THE EXTRACTION, PURIFICATION AND SOME CHEMICAL PROPERTIES OF ACTIN

by

T.-C. TSAO AND K. BAILEY

Biochemical Laboratory, Cambridge (England)

Most of the physico-chemical studies on actin have been made on preparations based on the original or modified procedures of STRAUB (STRAUB[1,2]; FEUER, MOLNÁR, PETTKÓ AND STRAUB[3]: GUBA AND SZENT-GYÖRGYI in SZENT-GYÖRGYI[4]). More recent work of WEBER[5], SPICER AND GERGELY[6], MOMMAERTS[7] and A. G. SZENT-GYÖRGYI[8] has shown, however, that these preparations may contain as much as 40–60% impurity, though admittedly much of this is inactive actin. The purification of actin is rendered difficult by the tendency to become inactive, *i.e.*, to lose the ability of forming F-actin. Since the demonstration by STRAUB AND FEUER[9] and LAKI, BOWEN AND CLARK[10] that the prosthetic group of actin is a nucleotide and that its integrity is essential for the stability of the protein, it has been possible to protect actin (by the addition of adenosine triphosphate) during preparation. This protection has assisted in the purification of actin by two independent methods, one an ultracentrifugal procedure of MOMMAERTS[7], and the other the potassium iodide procedure of A. G. SZENT-GYÖRGYI[8]. The present investigation arose from a similar attempt at purification, with a view to obtaining preparations for the characterisation of the molecular dimensions of actin. A new modification of STRAUB's procedures has been developed to yield an electrophoretically homogeneous preparation of actin.

In this paper, the criterion of purity will first be discussed followed by some preliminary observations on the impurities and the extraction of actin which led to the introduction of modifications and which may be of some interest in the understanding of the properties of actin in relation to the structure of muscle. Finally, the procedures for the preparation of an electrophoretically homogeneous solution of actin will be described. The results of physico-chemical studies of the size, shape and aggregation of actin and of the interaction of actin, myosin and adenosine triphosphate will be given in the accompanying papers[11,12].

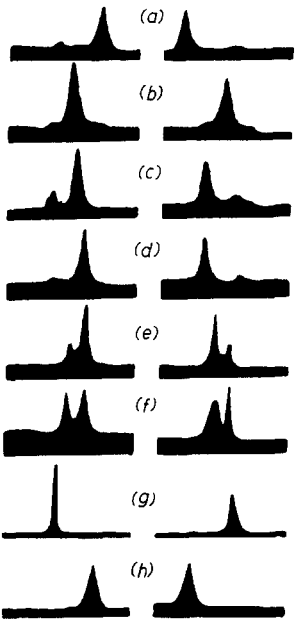
EXTRACTION AND PURIFICATION

Electrophoresis and criterion of purity

Electrophoresis was chosen to test the physico-chemical homogeneity of actin preparations. Because of the necessity of using a buffer system of considerable ionic strength for such experiments, it was necessary to employ depolymerising media to prevent the formation of the F-form. Earlier experiments with F-actin showed that the Schlieren diagrams consist of some 20 or more indefinite peaks (see also SPICER AND GERGELY[6]), that the ascending and descending peaks do not exactly correspond and that two experiments under identical conditions do not yield identical diagrams. It was possible to generate an indefinite number of peaks by the very careful mechanical displacement

References p. 113.

of the boundaries without the application of electric field. These phenomena are consequent upon the fact that the system is thixotropic. It is possible to reduce these anomalies by diminishing the concentration of the protein, but the gel structure of F-actin persists down to concentrations very much lower than 0.05% and could easily be seen when an apparent solution was allowed to flow down the wall of a test tube. The point of view that all the physical properties of the F-actin system are due to structures in solution rather than to filamentous polymers will be discussed in another paper[12]. It is sufficient to mention here that the number of peaks in the Schlieren diagram of F-actin appears in no way to be a guide to the true number of different protein components, nor of species of F-actin fibrils of different degree of polymerisation (SPICER AND GERGELY[6], DUBUISSON[13]).



In the present investigation, the aggregating influence of the salt ions was countered by the disaggregating effect of dilute alkali (pH 10) or acid (pH 2). Under these conditions, the electrophoretic patterns of actin were found to be extremely simple and definite (Fig. 1a, b) and tallying well with information obtained from fractionation.

