STUDIES ON THE ENZYMIC INTERACTIONS OF THE BOUND NUCLEOTIDE OF THE MUSCLE PROTEIN ACTIN

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SUMMARY

1. A reversible depolymerization of F-actin has been obtained by dialysis against a watery solution of creatine-phosphate. The resulting preparation, free of any exogenous nucleotide, enables an accurate determination of the changes in bound nucleotide both in actin–actin and actin–myosin interaction.

2. The depolymerization of F-actin in the presence of creatine-phosphate is accompanied by the phosphorylation of the bound ADP. Such transphosphorylation is shown to be dependent on the state of polymerization of the actin.

3. Complex formation between G-actin and H-meromyosin is indicated by several physical methods. Complex formation is accompanied by the splitting of the ATP bound to the G-actin.

4. In comparing G-actin–H-meromyosin complexes with F-actin–H-meromyosin complexes, it has been shown that only in the former is the nucleotide center of the actin component able to function as a center of "high energy" phosphate turnover in the presence of the phosphorylating system creatine-phosphate and ADP-CP transphosphorylase.

INTRODUCTION

The protein actin accounts for some 25 % of the total dry weight of the myofibril[1]. As such it is the second most abundant structural protein of striated muscle. It is somewhat surprising therefore that many theories of muscle contraction are couched almost exclusively in terms of intramolecular changes within the myosin filaments, and all either neglect the actin component or consider it as serving some subsidiary function. This somewhat paradoxical situation has been cited by BAILEY[2], who has called for evidence which would bear on the question of whether the molecular changes in contraction are associated with the actin, the myosin, or both.

The nature of the implication of actin in a reversible contraction is, of course, an open question. A possible role for actin might be suggested if it could be demonstrated that the bound nucleotide (ATP or ADP) of this protein is available for interaction with the various phosphorylating systems of muscle, or with myosin. Such

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evidence is lacking, and indeed from other experiments it has been inferred that this nucleotide is not reactive for "high-energy" phosphate turnover. Thus while the polymerization of G-actin is thought to be accompanied by the dephosphorylation of the bound ATP[3-6]:

$$\text{G-ATP} \longrightarrow \text{F-ADP} + H_3PO_4$$

a rephosphorylation of the bound ADP on depolymerization has not been demonstrated. The reversible depolymerization of F-actin in the presence of exogenous ATP has been interpreted by STRAUB AND FEUER[3] in terms of an exchange reaction:

$$\text{F-ADP} + \text{A'TP} \longrightarrow \text{G-A'TP} + \text{ADP}$$

At the same time, the non-reactive character of the myofibrillar-bound ADP, which nucleotide is associated with the actin component of the myofibrillar protein complement[7], has been demonstrated. PERRY[8] has shown that the myofibrillar ADP is stable in the presence of creatine-phosphate and ADP-CP transphosphorylase (CP-kinase), 5-adenylic deaminase, myokinase, and the myofibrillar-ATPase; a reaction system which rapidly converts any free ADP or ATP to inosinic acid, ammonia, and inorganic phosphate. This evidence militated strongly against the idea that the bound ADP of the actin could act as a center of phosphate turnover and the significance of the actin and its bound nucleotide remained and remains now beyond our appreciation.

It is nevertheless an interesting problem that ADP, bound at possible active sites, to one of the major structural proteins, is not in a reactive state within the "relaxed" myofibril. It also remains to be shown that while the myofibrillar-bound ADP is not susceptible to phosphorylation by creatine-phosphate as long as the myofibril remains uncontracted[8], this susceptibility may not in fact change on or during contraction, or during relaxation. It has been suggested[2,9,10] that during relaxation or contraction the bound nucleotide might undergo a cyclic phosphorylation, the level of phosphorylation being directed by the myosin or actomyosin ATP-ase activity. There is, however, as yet no *in vitro* evidence which would support this suggestion.

In view of the apparent relationship between the nucleotide of the myofibril and that of the actin, we have begun a study of the isolated actin with the aim of understanding the enzymic behavior characteristic of the myofibrillar nucleotide. Moreover, the motivation for the experimental approach here was a desire to define in clear terms some of the properties and *in vitro* activities of the actin. It was felt that the different states of aggregation or polymerization of this protein might be reflected in differences in the enzymic activity of the bound nucleotide. If such an *in vitro* relationship did, in fact, exist, then a study of this system might easily provide a basis for an experimental approach to the problem of the changes in state of aggregation of the actin, if any, during a contraction–relaxation cycle in living muscle.

In the experiments reported below it may be seen that a definite relationship exists between the state of polymerization of actin and the susceptibility of the bound ADP to phosphorylation by creatine-phosphate. This relationship carries over to the actomyosin complex. While complexes of F-actin–H-meromyosin are incapable of liberating creatine from creatine-phosphate, G-actin-H-meromyosin complexes of identical actin, and therefore nucleotide, content, under identical conditions, display large activities in this respect.

