Studies on the ATPase Activity, Relaxing Activity and Calcium Uptake of Rabbit Skeletal Muscle Microsomes

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骨骼筋 microsome의 ATPase 活性, 筋弛緩作用, 및 Ca 吸收作用에 關한 硏究

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摘 要

토끼의 骨骼筋 homogenate에서 23,000×*G*, 60分間의 遠心分離로 얻은 筋 microsome의 ATPase 活性, 筋收縮에 對한 弛緩作用, 및 Ca의 吸收作用을 여러가지 條件에서 測定하였다.

ATPase 活性은 Ca^{++} 및 Mg^{++} 兩이온의 存在에 依하여 活性化되며, 5m*M* Mg^{++}의 存在下에서는 Ca^{++}의 最適濃度는 0.1 m*M*이다. Oxalate의 存在下에서는 1 m*M*의 Ca^{++}이 最適濃度이므로 oxalate의 作用은 不溶性 Ca-oxalate를 microsome vesicle 內 및 medium 內에 沈澱시켜 遊離 Ca^{++} 濃度를低下시키는 것이라고 생각된다.

Microsome의 弛緩作用은 調製後 120時間까지 時間에 따라 減少되어 가나, 그의 ATPase 活性은 거의 變化가 없는 것으로 보아 $Ca^{++}+Mg^{++}$-依存性 ATPase는 弛緩作用에는 直接 關聯이 없는 것으로 해석된다.

Oxalate의 存在는 microsome의 Ca^{++} 吸收量을 顯著히 增大시키며 同時에 吸收飽和에 到達하는 時間을 遲延시킨다. Oxalate의 이러한 效果도 Ca-oxalate의 形成에 起因하는 것으로 해석된다. Microsome 內에 蓄積되는 Ca의 量은 ATP 濃度가 커질수록 많아진다. 그러나 蓄積된 Ca의 量과 ATP 濃度사이에 化學定量論的 關係는 없는 것 같다.

INTRODUCTION

One of the most remarkable discoveries in the study of the mechanism of muscle relaxation is probably the fact that skeletal muscle microsomes in the presence of Mg^{++} are able to transport Ca^{++} against concentration gradient hydrolysing ATP and utilizing its energy for the transport.

Early in the study of this line, Kielley and Meyerhof(1948) have found that the fraction obtained from the skeletal muscle extract by centrifugation at 18,000 x*G* for one or two hours had an ATPase activity. Marsh (1951) reported that the relaxing factor (Marsh factor) in the muscle contraction was a component of the protein obtained from muscle extract by centrifugation and Kumagai *et al.*(1955) found that this component was identical with the Kielley-Meyerhof's ATPase which could be sedimented by centrifugation at 18,000 x*G* for 1—2 hours. They suggested that this ATPase plays in some fashion a role in the physiology of muscle relaxation.

Shortly later it was known that the microsomes extracted from skeletal muscle homogenate concentrate Ca in the presence of ATP and Mg^{++} (Ebashi, 1960, 1961; Ebashi and Lipman, 1962; Ebashi, 1962; Hasselbach and Makinose, 1961, 1962). Hasselbach and Makinose (1961) observed that the presence of oxalate in the reaction mixture greatly increased the amount of Ca accumulated by the muscle microsome and reported that Ca was actively transported with the expenditure of the ATP energy. Hasselbach(1962) also reported that the energy required for the accumulation

of Ca is supplied from the hydrolysis of ATP by the Kielley-Meyerhof's ATPase. The presence of ATP is obviously an essential condition in the Ca-binding of microsomes since when ATP is decomposed Ca bound is released and when ATP is again added to the system Ca-binding activity is restored (Ebashi and Lipman, 1962, Ebashi and Endo, 1962). Ebashi (1961) reported that the relaxing activity of some chelating agents has a definite correlation with their Ca-binding activity and suggested that Ca is the regulatory factor in the contraction-relaxation coupling of skeletal muscle and that the Ca-bindding activity the of microsomes is itself the relaxing activity.

