PHOSPHATE TRANSFER TO MYOFIBRILS BY ATP-CREATINE TRANSPHOSPHORYLASE

KOICHI YAGI* AND LAFAYETTE NODA

Department of Biochemistry, Dartmouth Medical School, Hanover, N.H. (U.S.A.)

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SUMMARY

The following reaction cycle is observed in myofibrils from rabbit muscle treated with deoxycholate: In the absence of added nucleotides, ADP bound to myofibrils is phosphorylated in the presence of creatine phosphate and ATP-creatine transphosphorylase, resulting in the liberation of free creatine, and the ATP produced is hydrolyzed by the Mg-activated ATPase activity of the myofibrils. The same phenomenon also occurs with myofibrils isolated from glycerinated psoas fibers.

INTRODUCTION

Myosin and actin are considered to be the principal proteins involved in muscle contraction[1,2]. Isolated actin always contains tightly bound ADP[3] which can not be removed unless actin is denatured. In spite of much effort, the role of actin-bound ADP in the process of muscle contraction is still obscure[4–7].

STROHMAN[6] has reported that Cr-enzyme could catalyze the reaction between actin-bound ADP and added Cr-P. This transphosphorylation was accompanied by transformation of fibrous to globular actin. PERRY has reported that most of the ADP of myofibrils is bound to actin[4] and that the ADP of myofibrils cannot be phosphorylated[5] under conditions similar to those used by STROHMAN.

The failure to observe transphosphorylation under the conditions used by PERRY, might be explained perhaps by the inability of added Cr-enzyme to penetrate untreated myofibrils. We have found myofibrils treated by deoxycholate or glycerine to have a similar appearance in the microscope to untreated myofibrils. Most of the work reported here has been done on myofibrils treated with deoxycholate. A preliminary report has been previously presented[8].

MATERIALS AND METHODS

Preparation of myofibril

Myofibrils were prepared from rabbit back muscle by the method of PERRY[9], except that 4 m*M* $MgCl_2$ equal to the concentration of EDTA was added. Such myo-

Abbreviations: Cr-enzyme, ATP-creatine transphosphorylase (creatine kinase); Pi, inorganic orthophosphate; Cr-P, creatine phosphate; AMP, adenosinemonophosphate; ADP, adenosinediphosphate; ATP, adenosinetriphosphate; EDTA, ethylenediaminetetraacetate; ATPase, adenosinetriphosphatase; *p*-CMB, *p*-chloromercuribenzoate; TCA, trichloroacetic acid.

* Present address: Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo, Japan.

fibrils show no Cr-enzyme activity and the sarcomere length is 2.6 to 2.8 μ. If magnesium and EDTA are omitted from the wash solution, the sarcomere lengths are much shorter and more random. The concentration of myofibrils is expressed as protein, determined by the biuret procedure of GORNALL, BARDAWILL AND DAVID[10] using crystalline bovine serum albumin as a standard. Myofibrils were generally stored at 0° as a very thick paste (30 to 35 mg/ml) and portions were weighed for use assuming a density of 1.

Deoxycholate treatment of myofibrils

Commercial deoxycholate was recrystallized twice from methanol. A stock 5 % solution (approx. 0.125 *M*) was prepared after adjusting the pH to 7.8 with KOH. Myofibrils (3 volumes) were first mixed with an appropriate amount of deoxycholate–borate KCl mixture (4 volumes) and thoroughly stirred for 1 min at 0°. Then the mixture was washed 3 times, each with a volume of 0.04 *M* borate–0.03 *M* KCl, pH 7.15, equal to 5 to 6 times the volume of the original myofibril–deoxycholate suspension. The myofibrils were collected each time by centrifugation at 0°, 5000 $\times g$ for 15 min and finally suspended in the borate–KCl solution. Myofibrils treated with deoxycholate were used immediately.

Preparation of myofibrils from glycerinated muscle fibers

Glycerinated fibers were prepared from rabbit psoas muscle as follows: Muscle strands about 2 mm in diameter were partially dissected out and tied *in situ* with cotton thread to glass rods in order to maintain the rest length; the fibers attached to glass rods were allowed to stand at 0° in 50 % glycerine which was changed daily; beginning the third day the fibers in fresh 50 % glycerine at 0° were transferred to a deep freeze at about. 15° and stored for more than two months without change of solution. Glycerinated fibers were cut with scissors to about one-inch length and treated as in the preparation of fresh myofibrils.