A preliminary report on some of this work has already been published[11].

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MATERIALS AND METHODS

Myosin and H-meromyosin

Myosin was prepared according to PERRY[12], and after the third precipitation was stored at —15° in a 50 % 0.5 *M* KCl–glycerol mixture. The glycerol was removed by a subsequent precipitation of the myosin in 0.04 *M* KCl.

H-meromyosin was prepared according to SZENT-GYÖRGYI[13] and was used immediately or after a maximum storage at 0° of 48 h.

Actin

Actin was prepared according to a modification of the method of MOMMAERTS[14]. The acetone powder of rabbit muscle was made according to STRAUB[15], and the G-actin plus impurities of tropomyosin, soluble enzymes, and loosely associated nucleotides were extracted from this with CO_2-free water at room temperature. After polymerization induced by the addition of 0.001 *M* $MgSO_4$ and 0.1 *M* KCl, the F-actin was separated out by centrifugation at 30,000 rev./min for 2 h using the number 30 rotor in the Spinco model L preparative ultracentrifuge. The plugs of F-actin from the centrifugation were suspended in 0.1 *M* KCl and a partial solution was brought about by gentle manipulation in a glass homogenizer. The F-actin at this stage is refractory to solution but dissolves completely during subsequent dialysis and depolymerization (see text). This is the stock F-actin which may be stored for two days at 0°.

ADP-CP transphosphorylase

The enzyme was prepared according to NODA, KUBY AND LARDY[16] and was taken to the stage of lyophilization. The resulting powder was stored at —15°. Solutions of the enzyme were made by dissolving the powder in water brought to neutral pH by the addition of $NaHCO_3$.

Nucleotide analysis

Separation of the nucleotides (ADP and ATP) from the actin or from actin–H-meromyosin mixtures was accomplished with 5 % perchloric acid at 0°. The precipitated protein was removed by filtration and the filtrate containing the nucleotides was immediately neutralized with KOH. The potassium salts were removed by centrifugation and the pH of that supernatant was adjusted to 8.0–8.5. The alkaline mixture of nucleotides was adsorbed onto Dowex-1 columns and elution carried out with NaCl–HCl mixtures according to COHN AND CARTER[17]. The entire procedure was carried out at 3°. This method separates quantitatively AMP, ADP, and ATP. Two hundred ml each of the eluting systems for the individual nucleotides were used in all cases with known mixtures being run in parallel. The resulting fractions within each system were then pooled, concentrated to a constant volume (10 ml) and nucleotide concentration was determined using the O.D. at 260 mμ and a molecular extinction coefficient of 14,200.

Creatine determination

Creatine was determined according to the method outlined by CHAPPELL AND PERRY[18]. Samples from the reaction mixtures were deproteinized with the barium–zinc mixture as described by SOMOGYI[19], and constant amounts of the filtrate were used

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for the analysis. It was unnecessary in these experiments to employ any –SH poisons to stop the reaction, the barium–zinc mixture being entirely adequate for this purpose.

Nitrogen determination

Nitrogen was determined by the micro-Kjeldahl method as described in KIRK[20]. A factor of 6.2 was used to convert to protein content.

Viscosity determination

Viscosity was determined using an Ostwald viscometer with an average flow time for water, under gravity, at 25°, of 70 sec.

Other reagents

The ATP and ADP were the crystalline sodium salts purchased from the Sigma Chemical Co. The sodium salt of creatine-phosphate, also from Sigma, gave negligible creatine blanks. All other chemicals were reagent grade and the water was distilled water further deionized by passing through a column of Amberlite MB-3-ion-exchange resin.

EXPERIMENTS AND RESULTS

Enzymic phosphorylation of actin-bound nucleotide

I. Reversible depolymerization with creatine-phosphate. Attempts were made to phosphorylate the bound ADP of F-actin under two conditions: (1) where the F-actin was maintained in the polymerized state and (2) where the actin was allowed to depolymerize during the gradual reduction in ionic strength accompanying dialysis. Solutions of stock F-actin in 0.1 *M* KCl were incubated with the enzyme CP-kinase, about 10 mg enzyme being added per 800 mg actin. The F-actin-enzyme mixture was then divided into equal fractions and depolymerized to G-actin by (a) dialysis against water containing 50–60 μg/ml CP and (b) by dialysis against water containing identical amounts of CP which had previously been hydrolyzed in hot HCl for 30 min. The latter depolymerization was run as a control for the CP requirement in reversible depolymerization. Another equal fraction was dialyzed against 50–60 micrograms/ml CP, but in the presence of 0.1 *M* KCl so that depolymerization of the F-actin did not take place. The pH of the dialysis was maintained at 7.0 with $NaHCO_3$, and was continued for 36 h at 3°. At this time depolymerization in cases (a) and (b) above was complete as judged by the reduction in viscosity and loss in double-refraction of flow of the solution. The F-actin dialyzed against the CP–KCl solution showed no change in these properties.