From electron microscopic studies the Kielley-Meyerhof's ATPase(EC 3.6.1.4.) preparation was shown as fragments of endoplasmic reticulum or sarcoplasmic reticulum (microsomes) of the muscle cell (Ebashi and Lipman, 1962). Since this membraneous component loses its enzymatic activity as well as its relaxing activity when treated with phospholipase C and since the resulting decomposed products do not affect the enzymatic activity at all, it is considered that the enzyme preparation consists of lipoprotein containing phospholipids, especially lecithins, the essential factor being presumably the lecithins. It is now generally accepted that the strong accumulation of Ca by microsomes in the presence of ATP is the essential nature of the mechanism of muscle relaxation.

Based upon these observations that described above, it is presently considered that the muscle contraction and relaxation processes proceed as follows: In resting muscle, Ca is highly concentrated in the endoplasmic reticulum; the concentration of Ca inside the myofibril is too low to cause the shrinking of contractile protein. When the muscle is excited, the concentrated Ca in some portion of the endoplasmic reticulum is released by the electrical influence due to the depolarization of the muscle membrane, and the Ca thus released causes in turn the shrinking of the actomyosin system. When the excitation is over, Ca release ceases and the liberated Ca is recaptured by the endoplasmic reticulum (the relaxing factor); consequently, the shrunken actomyosin system is restored to its relaxed state. These procedures are clearly conformed by the experiments carried out by Lee *et al.* (1966) using electric stimulus with skeletal muscle microsomes.

The present study was carried out to determine: 1) the ATPase activity of microsomes at various Ca concentrations, 2) the change in ATPase activity with the microsomal age, 3) the Ca uptake of microsomes, and 4) the effect of ATP concentration on the Ca uptake of microsomes.

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MATERIALS AND METHODS

Preparation of Microsomes: Microsomes were prepared according to the methods of Ebashi and Yamanouchi(1964). Skeletal muscle was taken out from an adult rabbit and fatty materials and nervous fibres were carefully removed. About 100 g of the white psaos muscle was homogenized in about 100 ml of 0.01 N NaOH solution with a Waring blender and the pH during the homogenation was kept at 6.8. The homogenate was centrifuged twice at 9,000 x*G* for 20 minutes and the supernatant was filtered with Toyo filter paper No. 5 A. The filtrate was centrifuged at 23,000 x*G* for 60 minutes and the precipitate collected was homogenized with a glass homogenizer and suspended in 0.02 *M* tris-maleate buffer (pH, 6.8) containing 0.05 *M* KCl. The suspension was centrifuged again at 2,000 x*G* for 20 minutes to remove any impurities formed and the supernatant was finally centrifuged at 23,000 x*G* for 45 minutes. The precipitated microsomes were resuspended in an aliquot amount of the buffer solution to make the protein concentration about 40—50 mg/ml and the suspension was homogenized and kept at 0°C until used. All the procedures were performed at 0°—4°C.

Protein concentration was determined by micro-Kjeldahl method and biuret photometry. The preparation was checked by Warburg's manometry for the possible contamination of mitochondria.

Determination of ATPase Activity: ATPase activity was determined at 25°C in an incubation medium (pH, 6.8)

of 0.02 *M* tris-maleate-NaOH+0.05 *M* KCl+2m*M* ATP, to which Ca^{++}, Mg^{++}, or $oxalate^{--}$ in final concentrations as specified in the figures were added. The reaction was started by the addition of ATP and was stopped by adding trichloroacetic acid solution in a final concentration of 5%. The ATPase activity was expressed as the μmoles inorganic phosphate produced/mg microsomal protein/minute. Quantitative determination of the inorganic phosphate (Pi) was done by Allen's method (1940) modified by Nakamura (1950).

Determination of Relaxing Activity: The relaxing activity of microsomes was determined by measuring the inhibitory activity of microsomes on the ATPase activity of actomyosin (Portzehl, 1957 a, b; Nagai and Akita, 1965). The per cent inhibition of the actomyosin ATPase by microsome granules was given by: $(1-[(A-C)/B])\times 100$, where A and B are the amount of Pi liberated by actomyosin ATPase with and without microsomes, respectively, and C is the amount of Pi liberated by the microsomal ATPase alone.

