THE RATE OF DEPHOSPHORYLATION OF ADENOSINE TRIPHOSPHATE AND THE SHORTENING OF GLYCEROL-WASHED MUSCLE FIBERS

by

WILLIAM J. BOWEN AND TIMOTHY D. KERWIN

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U.S. Department of Health, Education and Welfare, Bethesda, Maryland (U.S.A.)

The observation that magnesium greatly accelerates the shortening of myosin B threads caused by adenosine triphosphate (ATP) while not likewise accelerating the dephosphorylation of ATP by myosin B[1], has been interpreted differently in regard to this enzymic role of myosin B. One school believes that independence of the shortening process from the splitting of terminal pyrophosphate bonds is indicated[1,2]. The other does not accept this view and has aligned the evidence in support of the dependence of the two processes[3]. This problem has been further investigated in our laboratory using glycerol-washed rabbit psoas muscle[4] as models of muscular contraction (muscle models) and as the enzyme for dephosphorylation of ATP. It has been found that in 0.001 *M* $MgCl_2$ and 0.02 *M* tris (hydroxymethyl) aminomethane (THAM) variation of the concentration of KCl influences the rate of dephosphorylation of ATP in a manner divergent from the effect upon the shortening of muscle models.

EXPERIMENTAL PROCEDURE

Glycerol-washed psoas muscle was prepared according to SZENT-GYÖRGYI[4]. Bundles of fibers for shortening experiments were made about 40 mm long and 300 to 500 microns wide*. The process of shortening was observed on microscope slides which lay on a millimeter rule. The initial length of the fibers was measured in a solution containing 0.02 *M* THAM (pH 7.5), 0.001 *M* $MgCl_2$ and concentrations of KCl which varied from 0.00 *M* to 0.3 *M* in the individual solutions. Then the fibers were transferred to the same solution plus 0.00025 *M* K_4ATP and measurements made of the length at regular time intervals as shortening proceeded.

Preparations of glycerol-washed muscle to be used as enzyme were made by blending 0.2 to 0.4 g of muscle for 2 to 4 minutes in a Waring Blendor followed by homogenization in a teflon-glass tissue grinder. This suspension was analyzed for N-content and stored as a water suspension at 0°.

* Histologically prepared cross-sections of fiber bundles showed that bundles of these widths were composed of 25 to 40 fibers of an average thickness between 40 and 50 μ. Neither the bundles nor the fibers were circular in cross-section but were irregular ribbon shapes.

Such structure aids diffusion of ATP into the interior of the fibers. HASSELBACH AND WEBER (*Pharmacol. Revs.*, 7 (1955) 97) maintain that penetration of 0.005 *M* ATP does not exceed 10 μ at 20° C but they base their conclusions on calculations using the Myerhof-Schulz equation which applies when the outside concentration of ATP is great enough to saturate the inner core of a fiber or fiber-bundle. This equation, as will be shown by BLUM in a forthcoming publication, is much too stringent when interior concentrations considerably less than saturation are adequate for shortening. For these, and other reasons, we think that diffusion played no significant role in these experiments.

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Dephosphorylations of K_1ATP were measured in the same medium as were shortenings, except that the concentration of K_1ATP was 0.001 M instead of 0.00025 M*. Dephosphorylations were run for 10 minutes at 26 ± 1° with continuous shaking. They were stopped by the addition of 1 ml of 20% trichloroacetic acid to 3 ml of reaction mixture. The homogenate of muscle was then removed by centrifugation and 2 ml of the supernatant solution analyzed for orthophosphate by the Fiske-Subbarow method. The values presented are the amounts of orthophosphate calculated to be in 3 ml (Fig. 1) or 1 ml (Fig. 2) of reaction mixture.

RESULTS

The amount of ATP dephosphorylated in the presence of 0.001 M $MgCl_2$ per 10 min test increases when small quantities of KCl are added to the reaction mixture, but with further increases (above 0.02 M) the amount dephosphorylated decreases (Fig. 1). This influence of the concentration of KCl has been obtained with or without $MgCl_2$ on many occasions. The amount dephosphorylated at 0.3 M KCl is but 19% and 22% of that dephosphorylated at 0.02 and 0.0 M KCl, respectively. The results of time-course experiments with these mixtures at 0.0 M and 0.3 M KCl (Fig. 2) indicate that the proportions of ATP and myosin which were used maintained constant rates of dephosphorylation throughout the 10 minutes of the experiments of Fig. 1, except for some part of the first minute**. These results (Fig. 2) also show that dephosphorylation is more rapid in the absence of KCl than in the presence of 0.3 M KCl throughout the incubation.