The addition of 0.1 *M* KCl + 0.001 *M* $MgSO_4$ to the G-actin from the CP dialysis results in a rapid increase in viscosity as indicated in Fig. 1. The depolymerization by dialysis against CP is therefore perfectly reversible since the viscosity reaches the same high value as that of the original F-actin. F-actin depolymerized by dialysis against hydrolyzed CP is depolymerized irreversibly. Reversible depolymerization requires therefore the presence of a phosphate donor system.

II. Nucleotide change accompanying depolymerization. Table I shows the changes in level of phosphorylation of the bound ADP of the F-actin during reversible depolymerization in the presence of CP (columns 1–2–3), and also when the actin is dialyzed

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against CP but in the presence of 0.1 *M* KCl so that no depolymerization takes place (column 4).

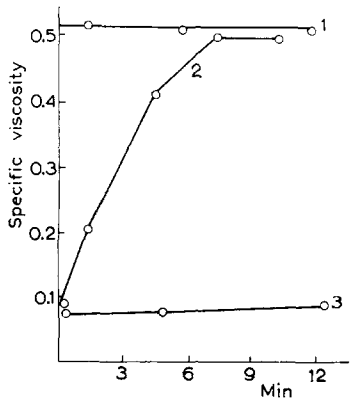

Fig. 1. Reversible depolymerization of F-actin with CP. Actin concentration is 0.6 mg/ml in all cases. (1) orig. F-actin in 0.1 *M* KCl + 0.001 *M* $MgSO_4$. (2) G-actin obtained from orig. F-actin by dialysis against CP. 0.1 *M* KCl + 0.001 *M* $MgSO_4$ added at 0 time. (3) G-actin obtained from orig. F-actin by dialysis against hydrolyzed CP. 0.1 *M* KCl + 0.001 *M* $MgSO_4$ added at 0 time.

TABLE I

ANALYSIS OF ACTIN NUCLEOTIDE DURING REVERSIBLE DEPOLYMERIZATION

Amounts in μmoles.

	(1) *Original F-actin*	*(2)* *G-actin from original F-actin by dialysis against CP*	*(3)* *F-actin by polymerization of actin in column (2)*	*(4)* *Original F-actin dialyzed against 0.1 M KCl + CP*
Prep. No. 1	20 ml	20 ml	20 ml	20 ml
ATP	0.220	1.400	0.260	0.480
ADP	1.080	0.160	0.980	0.940
Prep. No. 2	15 ml	15 ml	15 ml	15 ml
ATP	0.075	0.390	0.090	0.150
ADP	0.390	0.090	0.330	0.330
Prep. No. 3	15 ml	15 ml	15 ml	15 ml
ATP	0.150	0.702	0.148	0.227
ADP	0.753	0.080	0.670	0.540
Prep. No. 4	15 ml	15 ml	15 ml	15 ml
ATP	0.097	0.698	0.159	0.097
ADP	0.649	0.100	0.530	0.690
Average $\frac{\mu\text{moles/ml ATP}}{\mu\text{moles/ml ADP}}$	0.19	7.20	0.34	0.36

It is apparent that the bound ADP of the F-actin is converted to ATP during the depolymerization in the presence of CP. The susceptibility of the bound ADP to phosphorylation is, however, determined in some way by the degree of polymerization of the actin. The transphosphorylation takes place only during or after the depolymerization process. As column 4 of Table I shows, so long as the actin is

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maintained in the polymerized state then phosphorylation of the bound ADP does not take place. That the bound form of the nucleotide is undergoing phosphorylation by CP is indicated by the fact that the total amount of nucleotide before and after dialysis remains essentially unchanged. Had the nucleotide been dissociated from the protein prior to the reaction then an appreciable loss in nucleotide would be expected through the large volume of the dialysis fluid.

When the G-ATP actin obtained from the CP-dialysis is repolymerized the product is F-ADP actin (Table I, column 3). Since there is no nucleotide added to the actin at any stage of the preparation, except that originally bound to the actin, there can be no doubt that polymerization of the G-actin is accompanied by the dephosphorylation of the bound ATP. It is also to be noted in passing that the polymerization of the G-actin takes place in the presence of a large excess of CP. The tendency of the CP to maintain the nucleotide in the fully phosphorylated state is thus overcome by the polymerization process. Nor is polymerization induced by 0.1 *M* KCl inhibited by the presence of the CP, CP-kinase system.