Preparation of Actomyosin: Rabbit skeletal muscle was minced in the Weber-Edsall solution (0.6 *M* KCl+0.01 *M* Na_2CO_3+0.04 *M* $NaHCO_3$, pH 8.6) and the suspension was kept at 0°−4°C for 24 hours to extract actomyosin, diluted two-fold with 0.6 *M* KCl solution, and centrifuged at 900 x*G* for 20 minutes. The supernatant was transferred drop by drop into a double volume of water adjusting the pH of the suspension to 6.5, and was allowed to stand for 30 minutes to precipitate the actomyosin completely. The actomyosin precipitate was collected by centrifugation at 2,700 x*G* for 20 minutes and was dissolved in KCl solution of the concentration that would give final concentration of 0.6 *M* at pH 6.8. The solution was then centrifuged at 900 x*G* for 30 minutes to remove any denatured protein or impurities, the supernatant was diluted with water to make the concentration of K 0.2 *M* and to precipitate the protein and the precipitated actomyosin was collected by centrifuging the suspension at 5,000 x*G* for 30 minutes. The procedures were repeated three times and the actomyosin preparation thus obtained was dissolved in 0.6 *M* KCl solution and kept ice-cold until used.

The incubation medium used in the determination of the relaxing activity of microsomes consisted of 125 m*M* tris-acetate buffer (pH 7.1), 12 m*M* $MgCl_2$, 12 m*M* ATP, and K^+ ion concentration of 0.055 *M*. The reaction was conducted at 25°C for 2−15 minutes and the Pi produced was determined.

Measurement of Ca^{++} Uptake: The Ca^{++} uptake of microsomes was measured in the incubation medium of 0.02 *M* tris-maleate-NaOH buffer (pH 6.8)+0.5 *M* KCl+2 m*M* $MgCl_2$ containing a specified amount of ATP and microsomes at 24°C, in the presence or absence of oxalate. To the reaction mixture was added 0.1 m*M* $CaCl_2$ containing Ca^{45} with the specific activity of 1,000−5,000 cpm/ml reaction mixture. At end of incubation the reaction mixture was filtered with Millipore filter (Type HA, 0.45 μ diameter) to separate microsomes from reaction mixture. The filtration was completed within 2 seconds. The amount of Ca^{++} taken up into microsomes was calculated from the radioactivities of the reaction mixture, filtrate, and filter. The biuret test of the filtrate was negative showing no appreciable leakage of microsomal protein through the filter.

Radioactivity was counted on an Aloka FC-IE gas-flow type, windowless GM counter. Since microsomes are trapped on the filter disk, direct measurement of bound Ca can be made by estimating the Ca^{45} content of the filter and correcting it for the radioactivity of the microsome-free filtrate which adheres to the filter disk.

RESULT AND DISCUSSION

1. ATPase Activity at Various Ca^{++} Concentrations: The ATPase activity of rabbit skeletal muscle microsomes was determined at various concentrations of Ca^{++} in the presence or absence of Mg^{++} and $oxalate^{--}$ (Fig. 1). The ATPase activity was very low in the absence of Mg^{++} and the concentration of Ca^{++} seemed not to appreciably affect the enzyme activity, though the activity showed a tendency to slightly increase as the Ca^{++} concentration increased up to 1 m*M*. On the other hand, the presence of 5 m*M* Mg^{++} greatly stimulated the activity and the activity in this time was dependent upon the Ca concentration; it kept increasing as the Ca concentration increased up to 0.1 m*M* and dropped when Ca exceeded 0.1 m*M*. Obviously higher concentrations of Ca^{++} than 0.1 m*M* are inhibitive to the enzyme activity, contrast to the pattern of the activity change when no Mg^{++} is added to the system. It is

therefore apparent from Fig. 1 that the micosomes have an ATPase activity which is dependent upon both Ca^{++} and Mg^{++}. That the ATPase activity of skeletal muscle microsomes is dependent on both Ca^{++} and Mg^{++} is also shown in Fig. 2, which illustrates the change in ATPase activity when Ca^{++}(Fig. 2 A) or Mg^{++}(Fig. 2 B) was added during the incubation. As shown in the figure, the activity sharply increased when either Ca or Mg was added to the reaction mixture.