Preparation of [^{32}P]creatine phosphate

A mixture of [^{32}P]ADP and [^{32}P]ATP was prepared from radioactive inorganic phosphate using liver mitochondria[11] and the two nucleotides were purified by the procedure of PONTIS[12] using calcium chloride on a Dowex-1 column, but modified for batch operation—0.05 *M* NH_4Cl was used to wash the column, 0.01 *N* HCl to elute AMP and 0.035 *M* $CaCl_2$, pH 1.85, to elute ADP + ATP. [^{32}P]Cr-P was prepared from the calcium-free mixture of labeled adenine nucleotides by incubating 30 min at room temperature at pH 9.5 with excess creatine and purified preparations of Cr-enzyme and myokinase. [^{32}P]Cr-P was easily isolated by ion-exchange chromatography on a Dowex-1 chloride column (1 cm × 3.5 cm) by eluting with 0.025 *M* calcium chloride, pH 4.2. The effluent was lyophilized, the dry powder was dissolved in 1 m*M* NH_4OH and calcium was removed by the use of a Dowex-50 X Na^+-form column. The solution of [^{32}P]Cr-P contained less than 5 % Pi and showed no absorption at 260 mμ. The overall yield from starting compound, $Na_3{}^{32}PO_4$, to [^{32}P]Cr-P was 38 %.

A Nuclear Chicago Geiger tube and scaler were used for monitoring radioactivity and samples were plated and counted using a gas flow counter.

Determination of creatine liberating activity

The reaction mixture was composed of 0.300 ml 0.10 *M* buffer (generally histi-

dine buffer, pH 7.0), 0.100 ml 0.01 *M* $MgCl_2$, 0.050 ml or more of Cr-enzyme (generally, 45–50 mg crystallized enzyme/ml in 0.001 *M* glycine, pH 9), myofibril stock (0.100–0.500 ml of myofibril suspension containing about 10 mg/ml in borate–KCl solution; biuret protein determination is made) and 0.04 *M* borate–0.03 *M* KCl, pH 7.15, to a total volume of 0.900 ml. The reaction (incubated in 30° bath) was started by adding 0.100 ml Cr-P to a concentration of 1–10 m*M* and stopped by the addition of 2.5 m*M* *p*-CMB to a final concentration of 0.5 m*M* followed by cooling in an ice–water mixture. Protein was precipitated using barium hydroxide and zinc sulfate as described by SOMOGYI[13]. The supernatant from centrifugation, 5,000 × *g*, 0°, 15 min, was used for creatine determination by the method of ENNOR AND STOCKEN[14].

Determination of ATPase activity

Incubation at 30° was carried out in the presence of 1 m*M* ATP, 5 m*M* $MgCl_2$, 50 m*M* histidine, pH 7, and the Pi liberated was determined by the method of FISKE AND SUBBAROW[15].

Determination of radioactive phosphate incorporation

Incubation conditions were the same as in the determination of creatine liberation except that 5 times larger quantities were used. The reaction was stopped and the nucleotide liberated by adding cold 3 % TCA, and allowing the mixture to stand in an ice bath for 20 min. The mixture was centrifuged at 0° and the precipitate was once washed with 1 % TCA. The combined supernatant solutions were adsorbed on a Norite column (25 mg Norite A and 15 mg cellulose powder). To reduce the ^{32}Pi and [^{32}P]Cr-P contamination, the column was washed 3 times with a total of 30 ml of 0.1 *M* NaH_2PO_4. The Norite A column was transferred to a planchet and dried for counting.

RESULTS

Morphological structure of myofibrils

Myofibrils prepared as described above have been found to be heterogeneous in size, but the sarcomere lengths have been found to be almost uniform. Myofibrils washed with deoxycholate solution showed a morphological structure similar to untreated myofibrils except that the I band was somewhat shorter, the Z membrane was difficult to see even at 1000 × magnification, and the outer boundaries of the myofibrils were less distinct.

At low ionic strength in the absence of Mg^{++}, myofibrils shortened in 5 m*M* ATP and showed a simple pattern of alternating dark and light bands.

Myofibrils prepared from glycerinated muscle fibers appeared to have a more distinct band pattern than myofibrils prepared from untreated muscle myofibrils.

