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The impact of the paper of Edsall and Mehl (1940) on actin and actomyosin research

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Abstract

The paper of Edsall and Mehl, 'The effect of denaturing agents on myosin, II. Viscosity and double refraction of flow', J. Biol. Chem. 133 (1940) 409–429, inspired our research on actin and actomyosin. It led to the specific purification of actin with magnesium ions and to the demonstration of the central role of the Mg^{2+}-activated actomyosin ATPase in contraction of live muscle.

Keywords: Actin; Actomyosin; Muscle; Magnesium; Urea

1. Introduction

Protein chemistry in the first half of the 20th century focused on the characterization of proteins from blood, egg white, muscle, or plant seeds. In 1930, John Edsall [1] was the first to study the physical chemistry of the muscle globulin, myosin. It was his theory that the physicochemical properties of isolated myosin can be related to 'its function within the muscle fiber'. In two classical papers, von Muralt and Edsall [2,3] described the asymmetry of the myosin particles determined by flow birefringence and viscosity. They also noted that both acid and alkali destroyed the flow birefringence. Ten years later, Edsall and Mehl [4] reported the irreversible loss of flow birefringence of myosin solutions upon addition of various organic and inorganic compounds.

With the discovery of actomyosin by Albert Szent-Györgyi and collaborators [5] in 1942, a new area started in myosin research. It became evident that the myosin solutions used by previous investigators were actually actomyosin solutions with varying actin contents. This urged us to reinterpret the findings of Edsall and Mehl [4]. We found that Table I of Edsall and Mehl [4] was a rich source of information for investigating the properties of actin and the interaction of actin with myosin.

2. Results and discussion

2.1. Background

Our experiments, described in this paper, were carried out between 1950 and 1960. At this time,

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Table 1
The effect of various inorganic salts on the flow birefringence or viscosity of actomyosin solutions

Edsall and Mehl [4]			Bárány et al [6]		
Salt	Concentration (M)	Time for loss of FB	Concentration (M)	ATP-sensitivity	Status of AM
KCl	0.5	2 weeks	0.5	66	Clear
KCl	2.0	40 h	2.0	60	Clear
$MgCl_2$	0.4	5 min	0.5	0	Fine precipitate
$CaCl_2$	0.54	10 s	0.5	0	Fine precipitate
$BaCl_2$	1.1	10 s	0.5	0	Fine precipitate
KSCN	0.3	10 s	0.5	0	Clear

FB is the abbreviation for Flow Birefringence. AM is actomyosin. ATP sensitivity [7,12] is defined as: $\{(\text{logviscosity}_{\text{rel}} - \text{logviscosity}_{\text{relATP}})/\text{logviscosity}_{\text{relATP}}\} \times 100$, where $\text{logviscosity}_{\text{rel}}$ and $\text{logviscosity}_{\text{relATP}}$ are the logarithm of the relative viscosities of the actomyosin solution before and after addition of ATP, respectively. Essentially, the ATP sensitivity measures the actin content of the undissociated actomyosin, i.e. the higher the actin content the higher the ATP sensitivity.

muscle biochemistry focused on the relationship between contraction of water–glycerol extracted muscle fibers and the Mg^{2+}-activated ATPase activity of the fibers. The superprecipitation of actomyosin was another contractile system studied. Actomyosin was prepared from freshly minced muscle (usually from rabbit) by extraction with an alkaline 0.6 M KCl solution in the cold room for 24 h. This actomyosin was called 'natural actomyosin' to differentiate it from the 'synthetic actomyosin', prepared by mixing pure myosin with pure actin. Actomyosin hydrolyzed ATP both in the presence of Mg^{2+} and Ca^{2+}, but only the Mg^{2+}-activated actomyosin ATPase at physiological ionic strength was contractile. Pure myosin possessed a very low ATPase activity in the presence of Mg^{2+}, called Mg^{2+}-moderated myosin ATPase, but upon addition of actin to the myosin its ATPase was enhanced greatly and this was called the actin-activated Mg^{2+}-ATPase of myosin. The mechanism of ATP hydrolysis by actomyosin or myosin was not known, and consequently nobody spoke about cross-bridge cycle, a key concept of today's muscle biochemistry.

