THERMAL EFFECT OF INTERACTION BETWEEN ATP AND RELAXED AND CONTRACTED GLYCERINIZED FIBERS

I. I. Ivanov,* N. V. Karsanov, V. A. Magaldadze, L. Yu. Razamat, and D. Ya. Rozenblyum

UDC 612.741:612.744.2].014.43

The heat production accompanying interaction between bundles of glycerinized fibers of rat striated muscles and ATP was measured. Interaction both of relaxed and of initially contracted bundles was investigated. The kinetic temperature curves show that during interaction between ATP and the uncontracted bundle a peak of thermal power is observed to coincide with the contraction process, whereas previously contracted fibers give no such effect. It is concluded that the level of ATPase activity is directly dependent on the conformation of the actomyosin system.

KEY WORDS: *muscles; ATPase; actomyosin; thermal kinetics.*

The most intensively researched views regarding the mechanism of contraction of muscle fibers at the present time are those in which a trigger role is ascribed to an increase in the Ca^{2+} concentration in the sarcoplasm of the fiber up to 10^{-6}-10^{-5} M at the moment of excitation [8, 9, 11]. However, besides this mechanism, another, perhaps even more fundamental, undoubtedly exists. For example, in some orders of insects the frequency of contraction of the wing muscles is much greater than the frequency of incoming nervous impulses. The rhythm of these muscles is myogenic and not neurogenic [12]. It has been shown [3, 10, 13, 14] that glycerinized fibers, under certain conditions and in the presence of ATP and Ca^{2+} in a concentration of over 10^{-7} M in the medium, can oscillate under a definite load, like living muscles. Since the Ca^{2+} concentration in the solution remains constant under these circumstances, the change in length during stretching of the fiber in these cases can be presumed to be automatically connected with the change in activity of the enzyme system utilizing ATP. The mechanism of oscillation of both glycerinized muscle models and of various cell organelles of motion can evidently be understood only by postulating the existence of a connection between activity of the ATPase system and the state (conformation) of the contractile protein [3, 4]. It has recently been shown that ATPase activity can be increased by stretching myosin filaments treated in a certain way [1].

In the investigation described below an attempt was made to determine the existence of a connection between the ability of the glycerinized muscle fiber to hydrolyze ATP with the liberation of heat and the conformation of the actomyosin system during interaction between the glycerinized fiber and ATP.

EXPERIMENTAL METHOD

Bundles of glycerinized rat striated muscle fibers (BGMF) were obtained by Szent-Györgyi's method as described previously [6]. The thermal effect of interaction between BGMF and ATP was measured in the IKhPS-1 microcalorimeter [2], the detector of which was modified in order to reduce the time constant [5]. The perturbation of the system did not exceed $2 \cdot 10^{-4}$ J. The construction of the arrangements for starting the reaction (contraction of BGMF in the microcalorimetric cell was isotonic) is illustrated in Fig. 1. Before starting (Fig. 1A), the cup (3) in the system separates the buffer medium, in which the BGMF

*Deceased.

S. M. Kirov Military Medical Academy, Leningrad. Georgian Center for Medical Biophysics, Tbilisi. (Presented by Academician of the Academy of Medical Sciences of the USSR I. I. Ivanov [deceased].) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 9, pp. 292-294, September, 1977. Original article submitted February 9, 1977.

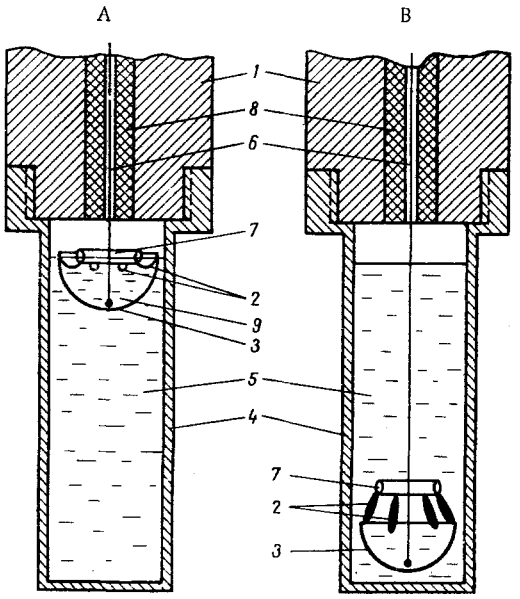

Fig. 1. Arrangements at start of reaction for recording thermal effect of interaction between BGMF and ATP during isotonic contraction: A) position before interaction; B) position during interaction; 1) holder of calorimetric container; 2) BGMF; 3) cup; 4) calorimetric container; 5) ATP solution; 6) starting thread; 7) float; 8) channel; 9) buffer solution.