Content of nucleotides of myofibrils

The nucleotides bound to myofibrils were eluted with 1 % $HClO_4$ and analyzed by ion-exchange chromatography on Dowex-1 according to the method of COHN AND CARTER[16] adapted to smaller quantities. Adenine nucleotides were further identified by optical density ratios 250 mμ/260 mμ = 0.22 and 280 mμ/260 mμ = 0.85. As seen in Table I the amounts of the nucleotides found were slightly lower than those reported by PERRY[4] (determined by a combination of phosphate analysis and enzyme assay).

TABLE I

ANALYSIS OF NUCLEOTIDES OF MYOFIBRILS BY ION EXCHANGE CHROMATOGRAPHY

	μmoles/g of myofibrils			Total	ADP + ATP	ADP / Total
	AMP	ADP	ATP			
Natural myofibrils	0.87*	2.02	0.42		2.44	
Natural myofibrils	0.14	2.26	0.30	2.70	2.56	0.84
Natural myofibrils (Perry)	0.88	2.7	0.47		3.17	
Myofibrils treated by						
0.7% deoxycholate	0.14	1.74	0.14	2.02	1.88	0.86
0.7% deoxycholate	0	1.64	0.34	1.98	1.98	0.83
0.7% deoxycholate	0.07	1.83	0.17	2.07	2.00	0.88

* Probably contaminated since ratio of absorbance at 250 mμ to 260 mμ was 1.37.

Accessibility of the creatine enzyme system to myofibrils

Cr-enzyme is specific for ADP and Cr-P[17]. Hence in a suitable mixture of myofibril, Cr-enzyme and Cr-P the liberation of creatine strongly suggests that the enzyme catalyzes the reaction between ADP bound to myofibrils and the added Cr-P. In agreement with the report[5] of PERRY, we have found that in a reaction mixture of untreated myofibrils, crystallized creatine enzyme, Mg^{++} and Cr-P there was only a slight liberation of creatine.

Myofibrils treated with deoxycholate, digitonin or ATP, or myofibrils isolated from glycerinated muscle fibers catalyzed the liberation of free creatine as shown in Figs. 1 and 2. No creatine liberation was observed even with these treated myofibrils if Cr-P, Cr-enzyme, or myofibril was omitted from the reaction mixture. The slow reaction observed in the absence of added Mg^{++} may be due to Mg^{++} bound to myofibrils[18]. An explanation for the effect of treating myofibrils with ATP is not

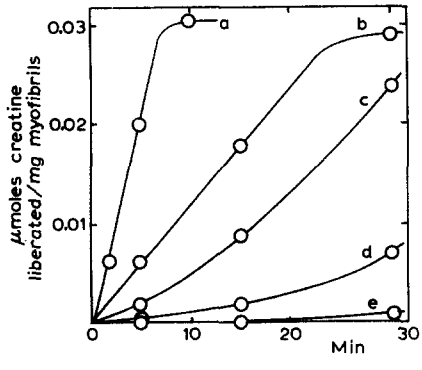

Fig. 1. Creatine liberating activity of myofibrils treated by different conditions. Creatine enzyme, 2.8 mg/ml. Creatine phosphate, 0.2 m*M*. a, myofibrils treated by 0.7% deoxycholate at pH 7.8, $\mu = 0.03$; b, myofibrils treated by 1.9% deoxycholate at pH 9.7, $\mu = 0.03$; c, myofibrils treated by 5 m*M* ATP at pH 7.0, $\mu = 0.02$; d, myofibrils treated by 1.5% digitonin at pH 7.0, $\mu = 0.47$; e, control.

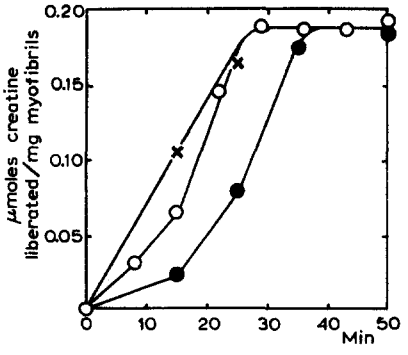

Fig. 2. Creatine liberating activity of myofibrils isolated from glycerinated psoas fibers. Creatine phosphate, 0.6 m*M*. ○, myofibrils isolated from glycerinated muscle fibers, 3.1 mg/ml. Creatine enzyme, 2.4 mg/ml. ●, same myofibrils, but 4 days after the preparation, 3.1 mg/ml. Creatine enzyme, 2.9 mg/ml. ×, same myofibrils as in ○. Myofibrils, 3.1 mg/ml. Creatine enzyme, 5.6 mg/ml.

apparent. In the case of myofibrils isolated from glycerinated fibers, the liberation of creatine showed a lag phase (Fig. 2). The lag phase was increased by aging of the myofibrils and decreased by increasing the ratio of the amount of creatine enzyme to myofibrils.