Natural actomyosin, of course, did contain troponin and tropomyosin, and the glass-distilled water used to prepare solutions did contain trace amounts of Ca^{2+}. However, it was not realized that these substances are essential for the regulation of muscle contraction.

2.2. Actin

We studied the mechanism of the loss of flow birefringence of 'myosin', actually actomyosin, upon addition of $CaCl_2$, $MgCl_2$, $BaCl_2$ and KSCN, as described in Table I of Edsall and Mehl [4]. The experiments of Edsall and Mehl were repeated [6], but ATP sensitivity [7], a viscometric test, was used instead of flow birefringence to follow the state of aggregation of 'myosin'. Table 1 compares the results of Edsall and Mehl with ours. Two weeks or 40 h were needed to abolish the flow birefringence of 'myosin' solutions kept in 0.5 or 2.0 M KCl, respectively. At the same time, a high ATP sensitivity was recorded of these 'myosin' solutions, indicating a high actin content. Note, that the 'myosin' solutions in KCl remained clear. On the other hand, alkaline earth metals and KSCN decreased the flow birefringence in minutes or seconds, and abolished the ATP sensitivity completely. In the presence of alkaline earth metals a fine precipitate was formed in the 'myosin' solutions, whereas in the presence of KSCN the solution remained clear. The precipitate could be easily spun down and when analyzed it was found to be composed of actin and alkaline earth metals. In case of KSCN we found that it depolymerized F-actin [6].

The precipitation of actin from 'myosin', actually actomyosin, with Mg^{2+}, Ca^{2+}, or Ba^{2+} was

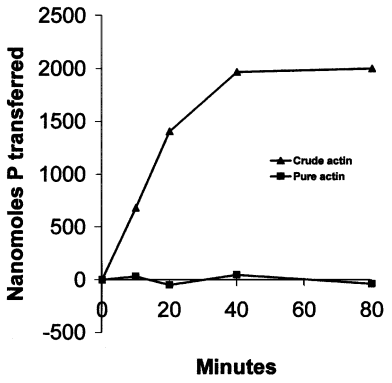

Fig. 1. Comparison of creatine kinase activity of crude and Mg^{2+}-purified actin [8,10].

of special interest: it demonstrated that alkaline earth metals have higher affinity to actin than myosin itself. This opened the way to a specific purification of actin with Mg^{2+} at a concentration as low as 0.02–0.025 M [8]. The purified actin was free of creatine kinase, adenylate kinase, or AMP-deaminase, which were present in the crude actin extract. Fig. 1 shows the creatine kinase assay, using approximately 20 mg protein of either crude or pure actin. No creatine kinase activity was detected in the pure actin preparation for as long as 80 min at 30 °C, whereas under the same conditions, the crude actin transferred 2000 nmoles of phosphate from phosphocreatine to ADP. Similarly, no adenylate kinase or AMP-deaminase activity appeared in the Mg^{2+}-purified actin. The absence of enzymes involved in turnover of ATP or its degradation products in the purified actin supported the idea of Straub and Feuer [9] that the hydrolysis of the actin-bound ATP to actin-bound ADP + Pi was catalyzed by the actin protein itself.

Further analysis of the interaction of actin with Mg^{2+} [10] revealed that 13 moles of Mg^{2+} were bound per actin monomer when actin was precipitated with Mg^{2+}. From the work of Straub [11], who discovered actin, it was known that actin was precipitated from its solution at pH 5. Accordingly, we postulated [10] that Mg^{2+} precipitates actin by binding to its dissociated carboxyl groups and thereby shifting the isoelectric point of actin from pH 5 to pH 7.