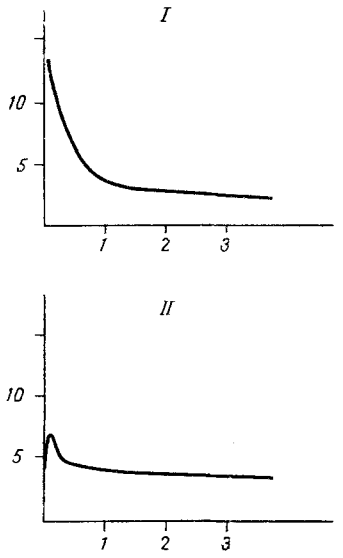

Fig. 2. Thermokinetic curves of interaction between BGMF and ATP: 1) interaction between ATP and relaxed BGMF; II) interaction between ATP and contracted BGMF. Abscissa, time (in min); ordinate, value of $(dQ/dt) \cdot 10^{-6}$ (in J).

are placed (2), from the ATP solution (5) made up in the same buffer and filling most of the capacity of the calorimetric container (4). The reaction was started by releasing the start thread (6), so that the float and BGMF could be immersed in ATP solution (Fig. 1B). The Archimedes' force acting on the float stretched all the BGMF, glued with cellulose nitrate, with a force of $2 \cdot 10^{-4}$ N per bundle. This force was constant throughout the period of contraction and created strictly isotonic conditions. The calorimetric measurements were made at 25°C after a period of 2 h at constant temperature and stabilization of the experimental zero. The ATP and Mg^{2+} concentration was 5 mM. From 6 to 10 BGMF 7-9 mm long were immersed simultaneously in the calorimeter. The ATP solution was made up immediately before the experiment and titrated to pH 7.20 ± 0.02. The course of heat production during interaction between the BGMF and ATP was calculated by graphic subtraction of the calibration curve (reflecting the kinetics of the thermal effect connected with dissolving the ATP, with mechanical displacement, and so on) from the experimental curve. The thermal effect of interaction between BGMF and ATP was determined twice in each experiment. In the first experiment ATP interacted with an ordinary relaxed BGMF, whereas in the second experiment it acted on the same bundle after its contraction and subsequent rinsing for 1 h in the same buffer solution. The resultant curves were averaged for each coordinate and analyzed in order to obtain a nearly true thermal kinetics [7].

EXPERIMENTAL RESULTS

The thermokinetic curves obtained are shown in Fig. 2 (I and II, respectively). The curve for interaction between the relaxed BGMF and ATP clearly preserved the character of the curves obtained previously with myocardial BGMF [6]. A peak heat production, corresponding to the stage of contraction, followed by stationary heat production (stage II) can be clearly distinguished. Meanwhile, in curve II, reflecting the thermokinetics of interaction between contracted BGMF and ATP, the peak stage I is ill-defined.

The heat produced by interaction between BGMF and ATP, as special control measurements showed, is due virtually entirely to the hydrolysis of ATP. The very small amount of heat

liberated in stage I (peak) in the experiments with previously contracted BGMF and the considerable liberation of energy in that same stage in the experiments with uncontracted (relaxed) BGMF, coinciding in time with its contraction, undoubtedly confirms the hypothesis stated above [4] that the level of ATPase activity of the actomyosin system is related to its conformation. These observations thus shed some light on the mechanism of oscillation of both muscle cells and of intracellular organelles of motion in a medium containing a constant concentration of Ca^{2+}.

LITERATURE CITED

1. A. E. Bukatina and V. N. Morozova, in: The Biophysics and Biochemistry of Muscles [in Russian], Tbilisi (1974), p. 120.
2. A. A. Vichutinskii and B. Yu. Zaslavskii, Zh. Fiz. Khim., 46, 259 (1972).
3. I. I. Ivanov and G. P. Pinaev, Dokl. Akad. Nauk SSSR, 115, 763 (1957).
4. I. I. Ivanov and V. A. Yur'ev, The Biochemistry and Pathobiochemistry of Muscles [in Russian], Leningrad (1961).
5. N. V. Karsanov, A. A. Vichutinskii, V. A. Magaldadze, et al., in: The Biophysical Basis of the Pathological State of Muscles and the Supply of Energy for the Contractile Apparatus [in Russian], Tbilisi (1973), p. 99.
6. N. V. Karsanov, A. A. Vichutinskii, V. A. Magaldadze, et al., in: The Biophysical Basis of the Pathological State of Muscles and the Supply of Energy for the Contractile Apparatus [in Russian], Tbilisi (1973), p. 104.
7. E. Calvet and M. Camia, J. Chim. Phys., 55, 818 (1958).
8. S. Ebashi and M. Endo, Prog. Biophys., 18, 123 (1968).
9. N. Hasselbach, Fed. Proc., 14, 167 (1964).
10. L. Lorand and C. Moas, Nature (London), 177, 1239 (1956).
11. H. Portzehl, Verh. Schweiz. Naturforsch. Ges., 206 (1965).
12. J. W. Pringle, Prog. Biophys., 17, 1 (1967).
13. R. E. Ranney, Am. J. Physiol., 179, 99 (1954).
14. J. C. Ruegg and K. T. Treagear, Proc. Roy. Soc. B, 165, 497 (1966).