Actin-H-meromyosin interaction

I. Complex formation. When F-actin and H-meromyosin are mixed in 0.1 *M* KCl, the resulting solution displays a high viscosity which is immediately but reversibly lowered on the addition of 1 m*M* ATP. This effect has been interpreted by A. G. SZENT-GYÖRGYI[13] as a reversible dissociation of an F-actin–H-meromyosin complex by the ATP. Fig. 2 shows the viscosity characteristics of both G- and F-actin–H-meromyosin mixtures of identical protein content, the only difference being that 10 min prior to the experiment the F-actin used was obtained from the stock G-actin by polymerization in the presence of 0.1 *M* KCl + 0.001 *M* $MgSO_4$ (Fig. 3). The F-actin–H-meromyosin (F–H) system shows the typical high viscosity and response to ATP. The G-actin–H-meromyosin (G–H) system displays only a slight rise in viscosity on mixing the two proteins in a salt-free medium. After 15–20 min incubation the addition of Mg^{++} and K^+ to the same final concentrations as in the F–H systems

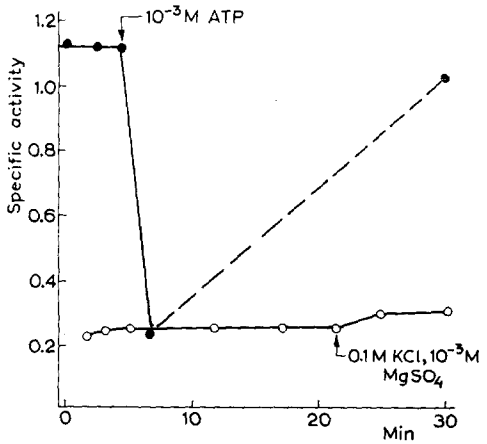

Fig. 2. Viscosity characteristics of G- and F-actin-H-meromyosin. (1) Closed circles: 0.6 mg F-actin + 3 mg H-meromyosin in 0.1 *M* KCl + 0.001 *M* $MgSO_4$; ATP added as indicated. (2) Open circles: 0.6 mg G-actin + 3 mg H-meromyosin in salt-free medium; KCl and $MgSO_4$ added as indicated.

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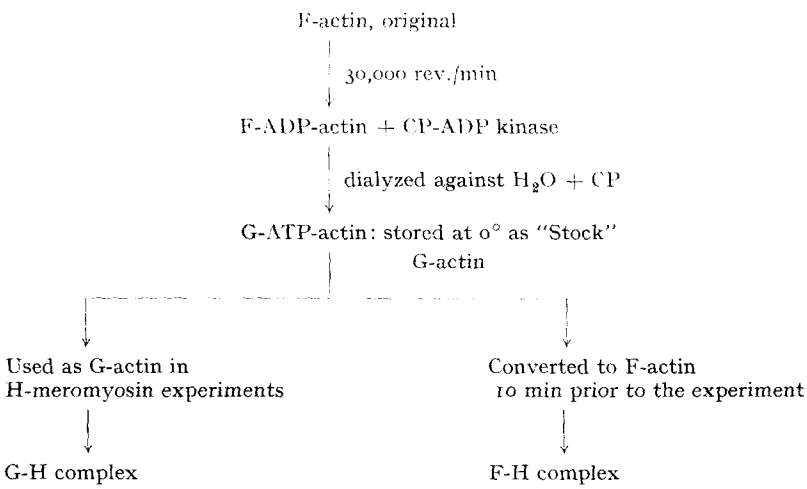

Fig. 3. Preparation of the G- and F-actin

fails to induce any significant rise in viscosity. The same amount of G-actin alone in the presence of these salts polymerizes rapidly and the failure of the viscosity response in the G–H system suggests that the G-actin has been complexed to the H-meromyosin and within the complex is incapable of polymerization. This effect and interpretation has been previously cited by A. SZENT-GYÖRGYI[21] for a G-actin-whole myosin system, but in the presence of 0.6 *M* KCl which by itself has an inhibitory effect on actin polymerization. Another explanation for the inability of the G-actin, in the G–H system, to polymerize would be that the H–meromyosin is converting the bound ATP to ADP. It has been shown by LAKI *et al.*[22] that the polymerization of G-ADP is seriously inhibited.

That a complex of G-actin and H-meromyosin is actually formed is also strongly indicated by the changes in the sedimentation pattern seen when these two proteins are mixed under conditions identical to those in the viscosity experiments. H-meromyosin sediments with an $S_{20} = 6$ or 7[13] and G-actin with an S_{20} = about 2[23]. When G-actin is mixed with the H-meromyosin a rapidly sedimenting peak is observed which is characteristic of a unit with a mol. wt. greater than either of the two components alone. This rapidly sedimenting fraction is assumed then to be the G–H complex. When the concentration of the H-meromyosin is held constant and the relative concentration of G-actin in the mixture is increased, then the fast moving peak is seen to increase in area while the H-meromyosin peak is correspondingly reduced. Such changes are best interpreted in terms of a formation of a G–H complex (Fig. 4).

II. Effect of complex formation on the ATP of G-actin. G-actin and H-meromyosin were mixed in the same relative concentrations used in the viscosity experiments and after 20 min incubation at room temperature, at which time there is no further change in viscosity, the nucleotide bound to the protein was separated out with perchloric acid at 0° and analyzed on the dowex columns. G-actin preparations in identical amounts used in the G–H mixtures were analyzed separately in parallel.