2.3. Actomyosin

The Mg^{2+}-precipitation of actin from actomyosin suggested the involvement of ionic bonds in the combination of actin and myosin. We attempted to test this idea on the Mg^{2+}-activated ATPase activity of actomyosin, which is the enzyme functioning during muscle contraction. We thought multivalent cations would inhibit this ATPase, because the hydrolysis of ATP involves the reversible interaction of actin with myosin. Indeed, working in Professor Ephraim Katchalski's laboratory at the Weizmann Institute we found that polylysine inhibited the Mg^{2+}-activated actomyosin ATPase specifically, i.e. Ca^{2+}-activated actomyosin ATPase, Ca^{2+}- or K^{+} (EDTA)-activated myosin ATPase, and the Mg^{2+}-moderated myosin ATPase were not affected [12]. This work was continued in Professor Hans H. Weber's laboratory in Heidelberg where it was found that protamine, a small basic protein, reproduced the polylysine effect [13]. Moreover, not only polycations but polyanions, such as heparin, were also found to be specific inhibitors of the Mg^{2+}-activated ATPase activity of actomyosin [13]. Furthermore, these polyelectrolytes inhibited the ATP-induced contraction of water–glycerol-extracted psoas fibers and relaxed these fibers in the presence of ATP [14]. These data provided evidence for the involvement of electrostatic forces in the actin and myosin interaction. These specific polyelectrolytes were called by Professor Weber 'interaction inhibitors', because they inhibited the enzymic interaction between actin and myosin.

We were interested to bring the interaction inhibitors into the intracellular water of live muscle fibers, in order to prove the central role of the Mg^{2+}-activated actomyosin ATPase in muscle contraction. We found that at least 4 charges on a molecule were needed for the interaction inhibitor effect. However, such charged compounds did not permeate through the plasma membrane of excised frog or rat muscles. After weeks of frustration we considered again the kind of substance, that could

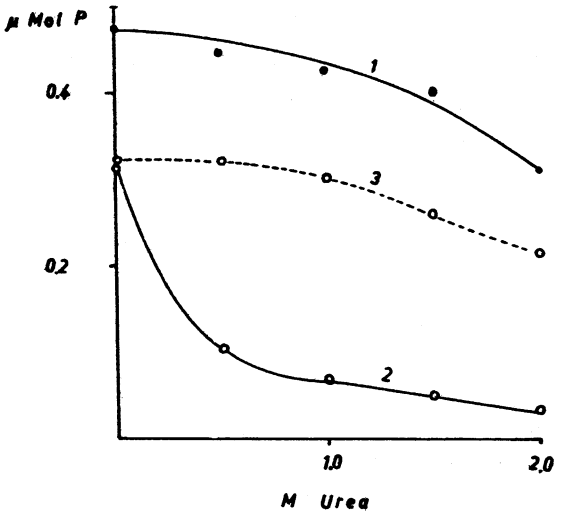

Fig. 2. ATPase activity of frog myofibrils as function of urea concentration [15]. Curve 1, in the presence of Ca^{2+}; Curve 2, in the presence of Mg^{2+}; Curve 3, in the presence of Mg^{2+} after removal of urea.

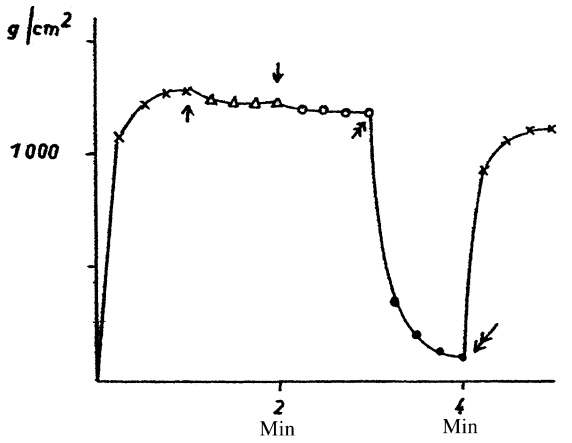

Fig. 3. Relaxation of water–glycerol extracted rabbit psoas muscle fibers in the presence of urea and ATP [15]. Ordinate: Tension; Abscissa: Time. All solutions contained 0.05 M KCl, 0.01 M phosphate buffer, pH 7.0 and 3 mM $MgSO_4$. Room temperature. At 0 min the fiber was contracted by 3 mM ATP; at the upward arrow the ATP was washed out; at the downward arrow urea was added to 1.0 M; at the upper double arrow 1.0 M urea and 3 mM ATP were added; at the downward double arrow the urea was washed out and 3 mM ATP was added.

