The contraction of muscle fiber and myosin B thread in KI and KSCN solutions

Models of muscular contraction made from glycerol-washed rabbit psoas muscle or extruded myosin B solution can be made to contract by adenosine triphosphate (ATP)[1] or inosine triphosphate[2] when certain ions are also present. The kind of ions is not specific but ATP and ITP are the only substances previously known to cause this contraction[1]. It is an observation of long standing that neutral Nessler's reagent (HgI_2 in KI solution) produces contraction of tendon[3,4,5]. We have found that this reagent also causes muscle fibers as well as myosin B threads to shorten.

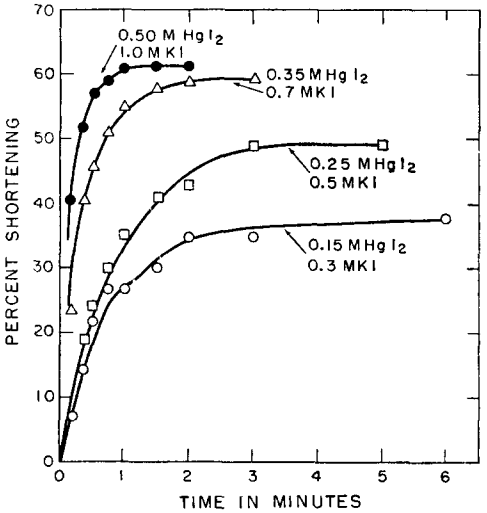

Fig. 1. The shortening of psoas muscle fibers in various concentrations of neutral HgI_2-KI solution plotted against time. "Shortening" is the ratio of lost length to initial length expressed as percent.

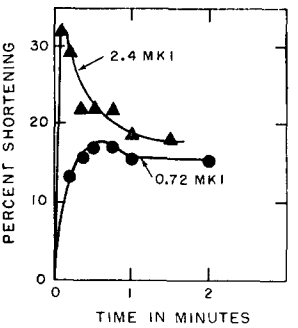

Fig. 2. The shortening of psoas muscle models in two concentrations of KI plotted against time.

Fig. 1 shows the shortening effect of various concentrations of the reagent. The speed as well as the extent of shortening depends on the concentration. In addition, the extent of shortening was found to decrease when the amount of HgI_2 present in a given KI solution was decreased. Nevertheless, even in the absence of HgI_2, there is a fast shortening but it is followed by an elongation (Fig. 2). It appears, therefore, that the contracting agent is the KI but its action is soon counterbalanced by another process which causes the fibers to elongate. The presence of HgI_2 eliminates this process so that the fiber contracts without the subsequent elongation. Many of these time-shortening curves in the presence of HgI_2-KI solution approximate the course of a first-order reaction.

HgI_2 dissolved in KSCN has similar effects. Here again, HgI_2 prevents the elongation following the fast contraction elicited by KSCN.

In these experiments pH values of the reagent between 7 and 10 had no effect on either the speed or the extent of shortening.

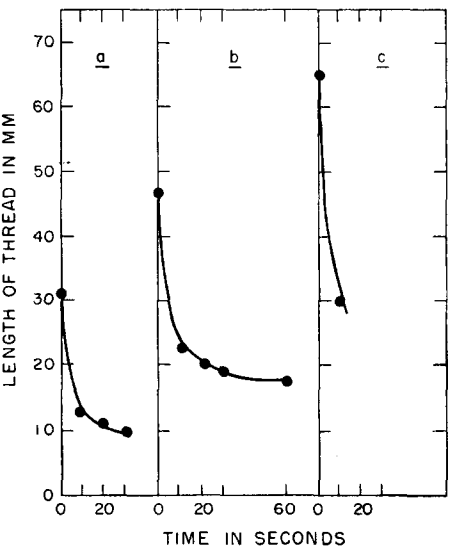

Fig. 3. The shortening of myosin B threads in 0.44 *M* HgI_2–1.25 *M* KI solution. The initial shortening (a) was followed by stretching and setting as described in the text and then reimmersion in the solution. After the second shortening (b) the thread was stretched to a still greater length and shortened a third time (c).