Table II shows the change in level of phosphorylation of the actin-bound ATP on incubation with H-meromyosin. In all preparations thus examined it is apparent

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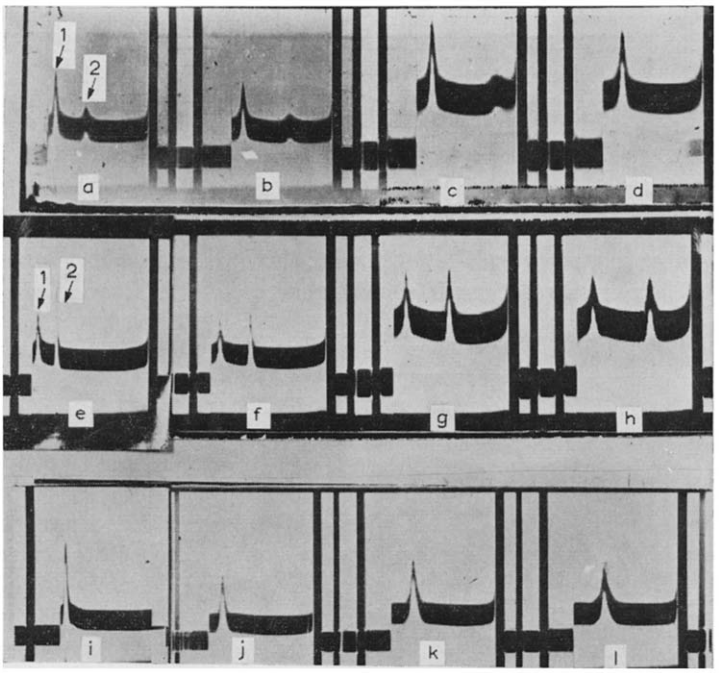

Fig. 4. (a)–(d): 4 mg/ml H-meromyosin + 0.5 mg/ml G-actin. Peak 1. = H-meromyosin; peak 2. = G–H complex. (a) Bar angle 60°, 5 min after reaching 59,700 rev./min; (b) Bar angle 60°, after 9 min; (c) Bar angle 40°, after 13 min; (d) Bar angle 40°, after 17 min.
(e)–(h): 4 mg/ml H-meromyosin + 1.0 mg/ml G-actin. Same time intervals and bar angles as above. 59,700 rev./min. The slower movement of the G–H complex (peak 2) results from the increased viscosity with increased actin addition.
(i)–(l): 6 mg/ml H-meromyosin only. Same time intervals as above. 59,700 rev./min. Bar angles of 65, 65, 60, 60.

TABLE II

BOUND NUCLEOTIDE CHANGE ACCOMPANYING G–H COMPLEX FORMATION

Prep.	*μmoles*	*G-Actin*	*G-Actin + H-meromyosin*
1	ATP	0.392	0.100
	ADP	0.140	0.430
2	ATP	0.350	0.110
	ADP	0.094	0.350
3	ATP	0.450	0.130
	ADP	0.094	0.480

that the formation of the G-H complex results in the dephosphorylation of the ATP of the actin, presumably under the influence of the H-meromyosin ATPase activity. The ADP of the F-actin remains unaffected by the formation of the F-H complex. It is thus possible to form complexes of H-meromyosin with both G- and F-actin, the two complexes being stable, by criteria of viscosity, at KCl concentrations up to 0.1 *M* KCl.

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$$\text{G-ATP} + \text{H} \longrightarrow \text{G-ADP-H}$$
$$\text{F-ADP} + \text{H} \longrightarrow \text{F-ADP-H}$$

The above reactions are depicted schematically and do not infer that complex formation is through the nucleotide center.

The nucleotide of actin as a center of phosphate turnover

Within both the G-H and the F-H complex the actin-bound ADP could possibly function as a center of phosphate turnover in the presence of a phosphate donor such as CP. The overall reactions in such systems would be, *schematically*:

$$\text{F-ADP-H} + \text{CP} \xrightarrow{\text{CP-kinase}} [\text{F-ATP-H}] + \text{creatine} \qquad (1)$$

$$\text{G-ADP-H} + \text{CP} \xrightarrow{\text{CP-kinase}} [\text{G-ATP-H}] + \text{creatine} \qquad (2)$$

$$\text{PO}_4\text{H}_2$$

and in the presence of excess CP, the liberation of free creatine from the CP would be a measure of the susceptibility of the bound nucleotide both to phosphorylation by CP and to dephosphorylation by the H-meromyosin, or actin-H-meromyosin ATPase activity. It has been demonstrated by CHAPPELL AND PERRY[18] that a system containing an ATPase, and CP + CP-kinase, can be used as an extremely sensitive assay for small amounts of either ADP or ATP, since either would function as a cofactor in liberating creatine from creatine-phosphate.