Kielley-Meyerhof's enzyme has been known to be activated only by Mg^{++} and independent of Ca^{++}. Ebashi and Lipman(1962), however, reported that the relaxing factor (microsomes) when moderately denatured showed an increased ATPase activity in the presence of Ca. Ebashi and Yamanouchi (1964) observed that glycoletherdiaminetetra-acetic acid (GEDTA), a specific chelating agent for Ca, had a more strong inhibitory effect than ethylenediaminetetra-acetic acid (EDTA) on the ATPase activity and suggested that the ATPase activity is dependent on the presence of Ca. In the present study it was shown that in the absence of Ca, the ATPase activity was very low even in the presence of 5 m*M* Mg^{++}, and that a small amount of Ca, even in the concentration of 0.01 m*M*, greatly enhances the ATPase activity in the presence of Mg, thus indicating that Ca^{++} is needed for the ATPase activity of the microsomes. That the ATPase activity of the relaxing factor which had been treated with a detergent and denatured moderately was stimulated by the addition of Ca is considered to be due to that the Ca concentration in the medium is lowered by the detergent to below the minimum concentration required for the activity, the addition of Ca to the medium hence restores the activity, and this restored activity might have been interpreted as if the microsomal ATPase is stimulated by the added Ca.

Hasselbach and Makinose (1961, 1962) have shown that the addition of a small concentration of Ca (10 μM) to a microsome suspension which contains $MgCl_2$, ATP, and oxalate results in a transient activation of the ATPase (extra-ATPase) which coincides with the uptake of Ca^{++} by the relaxing factor. These workers attributed the activation of the ATPase by Ca^{++} to the hydrolysis of ATP by the Ca^{++} transport system. It is interesting, however, that continued addition of small concentration of Ca^{++} will eventually lead to permanent activation of the ATPase (Ebashi, 1961), which is not necessarily accompanied by Ca uptake. In the present experiment, no extra-ATPase was observed (Fig. 2 A), presumably because, though originally no Ca was added, the ATPase had been already activated by the Ca^{++} introduced into the system as contaminants.

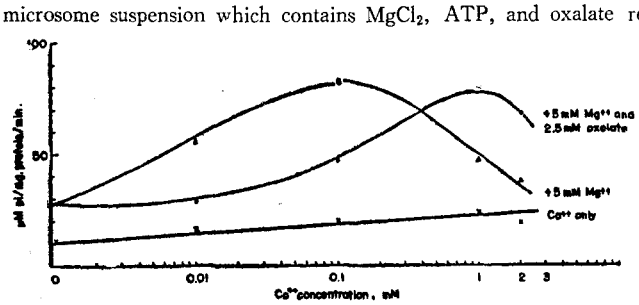

Fig. 1. Effects of Mg^{++} and Mg^{++} plus oxalate on the ATPase activity of rabbit skeletal muscle microsomes at various Ca concentrations. Incubation medium(pH, 6.8) consisted of 0.02 *M* tris-maleate buffer+0.05 *M* KCl+2 m*M* ATP +the indicated ions. Incubation was carried out at 24°C for 15 minutes. The concentration of microsomes was 0.03−0.1 mg protein/ml suspension. Each point in the figure represents an average of triplicate determinations.

Martonosi and Feretos (1964) described that one of the effects of Ca^{++} is the inhibition of ATPase at Ca concentration greater than 0.1 m*M* and the inhibition is practically complete at Ca concentration of 2 m*M*. They concluded that the Mg^{++}— or Mn^{++}—moderated ATPase is regulated by medium Ca concentration and the opitmal concentration is 5−50 μM at 0.1 *M* KCl, 5 m*M* histidine, 4-5 m*M* ATP and 4−5 m*M* $MgCl_2$. In the present experiment, the inhibition of the ATPase activity was also seen at the Ca concentration of greater than 0.1 m*M* when Mg was present.

