
0.001	###

Note: + 5~10%, ## 20~30%, ### 40%~

When a constant current was sent into the myofibril through a pair of these

Table 4.

(HCl, NaOH) pH	% of contraction
1.2	⫫
3.2	⫫
4.2	⫫
8.2	⫫
9.2	⫫
9.6	⫫
10.0	+→ relaxation

electrodes, the part lying beneath the anode contracted quickly while the part beneath the cathode contracted slowly. The grade of contraction became stronger according to the increase of the intensity of current on anodal side whereas not so marked on the cathodal side. On breaking the current relaxation quickly occurred only on the former side, and the cycle of contraction could be repeated on this side, for a number of times by repeated appliance of the current.

Discussion

The elastic system of the muscle fibre may be considered to be integrated with the elastic properties of the connective tissue covering, sarcolemma, sarcoplasm and myofibrils. A previously proposed view that the sarcolemma plays a conclusive part in the function of the elastic system of the muscle fibre seems to be highly questionable from our experimental point of view. It was first a very interesting question to what extent the myofibrils isolated in an oil bath are removed from the living fibre. With respect to the visco-elasticity, Table 1, illustrating closely simulating extension rates within 0 to 40% stretching, will give a definite answer. Namely, it may be inferred that the visco-elasticity of the muscel fibre in extension (or contraction) within this physiological range is entirely due to the myofibrils, but not to the sarcolemma, though the latter might behave as a protector when the muscle is stretched out to a higher degree (over 40%). This is further-more affirmed from our thermo-elastic studies (previously reported) which proved that the lineer expansion coefficient of the muscle fibre as well as the myofibril was of negative sign at and above 8°C, that of the sarcolemma being of positive[1]. In other words, it may not be so hazardous to assume that the myofibrils isolated in oil possess identical visco-elastic properties to those which the myofibrils in the intact fibre would have.

The behaviours of the myofibrils isolated in oil in responses to the action of water, saline and other various solutions give some suggestions to a better understanding of the normal mechanism of contraction. From the present study, it is clearly indicated that whenever the environment of the liquous state surrounding the myofibrils was altered by applying any of these solutions, they always contracted irrespectical of the sort and concentration of the added solution. How-

ever, it is noted that the myobirils did not so clearly contract on adding solutions of high concentrations of K_2HPO_4, KCI, acid, alkali, *etc.* In these cases, the injurious effect of these solulions should be taken into account. The interaction of ATP and actomyosin molecule is undoubtedly involved in the mechnisms of contration[3], but such a view that only this interaction is the most essential element in contraction cannot be accepted from the results of the present experiments. Besides, resembling responses of the isolated myofibrlis to those of living fibre to the action of some alkaloids such as quinine, nicotine and others would offer an evidence that the contractile proporties of these isolated myofibrils might not be so much different from those of the myofibrils in the living fibre.

The myofibrils contracted at the anodal side on passage of electric current, just reversal of what the living fibre does. To explain this reversed response, it will be first important to make clear the respective variations of ionic distribution on the surface of the bared myofibrils and the sarcolemma of the intact fibre on applying current. However, since the liquor environment surrounding the isolated myofibril must likewise be altered by electric current, the mechanism involved in this anodal contraction and relaxation would be considered essentially identical to that in contraction by water and other various solutions.

If it were permitted to speculate that when any substance, whatever it may be, associates with the side linkages of chain molecules of protein of the myofibril, the contraction develops and when dissociates from, the relaxation does (or *vice versa*), since, when a change occurs in the constituents of the liquid surrounding myofibrils, ionic linkages, hydrogen bonds and the linkage of VAN DER WAALS between chain molecules may accordingly varied, the changes of the myofibril as in above experiments would occur. To restore the original state from this association the development of some chemical processes in the constituents of the surrounding fluid would be very advantageous. If such a speculation were correct, a fact that the relaxation was developed less completely by some substances with a high adsorption ability could be plainly understandable. Although, of course, this speculation cannot be verified by only the results of the present experiments, it would at least be true that a transient alterration of the liquor constituents arround the myofibrils may have an important significance to the twitch-like contraction.

Summary

An ideal preparation of the myofibrils was produced by isolating in an oil bath. In respect of the viscoelasticity this preparation proved the highest similarit-

to the living fibre. It contractes and relaxed quickly like a twitch when a small amount of water or any of the solutions with varying concentrations of NaCI, KCI, $MgCI_2$, $CaCI_2$, $AlCI_3$, $CuCI_2$, K_2HPO_4, ATP, nicotine, quinine, caffeine, acid, alkali, *etc.* is applied, through it responded imcompletely to some of them. This twitch-like contraction could be repeated as often as desired in all cases. On applying an electric current, the site at the anode of the myofibril clearly contracted and on cutting it, relaxed; while the site at the cathode did not respond so clearly.

It is believed that these present studies on the myofibrils isolated in oil offered a better understanding of the normal mechanism of contraction, though further intensive investigation on this subject is needed.

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