Since any differences which might be found between the G–H and the F–H systems with respect to the availability of the actin-bound nucleotide to interact with the phosphorylating system might be referred to denaturation or differences in handling of the actin component, it must be emphasized that the F-actin used in these experiments was derived from the stock G-actin immediately prior to the experiment by polymerization induced by 0.1 *M* KCl (Fig. 3).

In the G–H systems the final salt concentrations used were obtained after the 15 to 20 min incubation in a salt-free medium. At this time (Fig. 2) the addition of KCl and $MgSO_4$ does not induce any polymerization in the actin, and the G–H complex has been formed. Thus both G–H and F–H systems were compared under identical conditions. The only source of the enzyme CP-kinase was that carried over with the actin component (Fig. 3).

The results of these experiments are given in Fig. 5, 6, and 7. Similar to the behavior of the myofibrillar-bound ADP, the ADP of the F-actin in the F–H complex is unable to liberate significant amounts of creatine from a reaction mixture which rapidly liberates creatine from CP in the presence of added ADP or ATP. On the other hand, G–H systems of identical actin, and therefore nucleotide, content rapidly liberate creatine under conditions which exactly duplicate those of the F–H systems (Fig. 5).

The liberation of creatine by the G–H system is calcium activated (Fig. 5) and this activation is inhibited by equimolar magnesium (Fig. 6). It must be concluded that the antagonistic action of these divalent cations is on the H-meromyosin ATPase activity since the enzyme CP-kinase is activated by either calcium or magnesium[24].

The decrease in activity of the G–H system to liberate creatine with increasing

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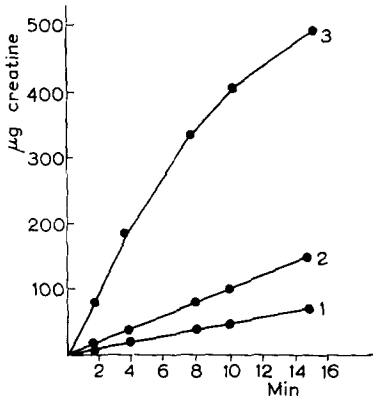

Fig. 5.

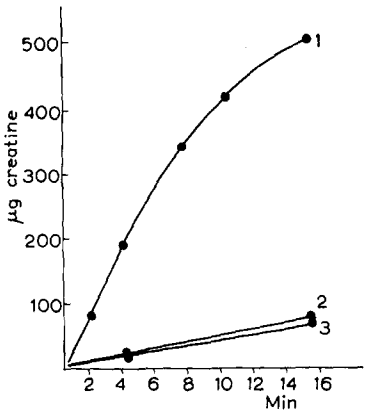

Fig. 6.

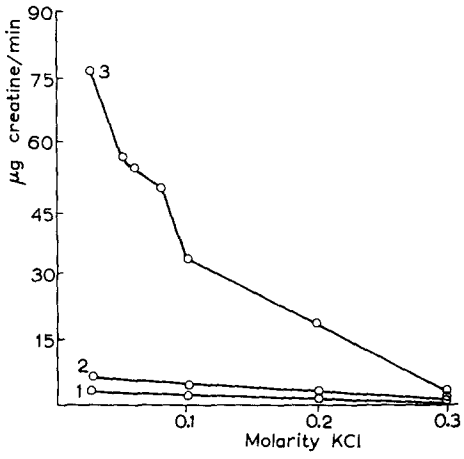

Fig. 7.

Fig. 5. Creatine release from G–H and F–H systems: Ca^{++} activation. 3 mg H-meromyosin, 0.6 mg actin, 0.1 *M* KCl, $5 \cdot 10^{-3}$ *M* Tris-malate buffer, pH 6.7, 10^{-3} *M* CP added at 0 time. (1) F-actin-H-meromyosin, 10^{-3} *M* $CaCl_2$. (2) G-actin–H-meromyosin, no calcium. (3) G-actin–H-meromyosin, 10^{-3} *M* $CaCl_2$.

Fig. 6. Creatine release: effect of Mg^{++}. 3 mg H-meromyosin, 0.6 mg actin, 0.1 *M* KCl, $5 \cdot 10^{-3}$ *M*. Tris-malate buffer, pH 6.7; 10^{-3} *M* CP added at 0 time. (1) G-actin-H-meromyosin, 10^{-3} *M* $CaCl_2$ (2) G-actin-H-meromyosin, 10^{-3} *M* $CaCl_2$ + 10^{-3} *M* $MgSO_4$. (3) F-actin-H-meromyosin, 10^{-3} *M* $CaCl_2$ + 10^{-3} *M* $MgSO_4$.

Fig. 7. Creatine release: effect of KCl. 3 mg H-meromyosin, 0.6 mg actin, $5 \cdot 10^{-3}$ Tris-malate buffer, pH6.7, 10^{-3} *M* CP. (1) F-actin–H-meromyosin, 10^{-3} *M* $CaCl_2$. (2) G-actin–H-meromyosin, 10^{-3} *M* $CaCl_2$ + 10^{-3} *M* $MgSO_4$. (3) G-actin-H-meromyosin, 10^{-3} *M* $CaCl_2$ only.