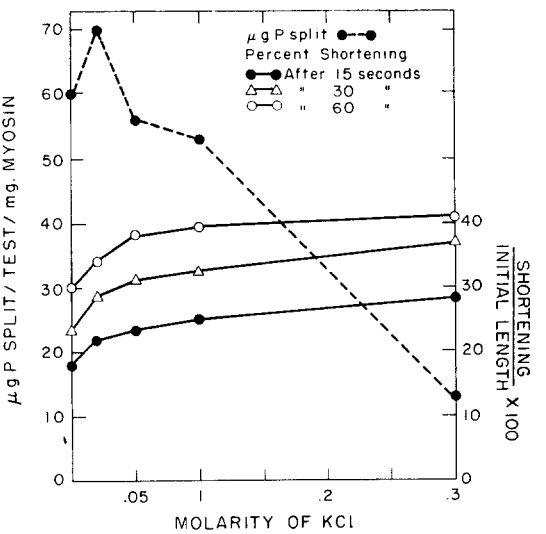

Fig. 1. The influence of the concentration of KCl upon the dephosphorylation of K_1ATP (left ordinate) by homogenized glycerol washed muscle fibers and upon the shortening of fibers (right ordinate) in 0.02 M THAM buffer, pH 7.5, and 0.001 M $MgCl_2$. "Shortening" is the length lost. Each point on the shortening plots is the average of records from 8 fibers.

Contrary to inhibiting dephosphorylation, increases of the concentration of KCl cause the fibers to shorten more rapidly (Figs. 1 and 3). In the experiments of Fig. 3 the shortening attained after 30 sec in 0.3 M KCl is greater than that in 0.0 M KCl by three standard errors of the difference***. Greater significance of this difference is shown by the results of experiments in which the time course of shortening was studied at each of the concentrations of KCl indicated in Fig. 1. The amount of shortening

* The concentration of K_1ATP in the dephosphorylation experiments was made 0.001 M so as to have greater amounts of phosphate to measure; however, in an experiment with 0.00025 M at 0.0 M and 0.3 M KCl, 36 and 13 μg of phosphate were split, respectively.

** WEBER AND HASSELBACH[5] have recently reported more rapid dephosphorylation during the initial phases of reactions between ATP and myosin. Fig. 2 shows, however, that when the over-all ate is greater with no KCl than with 0.3 M KCl it is likewise greater during the initial phase when both rates are undergoing change.

*** The failure of the fibers of Fig. 3 to approach the maximum shortening (80–90%) capable by such fiber-bundles is a manifestation of the direct relation of extent of shortening to concentration of ATP (W. J. BOWEN, *Am. J. Physiol.*, 179 (1954) 620).

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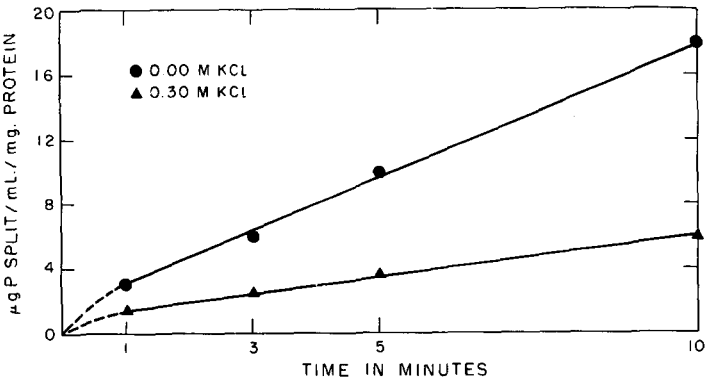

Fig. 2. The influence of 0.3 *M* KCl upon the dephosphorylation of K_4ATP by homogenized glycerol-washed muscle fibers in 0.02 *M* THAM buffer, pH 7.5, and 0.001 *M* $MgCl_2$.

after the lapse of each of 15, 30 and 60 second intervals became greater with each increase of the concentration of KCl (Fig. 1).

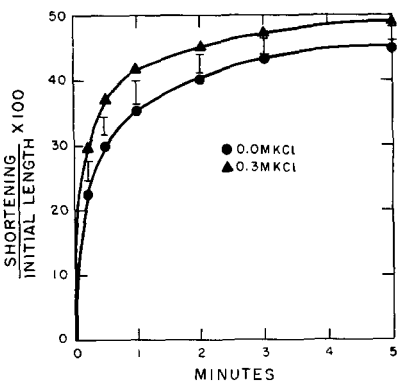

Fig. 3. The influence of 0.3 *M* KCl upon the shortening of glycerol-washed psoas muscle fibers. Each point is the mean of 24 fibers. The bars are one standard error of the respective differences.