enter into the muscle water, and still be an interaction inhibitor. To find an answer we studied the substances listed in Table I of the Edsall and Mehl paper [4]. We found that urea at concentrations of 1.1–2.0 M abolished the flow birefringence of the 'myosin' solutions in 2–20 min. Urea breaks hydrogen bonds in proteins and at the low concentrations used by Edsall and Mehl it dissociated actomyosin to actin and myosin, without affecting the subunit structure of myosin. It was reasonable to assume that hydrogen bonds also participate in the actin and myosin combination, in addition to ionic bonds. Furthermore, it was known that the small neutral urea molecule readily permeates into live muscle.

Accordingly, we tested the effect of low urea concentrations on the characteristic reactions of the actomyosin system [15]. Fig. 2 shows that 1.0 M urea inhibited the Mg^{2+}-activated ATPase activity of frog myofibrils to 80%, while only 10% inhibition was produced on the Ca^{2+}-activated ATPase activity. The effect of urea was reversible: After the urea was washed out from the myofibrils, the Mg^{2+}-activated ATPase activity returned to its normal value. Fig. 3 shows the ATP-induced contraction of water–glycerol extracted psoas muscle fibers, contraction was maintained when ATP was washed out and subsequently when the fibers were washed with 1.0 M urea. The fibers did relax when 1.0 M urea was added to the bath in the presence of ATP; after the urea was washed out from the fibers, addition of fresh ATP induced

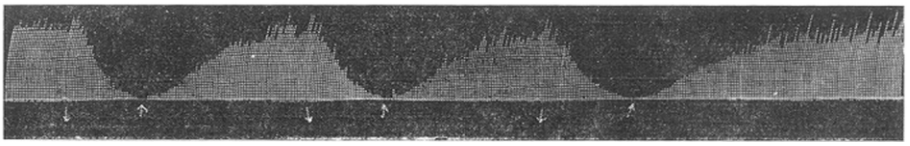

Fig. 4. Reversible inhibition of contraction of frog sartorius muscles by urea [15]. Muscles were stimulated in frog Ringer's solution electrically. At the downward arrow the Ringer's solution was exchanged for a Ringer's solution containing 1.0 M urea. At the upward arrow the urea-containing Ringer's solution was exchanged for normal Ringer's solution.

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April 1, 1960

Dr. Michael Bárány
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Dear Dr. Bárány:

We are most grateful to you for sending on your reprints to us in February, and they have had considerable use here.

I have taken note of your request that we send back the two papers "Untersuchung der Aktin-Aktinbindung I and II. However, since you will be in New York at the Institute for Muscle Disease after the first of May, I think it is perhaps best for us to hold these reprints here for the present and send them to your New York address after you arrive there.

I am glad to know that you will be in this country, and look forward to seeing you on visits here from time to time.

Best wishes,

Yours sincerely,

John Edsall

John T. Edsall

Fig. 5. Letter of Professor Edsall.

contraction again. Thus, the fibers underwent a reversible contraction–relaxation cycle, with a high Mg^{2+}-activated myofibrillar ATPase activity in the contracted state and with a urea-inhibited low Mg^{2+}-ATPase activity in the relaxed state. Fig. 4 shows that an electrically stimulated frog sartorius muscle relaxed when 1.0 M urea was added to the Ringer's solution, this was caused by the urea rapidly permeating into the intracellular muscle water and inhibiting the Mg^{2+}-activated myofibrillar ATPase activity. The muscle contracted again when the urea was washed out of the muscle and thereby the inhibition of the Mg^{2+}-activated myofibrillar ATPase activity was reversed. Reversible inhibition of live muscle contraction was repeated several times. Thus, with the help of the Edsall and Mehl paper one could prove that the Mg^{2+}-activated ATPase activity of actomyosin plays the key role in the mechanism of muscle contraction [15].