During the liberation of creatine, a gradual shortening of the myofibrils isolated from glycerinated fibers has been microscopically observed. Shortening occurs first in the H band and finally resulted in the formation of so-called contraction bands. The process seems to be very similar to the observation of HUXLEY[19] with glycerinated fibers.

Properties of creatine liberation by the myofibril-creatine enzymic system

As shown in Fig. 3, with increasing deoxycholate concentration in the washing of myofibrils, the creatine liberating activity increased to about 1 % deoxycholate and then decreased when the concentration of deoxycholate was greater than 2 %. There was variability in creatine liberating activity from preparation to preparation, especially when myofibrils were washed with deoxycholate concentrations greater than 0.5 %.

The Mg^{++}-activated ATPase activity of myofibrils showed a slight decrease with increasing concentration of deoxycholate up to about 1 % and at higher deoxycholate concentration the loss in ATPase activity was considerable (Fig. 3). There appears to be a relationship between creatine liberating activity and ATPase activity of myofibrils.

During deoxycholate treatment, large amounts of protein were extracted, as shown in Fig. 4. The nature of the protein extracted by deoxycholate has not been determined.

The creatine liberating activity of myofibrils washed by deoxycholate was not stable in borate–KCl solution stored at pH 7 and 0°. As shown in Fig. 5, stored myofibrils showed a lag phase not apparent with freshly treated myofibrils, but after overcoming the lag phase the rate of creatine liberation attained normal values. The lag phase observed with myofibrils prepared from glycerinated muscle fibers has already been pointed out (Fig. 2).

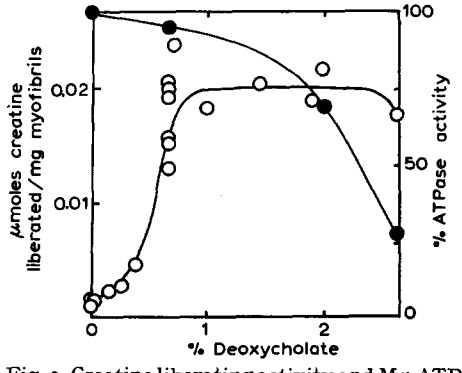

Fig. 3. Creatine liberating activity and Mg-ATPase activity of myofibrils treated by variable concentration of deoxycholate for 1 min, 0°. O, creatine liberation. Creatine enzyme, 2.8 mg/ml. Creatine phosphate, 0.2 m*M*. ●, Mg-ATPase.

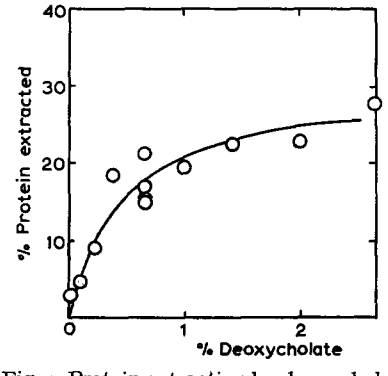

Fig. 4. Protein extraction by deoxycholate from natural myofibrils, pH 7.8, $\mu = 0.03 \pm 0.005$.

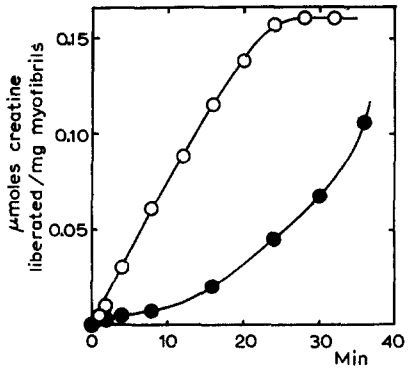

Fig. 5. Creatine liberating activities of myofibrils freshly treated by 0.7 % deoxycholate and the myofibrils stored overnight at 0°. ○, myofibrils freshly treated, 4.1 mg/ml. Creatine enzyme, 3.1 mg/ml. Creatine phosphate, 0.65 m*M*. ●, myofibrils stored, 3.9 mg/ml. Creatine enzyme, 3.1 mg/ml. Creatine phosphate, 0.7 m*M*.