Fig. 1. Electrophoretic diagrams of actin preparations:
a. prepared according to FEUER *et al.*[3], in 0.075 *M* glycine, 0.1 *M* KCl (titrated with HCl until pH reached 1.3); overall voltage 28 V, current 18.7 mA, 6 hours.
b. same as (a), in 0.1 *M* H_3BO_3, 0.1 *N* NaOH, 0.1 *M* KCl, pH 10; 48 V, 14 mA, $6^3/_4$ hours.
c. same as (a), plus tropomyosin.
d, e, f. prepared by butanol-acetone procedure, muscle mince pretreated at pH 6.5, 8 and 9 respectively. In 0.1 *M* glycine, 0.1 *M* KCl (titrated with HCl until pH reached 2).
g. prepared by butanol-acetone procedure at pH 7, using 30% acetone as extractant. In 0.1 *M* glycine, 0.067 *N* HCl, 0.1 *M* KCl, pH 2.3, 43 V, 14 mA, 6 hours.
h. same as (g), in 0.1 *M* H_3BO_3, 0.1 *N* NaOH, 0.1 *M* KCl, pH 10, 48 V, 14 mA, 4 hours.

Impurities in actin preparations obtained from the acetone procedure

Current estimates of the amount of impurities in actin, 40–60%, have been based upon the electrophoresis, sedimentation and the measurement of viscosity of F-actin, or from the determination of the ability of the sample to form the acto-myosin complex, a property which ultimately rests also on viscometry. While these criteria offer convenient means for the estimation of purity, the use of all these as strictly quantitative measures is subject to the uncertainty that one is here dealing with thixotropic gel systems, the quantitative behaviour of which are as yet very imperfectly known. It is probable that all these estimates are likely to be much too high (as indeed borne out partially by the observation of MOMMAERTS[7] that, even after purification, a portion of the protein does not sediment under the standard conditions).

The qualitative aspect of the nature of the impurities, however, is known with greater certainty, and the presence of enzymes, lipids, colouring material and inactive actin has been described. It will be seen from the present work that the chief protein impurity in actin solutions is tropomyosin, and there is evidence that a flavo-protein is also present. These impurities will be discussed below:

Enzymes. The activities of myokinase (LAKI AND CLARK[14]) and creatine phosphokinase (LJUBIMOVA AND POPOVA[15]) have been detected in actin preparations. The work of HOPKINS, MORGAN AND LUTWAK-MANN[16] has shown that after exhaustive extraction with salt solutions, rabbit muscle still contained dehydrogenases (succinic, glycerophosphate, and malic) in high concentration. Of these the succinic and glycerophosphate dehydrogenases have been released from the muscle fibril by treatment with organic solvent, *i.e.* butanol, and obtained in a water-soluble form. The possibility of the presence of these or similar enzymes in actin preparations has so far not been investigated.

Lipids. Aqueous solutions of actin are usually more or less cloudy resulting from the presence of lipids which are not removed by the acetone treatment. These could be reduced by repeated extraction of the dried muscle with acetone in a Soxhlet apparatus (FEUER *et al.*[3]) or by extraction with chloroform (BAILEY AND PERRY[17]).

Colouring matter and flavo-protein. Freeze-dried or concentrated aqueous solutions of actin are faintly yellow. This was found to be especially pronounced when an alkaline solution of actin was left standing in the air. When a concentrated solution of STRAUB's actin (made by dissolving the freeze-dried protein in *N* NaOH) was examined in the Beckmann spectrophotometer, the spectrum

References p. 113.

showed a strong, sharp maximum at 310 mμ and an extremely weak, broad maximum near 430 mμ (Fig. 2). After the solution was deproteinized by boiling with an equal volume of 10% trichloracetic acid, and the pH adjusted to 7, an approximately 10 fold reduction in light absorption intensity was observed, and the 310 mμ maximum was shifted to 330, whilst the 430 mμ maximum became more accentuated. In addition, there was a faint suggestion of a maximum at 360 mμ. The concentrated solution in *N* NaOH gave a weak blue fluorescence which became greenish and more intense when the solution was neutralised. The fluorescent light was strongly polarised, but after deproteinisation it was completely depolarised, indicating that the fluorochrome was originally in a bound form.