When Mg and oxalate in the concentrations of 5 m*M* and 2.5 m*M* respectively were present together, the ATPase activity showed a different pattern of increase with the concentration of Ca; in this case, the optimium concentration of Ca for the ATPase activity was 1 m*M* (Fig. 1). The difference in the optimal concentrations of Ca for the ATPase activity, in the presence and absence of oxalate, is considered to be due to the insoluble precipitate of Ca-oxalate formed in the medium. Thus, when oxalate is added to the reaction mixture containing Ca in the concentration of,

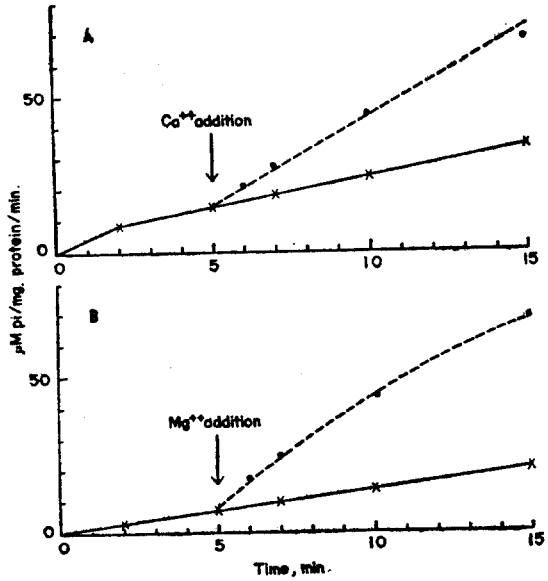

Fig. 2. Effects of Ca^{++} and Mg^{++} on the ATPase activity of rabbit skeletal muscle microsomes. Incubation was carried out in the medium consisting of 0.02 *M* tris-maleate buffer+0.05 *M* KCl+2 m*M* ATP, at 24°C with the microsomal concentration of 0.1 mg protein/ml suspension. In the A, Mg^{++} (5m*M*) was initially present, and $CaCl_2$(0.1 m*M*) was added at 5 minutes after the start of the incubation. In the B, Ca^{++}(0.1 m*M*) was present initially and $MgCl_2$(5 m*M*) was added as indicated by the arrow in the figure.

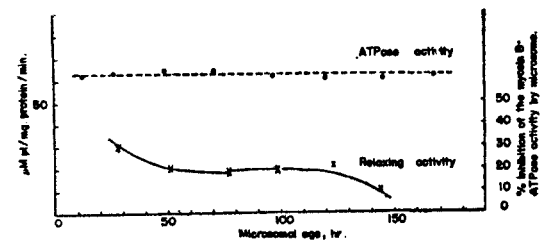

Fig. 3. Changes in relaxing activity and ATPase activity of rabbit skeletal muscle microsomes with the age. Relaxing activity was measured in the medium consisting of 125 m*M* tris-acetate buffer (pH, 7.2), 1.7 m*M* ATP, 0.06 *M* $MgCl_2$, and 0.06 *M* KCl, with or without 0.575 mg actomyosin protein/ml and 0.06 mg microsomal protein /ml suspension, at 24°C for 10 minutes. ATPase activity was determined in the medium consisting of 0.02 *M* tris-maleate buffer (pH, 6.8)+2 m*M* ATP+0.1 m*M* $CaCl_2$+5m*M* $MgCl_2$+2.5 m*M* oxalate+0.05 *M* KCl, at 24°C for 10 minutes. Microsomal concentration was 0.14 mg/ml suspension.

for example, 1 m*M* that significantly inhibits the ATPase activity, the insoluble precipitate of Ca-oxalate formed will lower the concentration of free Ca^{++} in the medium to such a level that will activate the ATPase. The activity curve obtained in the presence of oxalate in Fig. 1, therefore, is shifted to the right with regard to the one obtained in the absence of oxalate. Martonosi and Feretos (1964) reported that the addition of oxalate inhibits the ATPase activity in the medium containing 4 m*M* $MgCl_2$. They considered the inhibition as to be due to the lowering of medium Ca concentration below 1 μM by the added oxalate. They also reported that the optimal Ca concentration is 5–50 μM in the presence of 5 m*M* Mg^{++}, which is far below that of present experiment.