In addition to shortening, these reagents make the fibers extremely elastic. They can be stretched to two- or threefold their original length. When kept stretched the fibers remain "set" at that length if the reagent is washed out. Renewed immersion into the reagent causes contraction again. This

process can be repeated several times. Preliminary experiments show that these fibers not only shorten but develop tension comparable to that obtainable with ATP.

In the above experiments, bundles of 5–10 single fibers (35–40 mm in length) separated from glycerol-washed psoas muscle were used. After the length of these fibers had been measured they were transferred to reagent on a microscope slide, and the shortening followed by measuring the length at various time intervals on a millimeter rule lying under the microscope slide. The ratio of the lost length to the initial length expressed in percent is called the shortening (Figs. 1, 2).

Since these muscle fibers contain connective tissue, the possibility exists that the above reagents act on the collagenous rather than on the myosin component of the fibers. These reagents, however, also cause threads made from myosin B to contract (Fig. 3). In order to demonstrate this shortening effect, threads prepared from purified myosin B first were immersed in a dilute reagent. This altered the threads so that it was possible to extend them several times their original length. Then, the reagent was washed out by soaking the threads successively in versenate solution and distilled H_2O, a process which "set" the threads at an extended length. When such threads are immersed in a more concentrated reagent, they contract rapidly. The contraction of a "set" fiber may be repeated several times (Fig. 3, b and c) and the shortening appears proportional to the length at which the fiber was "set". Therefore, the behavior of these threads is analogous to the behavior of fibers and these reagents act on the myosin B component of the muscle fibers.

It is too early to speculate on the mode of action of these reagents but it might be pointed out that both KSCN and KI are known to depolymerize actin[6,7] and eliminate flow birefringence of myosin solutions[8].

K. LAKI
W. J. BOWEN

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.)

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The action of carboxypeptidase on ribonuclease*

A question which arises in attempts to determine the mechanism of action of enzymes, is whether the whole protein is necessary for catalytic activity. Crystalline ribonuclease (purchased from Worthington and Armour Laboratories) was selected as a suitable starting material since it is fairly stable, has a relatively simple structure, requires no co-enzyme or metal ion, is easily obtainable in electrophoretically homogeneous form, is assayed for fairly readily and is inexpensive.

Of the variety of tools available for degrading ribonuclease (RNase) the enzyme carboxypeptidase was the method of choice, since it is now used as a general method for determining the C-terminal end groups of a number of different proteins[1,2,3]. RNase was assayed using a modification of the method published by ANFINSEN *et al.*[4]. The amino acids liberated were separated by descending paper chromatography and quantitatively determined by direct photometry of the ninhydrin spots, according to the method of ROLAND AND GROSS[5].

Experiments were carried out by dissolving the substrate (RNase) in a small volume of phosphate buffer at pH 7.7 and adding carboxypeptidase to bring the total volume of the solution to 0.2 or 0.3 ml. As can be seen from Table I, the first amino acid which appears in any quantity is valine followed by leucine and phenylalanine. In addition, faint spots were discernible which corresponded to alanine and tyrosine (Table I, Experiment 1). The sixth amino acid to appear was methionine (Experiment 2). These results are exactly in accord with those obtained by ANFINSEN *et al.*[4]. Further increase of carboxypeptidase in these experiments gave no appreciable increase in digestion. Since 0.1 *M* phosphate buffer very strongly inhibited the action of carboxypeptidase[6], veronal buffer of the same pH was substituted for the phosphate. Adequate digestion took place, but under these conditions the veronal interfered with the chromatographic migration of the amino acids. However, in 0.01 *M* phosphate buffer, pH 7.8 or in alkaline water of the same pH more appreciable digestion took place (Table I, Experiments 1 and 2). Here again, valine, leucine, phenylalanine, alanine,

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