KCl concentrations is indicated in Fig. 7. While there are as yet no available data on the effect of high KCl concentrations on the association of G-actin with myosin or H-meromyosin, it is well known that such concentrations of KCl favor the dissociation of F-actomyosin. The decrease in activity may therefore simply reflect the requirement of complex formation for the reaction. Under all conditions investigated the F–H systems failed to liberate significant amounts of creatine from the reaction mixture.

DISCUSSION

All previously published methods for the purification of actin are based on the separation of the polymerized or F-actin from solution either by isolectric precipitation[25], by precipitation by divalent cations[26], or by centrifugation at high speeds[14]. The actin, once separated, is usually redissolved and depolymerized in the presence of exogenous ATP which is required for repolymerization and further purification. Depolymerization of F-actin in the absence of ATP has resulted in loss of activity of the protein[3].

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It is this requirement for exogenous ATP during depolymerization which has led to the divergent results reported from previous studies on the changes in level of phosphorylation of the bound nucleotide of the actin during changes in state of polymerization[3-6,27]. The reversible depolymerization of actin by dialysis against CP, as described in this paper, completely obviates the requirement for any added nucleotide during the preparation. The main advantage of such a preparation is that it permits a direct evaluation of the changes in the specifically bound nucleotide of the actin, uncomplicated by the presence of exogenous ATP, both in actin–actin and in actin-myosin interaction.

The results of this experimental survey are quite clear:

1. If the actin is maintained in the polymerized state (0.1 *M* KCl), then the bound ADP is not capable of interaction with the phosphorylating system CP + CP-kinase.

2. At some point during the depolymerization process, the bound ADP becomes susceptible to phosphorylation by the CP system:

$$\text{F-ADP-actin} \xrightarrow{0.1\ M\ \text{KCl} + \text{CP}} \text{F-ADP-actin} + \text{CP} \qquad \text{(i)}$$

$$\text{F-ADP-actin} \xrightarrow[\text{strength} + \text{CP}]{\text{lowering ionic}} \text{G-ATP-actin} + \text{creatine} \qquad \text{(ii)}$$

3. The depolymerization is completely reversible. The G-ATP-actin formed above (ii) repolymerizes immediately on the addition of appropriate salts, with the parallel dephosphorylation of the bound ATP to ADP.

$$\text{G-ATP-actin} \xrightarrow[\text{MgSO}_4]{0.1\ M\ \text{KCl} + 0.001\ M} \text{F-ADP-actin}$$

4. The transphosphorylation of the bound ADP takes place whilst the nucleotide remains bound to the protein. If phosphorylation took place after the nucleotide had been dissociated from the actin, then an appreciable loss of nucleotide would be expected to occur through the large volume of the dialysis fluid. In fact, the total amount of nucleotide before and after the dialysis remained essentially unchanged.

Of the above conclusions, point 4 seems to require some modification. It is well known from original work of STRAUB AND FEUER[3] that F-actin is reversibly depolymerized in the presence of free ATP. This depolymerization mechanism has been interpreted by STRAUB AND FEUER as an exchange reaction between the bound ADP and the ATP in solution during depolymerization. Such an interpretation based on a dissociation equilibrium between nucleotide and protein would seem incompatible with the above results on the CP depolymerization. It must be considered therefore that the binding constants of ADP and ATP to the actin differ at different stages of depolymerization. Thus, in an actin preparation which has undergone depolymerization to some small extent and is still ADP-actin (stage 2 below), the protein–nucleotide complex might be unstable, and in the presence of free ATP, an exchange equilibrium might well provide the mechanism for the formation of the G-ATP-actin. In the presence of a phosphorylating system such as CP, CP-kinase however, transphosphorylation would occur at the critical stage of the depolymerization (stage 1 below), giving rise to a stable actin–ATP complex. In this latter case the dissociation equilibrium between nucleotide and protein would strongly favor complex formation. The two possible mechanisms could be represented *schematically* as follows:

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$$\text{F-ADP} \longrightarrow \underset{\text{(stage 1)}}{\text{X-ADP}} \longrightarrow [\text{X-ADP} \rightleftharpoons \underset{\text{(stage 2)}}{\text{X}} + \text{ADP}] + \text{ATP} \longrightarrow \text{G-ATP}$$

$$\text{X-ADP} \xrightarrow{+\text{CP}} \text{X-ATP} \longrightarrow \text{G-ATP}$$

The experiments reported here do not allow a decision to be made concerning the mechanism but we are at present studying the reversible depolymerization of F-actin using [^{14}C]ATP both in the presence and absence of the phosphorylating system CP + CP-kinase. It is hoped that this kind of *in vitro* approach will also yield a methodology for an investigation of the behavior of the actin component *in situ* or *in vivo*.