The above results were obtained with one preparation of glycerol-washed muscles. The shortening of fibers from three other preparations was also faster in 0.3 *M* KCl than in 0.0 *M* KCl. The ages of the preparations at the time of use ranged from 2 to 105 days. Dephosphorylation with homogenates of the other preparations of fibers were affected by increases of KCl concentration in the manner shown in Figs. 1 and 2.

DISCUSSION

These results are another instance of nonparallelism of the effects of a given ionic milieu upon dephosphorylation of ATP and upon the shortening of muscle models which is caused by ATP. Instances previously reported from this laboratory are (a) that in which $CaCl_2$ was found to accelerate dephosphorylation of ATP by myosin B while depressing the shortening of myosin B threads[1] and (b) that in which 0.01 *M* $MgCl_2$ was found to have either no or a slight inhibiting influence upon dephosphorylation but to accelerate shortening of myosin B threads severalfold[1]. The significance of these two instances of absence of parallelism has been dismissed[3] as being the result of using myosin B in a partially dissolved gel-form for the dephosphorylation of ATP and, in the instance with $CaCl_2$, as a degradation in the efficiency of the transmission of energy. Subsequent to these first observations[1], however, the gel-form of myosin B threads was used as ATP-ase[6] and the effect of 0.01 *M* $MgCl_2$ upon the dephosphorylation of ATP was found to be of the same relative magnitude as with the suspended form. This is in accordance with the results of HASSELBACH's studies of the concentration of $MgCl_2$[7].

To construe the nonparallelism of the effects of calcium and magnesium as alter-

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ations of the efficiency of the transmission of energy[3] merely shifts the problem to that of devising a transmission mechanism at the molecular level. When one attempts to do this it is evident that consideration of the binding of ATP to myosin as a source of energy leaves less unexplained than does the splitting of ATP by myosin as the source of energy.

In addition to these three instances in which ionic conditions dissimilarly influence dephosphorylation of ATP by muscle protein and shortening of muscle models, the effects of 0.001 *M* $CoCl_2$, $MnCl_2$, and $SrCl_2$ on each process further indicate that the rapidity of shortening is independent of the rate of dephosphorylation[8]. Recently PORTZEHL has demonstrated that the inhibitive effect of salyrgan is not nearly as great on the splitting of ATP as on the shortening of fibers[9]. Such noncorrelations contradict the contention that it is the ATP-splitting *per se* which supplies the energy necessary for the shortening of these models and perhaps for muscular contraction. They are consistent, however, with the contention of MORALES, BOTTS, and their colleagues[2] that it is the energy released by the binding of ATP to myosin which produces shortening of these muscle models and the contraction of muscle.

SUMMARY

Increasing the concentration of KCl in reaction mixtures of ATP and homogenized psoas muscle fibers with $MgCl_2$ retards dephosphorylation of the ATP. Similar increases of the KCl accelerate shortening of psoas fibers. These dissimilar effects of KCl are interpreted as further evidence in support of the hypothesis that the binding of ATP to myosin provides the energy for the contraction of muscle.

RÉSUMÉ

Un accroissement de la concentration de KCl dans des mélanges d'ATP et de fibres de muscle psoas homogénéisées avec du $MgCl_2$ retarde la déphosphorylation de l'ATP. De semblables accroissements de la concentration du KCl accélèrent le raccourcissement des fibres de psoas. Ces différents effets du KCl peuvent être interprétés comme un argument de plus à l'appui de l'hypothèse suivant laquelle l'énergie nécessaire pour la contraction du muscle serait fournie par la liaison de l'ATP à la myosine.

ZUSAMMENFASSUNG

Eine Zunahme der KCl-Konzentration in Reaktionsgemischen von ATP und mit $MgCl_2$ homogenisierten Psoasmuskelfasern verzögert die Entphosphorylierung des ATP. Ähnliche Zunahmen der KCl-Konzentration beschleunigen die Verkürzung der Psoasfasern. Diese verschiedenen Wirkungen des KCl können als ein weiteres Argument zur Unterstürzung der Hypothese interpretiert werden, nach welcher die Energie für die Muskelkontraktion durch die Bindung des ATP an das Myosin geliefert wird.

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