These observations are still preliminary. Pending further qualitative and quantitative characterisation, it seems that the 360 mμ and 430 mμ maxima, the green, polarised fluorescence, and, furthermore, the non-dialysability are probably indications of the presence of flavo-protein (one possibility is diaphorase). The 310 mμ maximum probably arises from carotenoid material in muscle. On shaking with ether, however, the yellow pigment remained in the aqueous layer and this, together with the mode of preparation of actin and the non-dialysability, suggests that the carotenoid material exists in a strongly bound form. It is not known whether the flavin and carotenoid groups are linked to the same or several protein moieties.

Tropomyosin. The nature of the muscle stroma, left over from extraction with strong salt solutions, is very imperfectly known. The existence of tropomyosin could be easily demonstrated by extraction with *M* KCl following the procedure of BAILEY[18] for ethanol-ether dried muscle fibre. As tropomyosin is water soluble, it may be expected that during the extraction of actin, some tropomyosin may also escape into solution. This was indeed found to be the case.

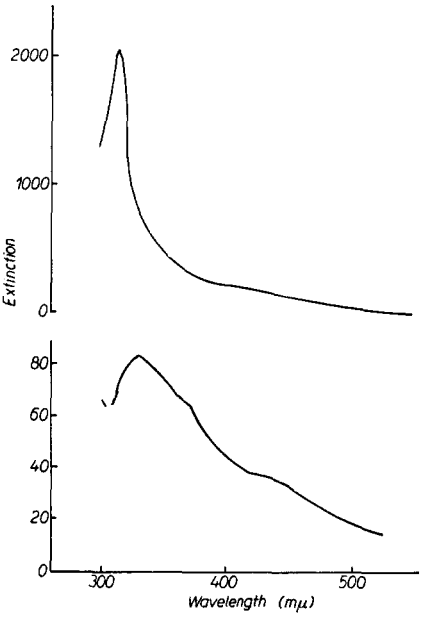


Fig. 2. Absorption spectra of a concentrated solution of Straub's actin:
upper curve: original solution
lower curve: after deproteinisation

Actin was extracted in the usual way from debris prepared by the acetone procedure. Solid ammonium sulphate was added to the extract until the ionic strength of the solution was about 1. The pH was then brought to 4.7. After half an hour, the precipitates were collected by centrifugation, and subjected to the usual salting out procedures for the preparation of tropomyosin. The products were identified as tropomyosin by crystallisation. From 400 ml of extract (concentration ~0.6%), 47 mg of tropomyosin were obtained representing about 2% yield. This is necessarily a minimum estimate.

STRAUB's earlier observation[2] that the grinding of the muscle powder before extraction, stirring during extraction or extraction over periods longer than 15–20 minutes resulted in partial polymerisation of actin as manifested in the increased viscosity of the extract can readily be interpreted by the alternative explanation of the increased extraction of tropomyosin under the above conditions, an interpretation borne out by electrophoresis or actual isolation.

The correlation of the impurities with the electrophoretic diagrams. The above survey does not cover inactive actin. Current views seem to be that the inactivation of actin as normally encountered results from changes in the prosthetic group or other minor changes in the molecules (STRAUB AND FEUER[9]). At pH 2 or 10, actin is inactivated so that the electrophoretic patterns (Fig. 1a,b) would not differentiate between molecules that were originally inactivated and those inactivated by acidity or alkalinity. However, for the purpose of testing homogeneity for the study of molecular dimensions, such a differentiation is not necessary. Earlier experiments with the electrophoresis of actin prepared under various conditions showed that the main peak of the electrophoretic diagram arose from actin. At pH 2 there are two fast-moving peaks. If tropomyosin was added to the solution, the area of the second one was enhanced (Fig. 1c). At pH 10,

References p. 113.

the electrophoresis of tropomyosin-rich actin gave a diagram relatively rich in the slow moving component.

It is possible to effect fractionation at 0 – —5° C and pH 9, ionic strength 0.02–0.08, with increasing amounts of acetone; tropomyosin is precipitated first, followed by actin in its inactive form, and finally a yellowish fraction containing all the carotenoid and flavin materials. By a process of elimination, these are therefore likely to be the components which give rise to the fast moving peaks at pH 2 and 10.

It is probable that the enzymes are present only in traces, and consequently may not show up in the electrophoretic patterns. In the purification of actin for the purpose of studying molecular dimensions, these impurities have therefore been neglected. The problem of purification then resolves itself into the elimination of lipids, tropomyosin and flavin and carotenoid materials under conditions where inactivation of actin is minimal. Judging from the electrophoretic patterns, the non-actin impurities do not seem to amount more than 10%.