In either case the above results indicate quite clearly that enzymic phosphorylation of the actin-bound nucleotide can take place, and furthermore, that it is the state of the actin polymerization which in some way regulates or directs the susceptibility of the nucleotide to such phosphorylation. The inability of the CP system to phosphorylate the ADP of F-actin is strongly reminiscent of the behavior of the myofibrillar-ADP as studied by PERRY[8]. It seems clear however that if a depolymerization of the actin is involved at any stage of a contraction cycle, then the ability of the nucleotide center of the actin to act as a center of phosphate interaction would be strikingly changed.

The ability of the nucleotide center of actin, within the actin–H-meromyosin complex, to function as a center of phosphate turnover seems to be again a strict function of the state of polymerization of the actin component. Thus an F–H complex fails to interact with the phosphorylating system employed, while G–H complexes of identical actin, and therefore nucleotide, content display large activities in this respect. The activation by calcium and inhibition by magnesium of this phosphate turnover reaction also seems significant in light of the profound effects of these divalent cations on both the ATPase and ability of muscle models to contract or relax. The possibility that actin depolymerization *in vivo* might function as a control mechanism for phosphate turnover at sites directly available to the enzymic centers of myosin—"built-in nucleotide centers" as envisioned by PERRY[10]—makes a further and detailed study of G-actin interaction with both myosin and H-meromyosin imperative.

These experiments merely suggest a specific functional behavior for the actin in a normal contraction situation. That actin participates in some way in contraction has been assumed for some time, notwithstanding the report of KAFIANI AND ENGELHARDT[28] that actin-free myosin fibers prepared according to HAYASHI[29] are intrinsically contractile. The recent work of HAYASHI *et al.*[30] has confirmed, however, the necessity for actin in contractile systems built from the surface-spread fiber. These workers have shown that actin-free myosin fibers are not contractile in the presence of ATP under a variety of conditions. As the relative concentration of actin in the actomyosin fiber is increased, the tension induced by the addition of ATP increased proportionally. The need for some type of actin–myosin interaction in model contraction seems apparent. The nature of that interaction remains unknown.

The importance of a further study of actin-H-meromyosin interaction is increased immeasurably by the recent findings of MARSHALL AND HOLTZER[31] and of INOUE[32]. They have reached the conclusion, from somewhat different experimental approaches

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that H-meromyosin and L-meromyosin are located in separate bands within the sarcomere of the isolated myofibril. The possibility that actin–H-meromyosin interaction occurs *in situ* must therefore be entertained more seriously.

Finally, it must be mentioned that if the ATP splitting which has been assumed to take place during contraction, takes place through a *cyclic* dephosphorylation of the actin-bound nucleotide, no free ADP then being liberated, then no immediate decrease in sarcoplasmic ATP would be expected to occur during a single twitch. Indeed, in some recent cases, where ATP hydrolysis has been investigated during the single twitch, it has not been found[33–35] (see however[36]).

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I would like to dedicate this simple effort to the memory of my late wife, JUNE MARGARET STROHMAN, whose love, understanding and magnificent determination was always a constant source of inspiration.

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EFFECTS OF CHLORAMPHENICOL ON RIBONUCLEIC ACID METABOLISM IN T2-INFECTED *ESCHERICHIA COLI*

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SUMMARY

The effect of chloramphenicol on RNA turnover in T2-infected *E. coli* was studied with the aid of ^{32}P-labeled orthophosphate. When chloramphenicol is added *before* infection, the subsequent distribution of radioactivity among RNA mononucleotides is different from the distribution found in control infected cells and resembles the distribution found in uninfected cells. When chloramphenicol is added *after* infection, the ^{32}P distribution in RNA is that typical of infected cells.

If the infected cells are inhibited before or *shortly* after infection, RNA turnover and DNA synthesis are inhibited. When chloramphenicol is added 9 min after infection, conditions that allow DNA synthesis, the rate of ^{32}P incorporation into RNA is increased and turnover of RNA now occurs in the presence of chloramphenicol. Thus RNA turnover is blocked under conditions that prevent DNA synthesis, but RNA turnover is not inhibited when DNA synthesis can proceed.

These observations are in agreement with the concept that some protein synthesis must precede or accompany formation of an RNA peculiar to phage-infected bacteria. It is also suggested that in the presence of inhibitor, RNA is synthesized by reversible reactions from precursor material also used for DNA synthesis.

INTRODUCTION

The ability of chloramphenicol (CP) to inhibit bacterial protein synthesis is well documented[1,2]. Chloramphenicol may also inhibit deoxyribonucleic acid (DNA) synthesis in bacteria infected with certain bacteriophages[3]. If chloramphenicol is added shortly after infection, complete inhibition of DNA synthesis is observed. With increased delay between infection and time of chloramphenicol addition, DNA syn-

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