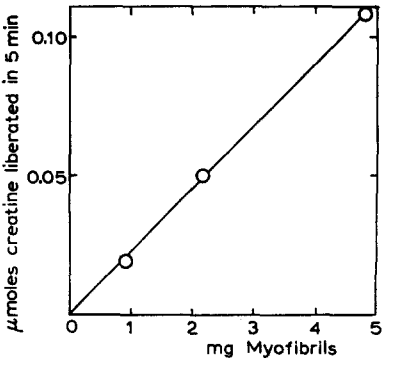

Fig. 6. The linear relationship between the activity of creatine liberation and the amount of myofibrils treated by 0.7 % deoxycholate. Creatine enzyme, 2.8 mg/ml. Creatine phosphate, 0.2 m*M*.

Fig. 6 shows the linear relationship between creatine liberating activity and the amount of myofibrils (freshly washed with deoxycholate).

Under our conditions the creatine liberating activity of myofibrils was not saturated with Cr-enzyme as shown in Fig. 7. For routine runs, Cr-enzyme was mixed with the reaction mixture shortly before the start of the reaction. When myofibrils were preincubated with Cr-enzyme in borate–KCl at pH 7 and at 0°, creatine was liberated only when further quantities of Cr-enzyme were added.

Fig. 8 shows the LINEWEAVER-BURK plots of creatine liberation. Maximum activity was reached at Cr-P concentrations greater than 1 m*M* and apparent K_m values for Cr-P of $1.05 \cdot 10^{-4}$ and $1.10 \cdot 10^{-4}$ *M* have been obtained from curves a and b respectively. However, the velocity of creatine liberation was directly proportional

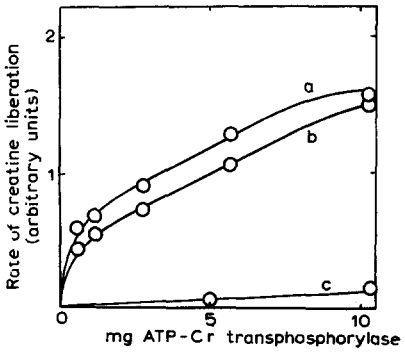

Fig. 7. The relation between creatine liberating activity and the amount of creatine enzyme on the myofibrils treated by 0.7 % deoxycholate. Creatine phosphate 0.2 m*M*. a, myofibrils treated by 0.7 % deoxycholate, 2.5 mg/ml. b, myofibrils treated by 2 % deoxycholate, 2.5 mg/ml. c, control with natural myofibrils, 3.2 mg/ml.

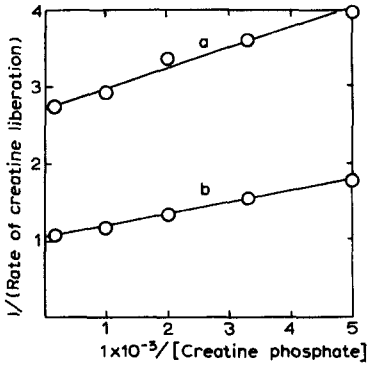

Fig. 8. LINEWEAVER-BURK plot of the inverse of the velocities of creatine liberation against the inverse of the concentrations of creatine phosphate. Myofibrils treated by 0.7% deoxycholate. a, creatine enzyme, 2.4 mg/ml. b, creatine enzyme, 4.9 mg/ml.

to the amount of Cr-enzyme. The results suggest that the rate determining step involves the reaction catalyzed by Cr-enzyme.

Behavior of ADP on myofibrils

The myofibrils were first incubated with the complete system (myofibrils, Cr-enzyme, Mg^{++}) at pH 7 for 20 min at 30° and then were washed by borate–KCl solution. The ADP of the myofibrils was still available for creatine liberation as shown by the creatine liberating activity of a second incubation which showed the same value as the first incubation (Table II, Expts. 1 and 4, column 8). Cr-enzyme once added in the first incubation was unavailable or lost for the second incubation, but ADP was still on the myofibrils and it functioned with the same efficiency as before. These results strongly suggest that the ADP is always situated in a convenient position both for phosphorylation by the Cr-enzyme Cr-P system and for dephosphorylation by ATPase.