2. Change in ATPase Activity with Time: Fig. 3 shows the change in ATPase activity with the microsomal age when microsomes were stored at 0° C for a week. As shown in the figure, the ATPase activity of microsomes showed essentially no change during the period of the storage. On the other hand, the relaxing activity of the microsomes, measured as the inhibition of the actomyosin ATPase by microsomes, decreased gradually with time up to 50 hours after the preparation of the microsomes, was relatively constant during the period of 50 to 120 hours showing a plateau which was very flat, and was finally lost.

Nagai and Akita(1965) reported that in aged preparation (more than ten days after the preparation) the microsomal ATPase increased progressively during standing period at 37°C and at the same time the inhibiting activity decreased. The microsomes they used were usually stored at -20°C, but when preserved at 0°C, inhibitory activity was gradually lost and the ATPase activity increased. Ebashi (1958) observed that if the microsomes were moderately denatured by storage at 0°C for 3-4 days, ATPase activity of the preparation increased concurrently with the decrease of relaxing activity. In the present experiment, the ATPase activity did not increase but was constant throughout the experiment while the relaxing activity decreased with time. It could be, therefore, concluded that there is no direct relationship between ATPase activity and the relaxing activity of the microsomes. This conclusion does not exclude the fact that the Ca

uptake and hence the relaxing activity requires the hydrolysis of ATP by the microsomal ATPase, but implies the possibility that the Ca^{++}- and Mg^{++}-dependent ATPase is not exactly the same enzyme as that which participitates in the relaxing activity.

3. Ca Uptake of Microsomes: Fig. 4 shows the Ca uptake of microsomes as determined with Ca^{45}. It is seen from the figure that Ca^{++} uptake was almost completed within 2-3 minutes. Carsten and Mommaerts (1964) reported that in the presence of 1m*M* ATP and 1.25 μM Ca (per mg protein), the Ca uptake was almost complete in 8 minutes with 0.08 mg microsomal protein/ml and after the 8 minutes the uptake continued very slowly. These workers also reported that the saturation required more than 2 minutes regardless of the microsomal concentration, even in the high concentration such as 1-3 mg/ml. Hasselbach and Makinose (1961, 1962) reported that the saturation of the uptake occurred within 2-15 minutes. Martonosi and Feretos (1964) also reported a similar result with that of Hasselbach and Makinose, while Ohnishi and Ebashi (1963, 1964) reported that the saturation was completed instantaneously. Ebashi and Yamanouchi (1964) reported that in the presence of 0.15 m*M* $CaCl_2$ the saturation of Ca uptake occurred in 5 minutes, and in the presence of 0.1 m*M* Ca it took 2-3 minutes. The concentration of microsomes they used was approximately the same as that employed in the present experiment. In the present determination where the Ca concentration was 0.1 m*M*, the saturation occurred at about 3 minutes. This result is in good agreement with that of Ebashi and Yamanouchi(1964). The presence of 1 m*M* oxalate in the reaction medium greatly increased the amount of Ca taken up by the microsomes as shown in Fig. 5. Hasselbach and Makinose (1961) and Martonosi and Feretos (1964) also reported that the Ca uptake was greatly stimulated by the presence of oxalate and that the active transport of Ca was performed by the ATP energy. Carsten and Mommaerts(1964)reported that the amount of Ca accumulated at saturation was 2.2 μM/mg microsomes in the presence of oxalate and 0.16 μM/mg microsomes in the absence of oxalate and that the Ca concentration at which the saturation occurred was 2 m*M* when oxalate was present and 0.2 m*M* when no oxalate was present. The amount of Ca accumulated in the presence of 1 m*M* oxalate as determined in the present experiment was about five times that obtained in the absence of oxalate, but was not as high as that Carsten and Mommaerts reported. The increasing effect of oxalate on the Ca uptake is considered to be due to the formation of Ca-oxalate precipitate, hence keeping the free Ca concentration low in the microsomes. Martonosi and Feretos (1964) measured the exchange of Ca bound to microsomes in the presence and absence of oxalate and found that Ca bound to microsomes do not exchange with medium Ca when 4-5 m*M* oxalate was present and concluded that oxalate inhibits the outflux of Ca, thereby decreasing the exchange of Ca bound to microsomes with medium Ca. They considered that oxalate decreases the exchange rate of Ca by precipitating the latter as Ca-oxalate and thereby decreasing the concentration of free Ca^{++} in the microsomes, as suggested by Hasselbach and Makinose (1961). The influx of oxalate along with Ca into microsomes to form the Ca-oxalate precipitate in the microsomes