2.4. Personal contact

Professor Ephraim Katchalski introduced one of us (MB) to Professor Edsall, while visiting the Biological Laboratories of Harvard University in 1958. Professor Edsall was very much interested in our advancing the results of the Edsall and Mehl paper and have asked for all our reprints that appeared in the Acta Physiologica Hungarica. Since we left Hungary as refugees without taking our publications with us, it took well over a year until we could send our reprints to Professor Edsall. Fig. 5 shows the Thank You Letter of Professor Edsall that he mailed to us just 1 month before we immigrated to the United States.

Subsequently, we have seen Professor Edsall from time to time. We were always impressed by his extensive knowledge of protein chemistry, and by his wisdom. Moreover, we were always impressed by his humanity and his ability to deal with people.

We dedicate this article to the memory of Professor Edsall.

References

[1] J.T. Edsall, Studies in the physical chemistry of muscle globulin, II. On some physicochemical properties of muscle globulin (myosin), J. Biol. Chem. 89 (1930) 289–313.
[2] A.L. von Muralt, J.T. Edsall, Studies in the physical chemistry of muscle globulin, III. The anisotropy of myosin and the angle of isocline, J. Biol. Chem. 89 (1930) 315–350.
[3] A.L. von Muralt, J.T. Edsall, Studies in the physical chemistry of muscle globulin, IV. The anisotropy of myosin and double refraction of flow, J. Biol. Chem. 89 (1930) 351–386.
[4] J.T. Edsall, J.W. Mehl, The effect of denaturing agents on myosin, II. Viscosity and double refraction of flow, J. Biol. Chem. 133 (1940) 409–429.
[5] I. Banga, A. Szent-Györgyi, in: A. Szent-Györgyi (Ed.), Studies from the Institute of Medical Chemistry University Szeged, Preparation and properties of myosin A and B, 1, S. Krager, Basel, 1941–1942, pp. 5–15.
[6] M. Bárány, N.A. Biró, J. Molnár, Uber die Reaktion zwischen Actin und zweiwertigen Kationen, Acta Physiol. Acad. Sci. Hung. V (1954) 63–78.
[7] H. Portzehl, G. Schramm, H.H. Weber, Aktomyosin und seine Komponenten, Z. Naturforschg. 5b (1950) 61–74.
[8] M. Bárány, N.A. Biró, J. Molnár, F.B. Straub, Darstellung enzymfreien Actins durch Umfällung mit Magnesium, Acta Physiol. Acad. Sci. Hung. V (1954) 369–381.
[9] G. Feuer, F.B. Straub, Adenosinetriphosphate the functional group of actin, Biochim. Biophys. Acta 4 (1950) 455–470.
[10] M. Bárány, G. Köteles, E. Nagy, J. Spiro, Modified purification method for actin. Relationship between actin precipitation and Mg^{2+}-binding (translated from Hungarian), Kisérletes Orvostudomány 5 (1956) 491–497.
[11] F.B. Straub, in: A. Szent-Györgyi (Ed.), Studies from the Institute of Medical Chemistry University Szeged, Actin, II, 3, S. Krager, Basel, 1941–1942, pp. 23–37.
[12] M. Bárány, K. Bárány, Studies on active centers of L-myosin, Biochim. Biophys. Acta 35 (1959) 293–309.
[13] M. Bárány, K. Bárány, Polyelektrolyte als Interaktions-Inhibitoren und die Bedeutung von Ca und Mg für die aktin-myosin interaktion, Biochim. Biophys. Acta 41 (1960) 204–216.
[14] M. Bárány, F. Jaisle, Kontraktionszyklus und Interaktion zwischen actin und L-myosin unter der Wirkung spezifischen interaktions-inhibitoren, Biochim. Biophys. Acta 41 (1960) 192–203.
[15] M. Bárány, K. Bárány, W. Trautwein, Die Hemmung der aktin-L-myosin interaktion in lebenden und extrahierten Muskeln durch Urea, Biochim. Biophys. Acta 45 (1960) 317–335.