TABLE II

THE EFFECT OF PRE-INCUBATION ON CREATINE LIBERATING ACTIVITY OF DEOXYCHOLATE TREATED MYOFIBRILS

	Reaction mixture of the first incubation						*Creatine liberating activity of the second incubations (μmoles creatine/ mg myofibrils/5 min, 30°)*	
Expt.	*Myofibrils treated by 0.7% deoxycholate (2.4 mg/ml)*	*Creatine enzyme (2.5 mg/ml)*	*$MgCl_2$ (1 mM)*	*Creatine phosphate (1 mM)*	*Temperature*	*Incubation time (min)*	*Complete system*	*Creatine enzyme is omitted from complete system*
1	+	+	+	+	30	20	0.054	0.003
2	+	—	+	—	30	20	0.048	< 0.001
3	+	—	+	—	0	20	0.052	None
4	+	—	—	—	0	—	0.055	None

In Table III are given some representative data from which the figures have been drawn for the comparison of the rate of ATP hydrolysis to the rate of creatine liberation. It is seen that the rate of ATP hydrolysis is 10 to 50 times greater than the rate of creatine liberation (phosphate transfer).

Phosphate incorporation in myofibrils

In early experiments untreated myofibrils were incubated with Cr-enzyme, [^{32}P]Cr-P and Mg^{++}, all adjusted to pH 7. The reaction was stopped by 50 % methanol which did not extract any nucleotides from myofibrils but almost completely inhibited the magnesium activated ATPase of the myofibrils. 1 ml of the methanol treated mixture was layered on 3 ml of 0.25 *M* sucrose in the tube of a Spinco swinging bucket rotor and immediately centrifuged at 20,000 × *g* for 3 min. The precipitated myofibrils were plated and the radioactivity was counted. As shown by control runs without creatine enzyme, the myofibrils were washed almost completely free of radioactive phosphate during the sedimentation through the sucrose layer. An excess of about $5 \cdot 10^{-4}$ μmoles of radioactive phosphate/g of myofibrils was observed when the reaction was stopped at 10 sec or 15 min. The results confirmed the previous observation of PERRY.

TABLE III

COMPARISON OF Mg-ATPASE AND CREATINE LIBERATING ACTIVITY OF MYOFIBRILS AT pH 7 AND 30°

Preparation	*Deoxycholate* %	*ATP-ase** *μmoles P/mg/min*	*Creatine liberating activity*			
			Myofibrils (mg)	*Cr-enzyme (mg)*	*CrP mM*	*μmoles Cr**/ mg/min*
7	0	0.34	5.5	2.8	2.2	< 0.0002
	0.02	0.35	5.4	2.8	0.2	0.0003
	0.13	0.35	5.5	2.8	0.2	0.0004
	0.26	0.31	5.1	2.8	0.2	0.0006
	0.39	0.36	5.1	2.8	0.2	0.0012
	0.70	0.33	5.0	2.8	0.2	0.003
	1.4	0.26	4.9	2.8	0.2	0.004
	2.0	0.27	4.7	2.8	0.2	0.004
	0.7		2.5	5.7	0.2	0.006
	0.7		2.5	11.4	0.2	0.007
8	0.7		2.65	2.4	5.0	0.013
	0.7		2.65	4.9	0.2	0.019
	0.7		2.65	4.9	1.0	0.019
	0.7		2.65	4.9	5.0	0.031

* Measured in the presence of 1 m*M* ATP and 5 m*M* $MgCl_2$.
** Measured in the presence of 1 m*M* $MgCl_2$.

In other experiments with myofibrils freshly washed with deoxycholate, the reaction mixtures (incubated in centrifuge tubes) were rapidly cooled in an ice–water bath, after the appropriate reaction time, and then rapidly centrifuged at 0°. The supernatant was sucked out and discarded. The bound nucleotides were extracted from sedimented myofibrils by the addition of cold TCA and were adsorbed on a column of 15 mg of Norite A mixed with cellulose powder (previously washed by acid, alkali and ethanol). The time lapse from the start of the reaction to addition of TCA was generally 20 to 25 min. However, even if *p*-CMB was added to 0.5 m*M* to stop the reaction or EDTA was added to 2 m*M* to overcome the ATPase activity by sequestering Mg^{++}[21] the amount of nucleotide containing phosphate adsorbed on Norite A did not significantly vary from $5 \cdot 10^{-3}$ μmoles/g of myofibrils.