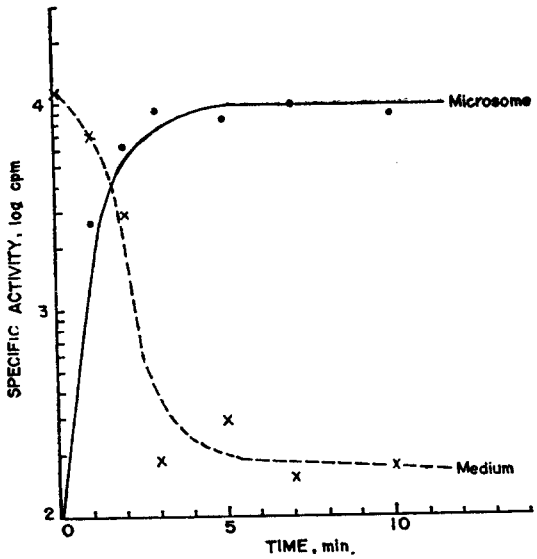

Fig. 4. Time course of the Ca^{++} uptake of rabbit skeletal muscle microsomes. The medium consisted of 0.02 *M* tris-maleate buffer+1m*M* ATP+2 m*M* Mg^{++}+0.05 *M* KCl+1 m*M* oxalate+0.1m*M* Ca^{++}(containing Ca^{45} with specific activity of 1.2 x 10^5 cpm/m*M* Ca). Microsomal concentration was 0.2 mg protein/ml suspension. Each point represents an average of three determinations.

was observed by Martonosi and Feretos (1964), who reported that the sarcoplasmic reticulum fragments show an ATP-, Mg^{++}-, and Ca^{++}- dependent transport of oxalate against concentration gradient, and that there is a stoichiometric correlation between Ca and oxalate uptakes with the ratio of 1.

There is an interesting difference between oxalate-containing and oxalate-free systems in the saturation time of Ca uptake. As shown in Fig. 5, oxalate-free system attained the saturation at about 30 seconds of incubation, whereas about 2 minutes were required in oxalate-containing system. This delay by the presence of oxalate is probably attributed also by the formation of Ca-oxalate. In the absence of oxalate the inside of microsomes is instantenously saturated by the influx of Ca^{++} and the net uptake soon reaches the limit, within 30 seconds, while in the presence of oxalate the influx continues until the equilibrium between the precipitate and the soluble Ca^{++} level in the microsomes so attained as to raise the free Ca^{++} concentration to a level of saturation.

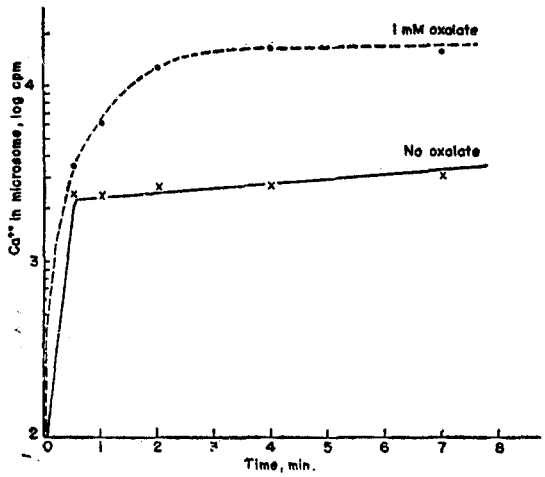

Fig 5. The effect of oxalate on the Ca^{++} uptake of rabbit skeletal muscle microsomes. Incubation medium was 0.02 *M* tris-maleate buffer+ 1 m*M* ATP+2m*M* Mg^{++}+0.05 *M* KCl+ 0.1 m*M* Ca^{++} Each points represents an average of three determinations.