In later experiments on phosphate incorporation by deoxycholate treated myofibrils, the procedure used was that described above. The curves indicating the amount of radioactive nucleotide incorporated against the reaction time were obtained as shown in Figs. 9 and 10. The incorporation rises to a maximum in 1 to 2 min and then shows a decline. The amount of radioactive nucleotide increased with increase in concentration of [^{32}P]Cr-P added (Fig. 10). The maximum incorporation of ^{32}P from [^{32}P]Cr-P that we have observed (conditions may not yet be ideal) has been 40% of the ADP and ATP bound to the myofibrils treated with deoxycholate. Untreated myofibrils or control runs in which the TCA was added before the substrate showed essentially no incorporation of the phosphorus isotope.

Identification of radioactive nucleotide

Ion exchange chromatography (Dowex-1) of nucleotides[22] extracted from myofibrils incubated with the complete system showed three peaks of AMP, ADP and ATP by u.v. absorption and the ATP peak contained essentially all of the radioactivity, excluding ^{32}Pi and [^{32}P]Cr-P (Fig. 11).

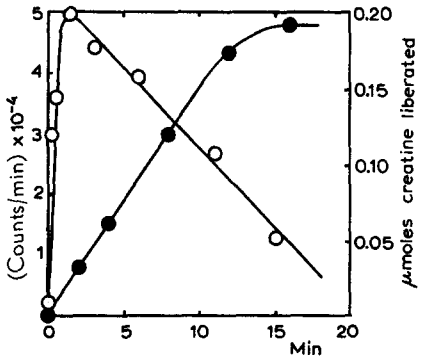

Fig. 9. Radioactive phosphate incorporation in the myofibrils treated by 0.7 % deoxycholate with creatine enzyme system, and the creatine liberating activity with the same myofibrils and the enzyme system. ○, phosphate incorporation. Radioactive creatine phosphate, 0.2 m*M* and $2.3 \cdot 10^7$ counts/min. Myofibrils, 19.5 mg/5 ml. Creatine enzyme, 15.4 mg/5 ml. ●, creatine liberating activity. Creatine phosphate, 0.2 m*M*. Myofibrils, 3.9 mg/ml. Creatine enzyme, 3 mg/ml.

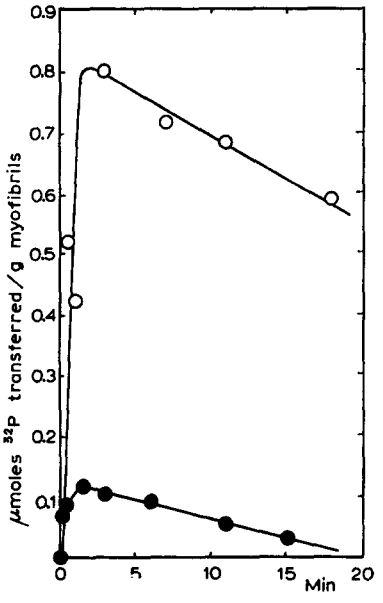

Fig. 10. Radioactive phosphate incorporation in the myofibrils treated by 0.7 % deoxycholate with creatine enzyme system at different creatine phosphate concentrations. ○, radioactive creatine phosphate, 0.75 m*M* and $1 \cdot 10^7$ counts/min. Myofibrils, 19.4 mg/5 ml. Creatine enzyme, 15.4 mg/5 ml. ●, radioactive creatine phosphate 0.2 m*M*. Myofibrils, 19.5 mg/5 ml. Creatine enzyme, 15.4 mg/5 ml.

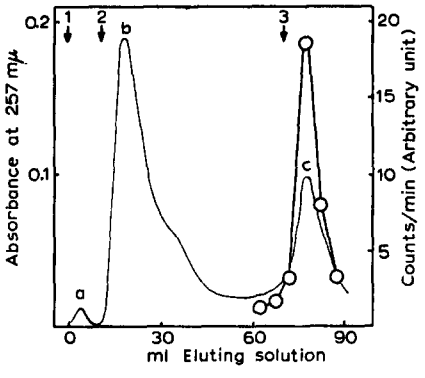

Fig. 11. Ion exchange chromatography of nucleotides extracted from myofibrils treated by 0.7 % deoxycholate. Radioactive creatine phosphate, 0.16 m*M* and $9.5 \cdot 10^6$ counts/min. Myofibrils, about 130 mg/15.5 ml. Creatine enzyme, 24.3 mg/15.5 ml. Eluent at 1 is 0.2 *M* formic acid–0.3 *M* ammonium formate; at 2, 2.5 *M* formic acid–0.3 *M* ammonium formate; and at 3, 4 *M* formic acid–0.3 *M* ammonium formate. Peak a, AMP; b, ADP; c, ATP. ○, radioactivity.

DISCUSSION

The reaction system containing Cr-enzyme and deoxycholate-washed myofibrils, liberated creatine and produced bound ATP without addition of ADP, *i.e.*, ADP bound to myofibrils was phosphorylated by the Cr-enzyme system and the nucleotide remained bound to the myofibril. Subsequently the bound ATP was hydrolyzed by the Mg-ATPase of myofibrils to bound ADP and inorganic phosphate so that the net reaction is the breakdown of Cr-P. The same Mg-ATPase activity of myofibrils was maintained during the reaction cycle. The highest phosphate incorporation observed by us was 40 % of the total amount of ADP plus ATP bound to myofibrils.

As shown in Table I, more than 80 % of the nucleotides of myofibrils was ADP. It is generally assumed that the nucleotides of myofibrils are bound to actin. It should be noted that preparations of myosin also contain ADP and ATP[23], but the amount

is too low to account for the observed amount of phosphate incorporation. The observed time course of phosphate incorporation (Fig. 9) indicates that there is an observable time interval between phosphate incorporation from Cr-P and the liberation of Cr. Thus it may be reasonable to conclude that ADP on actin of the myofibrils incorporated phosphate from the Cr-enzyme system and was converted to ATP which in turn was hydrolyzed and thus makes possible a cyclical process.

From these considerations the following reaction scheme is suggested: (a) Cr-enzyme catalyzes the reaction of Cr-P with ADP tightly bound to actin of myofibrils resulting in the production of bound ATP and the liberation of creatine. (b) The loosely bound ATP is transferred from actin to the ATPase active site of myosin in myofibrils or alternatively, the ATP induces a configurational change in the make-up of the myofibrils. (c) The ATP is hydrolyzed by the ATPase activity of myosin to loosely bound ADP and Pi and (d) the ADP formed is returned to the original state. We might speculate that in this system under consideration the creatine liberating activity may be limited by the degree to which the nucleotide sites of the myofibril are saturated with creatine enzyme, since (a) the myofibril system seems to become very rapidly saturated with regard to formation of bound ATP; and (b) increasing amounts of enzyme increased creatine liberating activity (Fig. 8) although even at low enzyme concentrations many-fold excess enzyme (on the basis of assay in a purified system) had been added for the observed activity.

The accessibility of myofibril for the Cr-enzyme system might depend on a steric interrelation of actin and myosin inside the myofibril and/or a diffusion of Cr-enzyme into the myofibrils. There is no evidence that proteins in glycerinated myofibrils have the same configuration as in the intact myofibrils, but the results from electron microscopy suggest the same structural interrelationship between actin and myosin in both kinds of myofibrils. However, myofibrils from glycerinated muscle fibers can liberate creatine from the Cr-enzyme system while natural myofibrils can not. This result seems to suggest that the effect of deoxycholate and glycerine on myofibrils may be on the penetration of the Cr-enzyme system into the myofibril.

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A RE-EXAMINATION OF THE MOLECULAR CHARACTERISTICS OF G-ACTIN

CYRIL M. KAY

Department of Biochemistry, University of Alberta, Edmonton, Alberta (Canada)

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SUMMARY

1. The molecular weight of ultracentrifugally homogeneous G-actin, prepared by the method of TSAO AND BAILEY, is 66,000 ± 2,000 by light scattering and 61,600 ± 6,000 by sedimentation viscosity.

2. The virtual identity of the extrapolated Hc/τ intercept found by light scattering for G-actin in either 0.5 *M* KI or H_2O-10^{-4} *M* ATP suggests that the same limit of dissociation is attained for the protein dissolved in either of these media.

3. The light scattering data do not support the earlier view, derived from osmotic pressure measurements, that both monomeric and dimeric G-actin exist in depolymerized actin systems.

4. The dispersion constant, $\lambda_0 = 223$ mμ, is unusually low for G-actin, reflecting an apparent lack of any extensive helical configuration in the molecule.

INTRODUCTION

The earliest attempts to characterize monomeric G-actin were limited by the lack of purity of the preparations used[1–3]. Most of these studies were carried out on preparations based on the original or modified procedures of STRAUB[4], and these were subsequently shown to contain as much as 40–60 % impurity[5,6]. In 1951 MOMMAERTS[5], using the method of successive polymerizations and ultracentrifugal separation of the