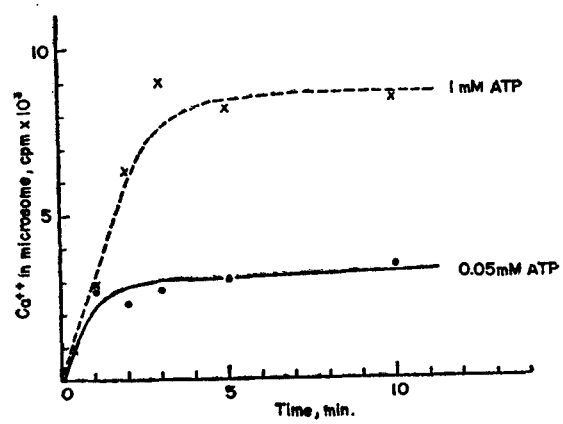

Fig 6. The effect of the concentration of ATP on Ca^{++} uptake of rabbit skeletal muscle microsomes. Incubation conditions are the same with those in Fig. 5 except the concentration of ATP.

4. ATP Concentration and Ca Uptake: The amount of Ca accumulated in the microsomes seemed to be dependent on the concentration of ATP as seen in Fig. 6. Microsomes in the medium containing 1 m*M* ATP accumulated much more Ca than in the medium where only 0.05 m*M* ATP was present. Hasselbach and Makinose (1961) reported that the relaxing granules actively incorporate Ca^{++} into their vesicular structure and that addition of small quantity of Ca to the reaction mixture always causes extra-splitting of ATP. They suggested that the microsomal ATPase might play a role of energy-provider for the incorporation of Ca and that the stoichiometric correlations between the Ca uptake and ATPase exist with the ratio of 1.2. Ebashi and Yamanouchi (1964), however, have shown that the ratio between Ca uptake and ATP breakdown has no definite stoichiometric correlations and increases with the decrease of ATP concentration. They explained that the ratio of 1.2 as Hasselbach and Makinose reported could be obtained only when high concentrations of ATP like 5 m*M* is employed, whereas if the ATP concentration is kept low (lower than 0.1 m*M*) and introducing an ATP-regenerating system, such as phosphoenolpyruvate-pyruvate kinase system, in the incubation medium, no stoichiometric correlation could be observed. In the present experiment, using 0.05 m*M* and 1 m*M* ATP, the results were in favor of the latter. The low uptake of Ca^{++} in the presence of 0.05 m*M* ATP is probably caused by the exhaustion of the ATP before the saturation of the Ca accumulation is reached.

SUMMARY

Measurements were made of the ATPase activity of rabbit skeletal muscle microsomes (fragments of sarcoplasmic reticulum) at various Ca concentrations, the change in the ATPase activity with the microsomal age, the Ca uptake of the microsomes, and the effect of ATP concentration on the Ca uptake of the microsomes and the results obtained are summarized as follows:

1. Skeletal muscle microsomes have an ATPase activity which is activated by Ca^{++} and Mg^{++}. The optimum Ca concentration for the activity is 0.1 m*M* in the presence of 5 m*M* Mg.

2. There seems to be no direct relationship between Ca^{++}– and Mg^{++}–dependent ATPase activity and relaxing activity of microsomes.

3. The presence of oxalate stimulates the Ca uptake of microsomes and delays the saturation time.

4. The amount of Ca accumulated in the microsomes depends on the concentration of ATP present, high ATP concentration giving larger amount of accumulation.

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