Effect of pH and organic solvent treatment on the extraction of actin

The essential steps in the classical procedure worked out by STRAUB and his collaborators involve the selective removal of myosin, treatment of the residue with an alkaline medium followed by drying with acetone. The recent work of A. G. SZENT-GYÖRGYI[8] and HASSELBACH AND SCHNEIDER[19] have demonstrated that the use of alkali and organic solvents is not essential for the liberation of actin, and the present work was guided by the idea that the release of actin might nevertheless necessitate the breakdown of lipo-protein complexes existing in the insoluble muscle after myosin extraction. A number of organic solvents have therefore been used under various conditions of pH and ionic strength with a view to liberating actin with minimum inactivation and maximum yield and purity. While this aim has in most cases not been attained, the use of butanol gave rise to some quite encouraging results. The effectiveness of butanol in the fission of lipo-protein complexes has already been demonstrated by MORTON[20] in the case of many enzyme systems.

If the procedures of STRAUB and his collaborators were followed as far as the washing with 0.4% $NaHCO_3$ and the muscle residue was washed twice for 15 minutes each with 10 vol of distilled water, macerated for 1 minute in the Waring blender with 3 vol of butanol, allowed to stand for half an hour and finally washed thoroughly with 3–4 changes of acetone, the resulting air-dried powder was distinctly different from that obtained without the butanol treatment. All the lipid material was liberated from the muscle fibril by butanol and came out with butanol or with the first acetone washing, from which it gradually separated out on standing as a white precipitate. Butanol and the first acetone washing also contained all the colouring matter. The aqueous extracts made from the dried powder therefore contained little lipid, carotenoid and flavin impurities. Such an extract was slightly viscous, and showed an initial drop in viscosity on the addition of neutral salts. It seemed that from this, and from the electrophoretic evidence (Fig. 1), that this was due to the extraction of more tropomyosin.

If the 0.4% $NaHCO_3$-washed residue was treated with acetate, glycine, phosphate, and bicarbonate buffers of pH from 5.5 to 10, washed with water, treated with butanol-acetone and then extracted in the usual way, the total yield of protein was increased with rising pH. There was also a gradual increase in the proportion of tropomyosin in the extract (Fig. 1d,e,f). Acetone alone produced only the first effect. The actins

References p. 113.

obtained from butanol-acetone treatment and from acetone alone showed different courses of "polymerisation" in the presence of neutral salts (Fig. 3). It would seem that in the presence of ~30% tropomyosin, the catalytic effect of Mg^{++} and inhibitory effect of Ca^{++} on the polymerisation of actin[3] were no more evident. It is not known as yet

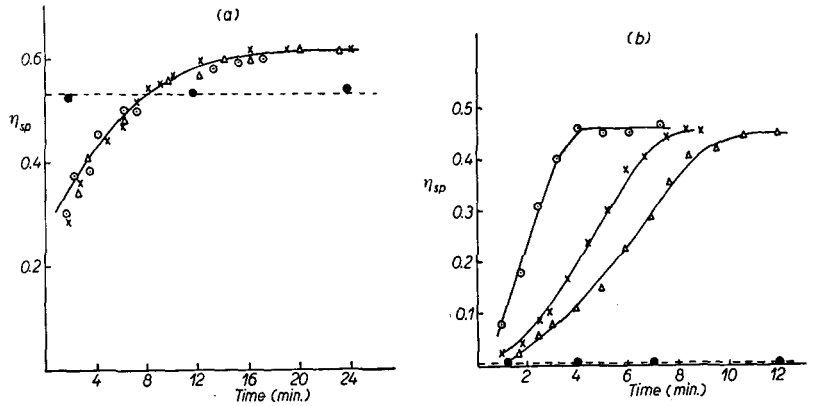


Fig. 3. Effect of neutral salts on the "polymerisation" of actin at pH 7.
a. actin prepared from butanol-acetone treatment, pH 9.
b. actin prepared from acetone treatment, pH 9.
------ viscosity behaviour in absence of neutral salts.
——— viscosity behaviour in presence of neutral salts.
"Polymerisation" induced by 0.1 *M* KCl (×), 0.1 *M* KCl + 0.001 *M* $MgSO_4$ (◉), or 0.1 *M* KCl + 0.001 *M* $CaCl_2$ (△).

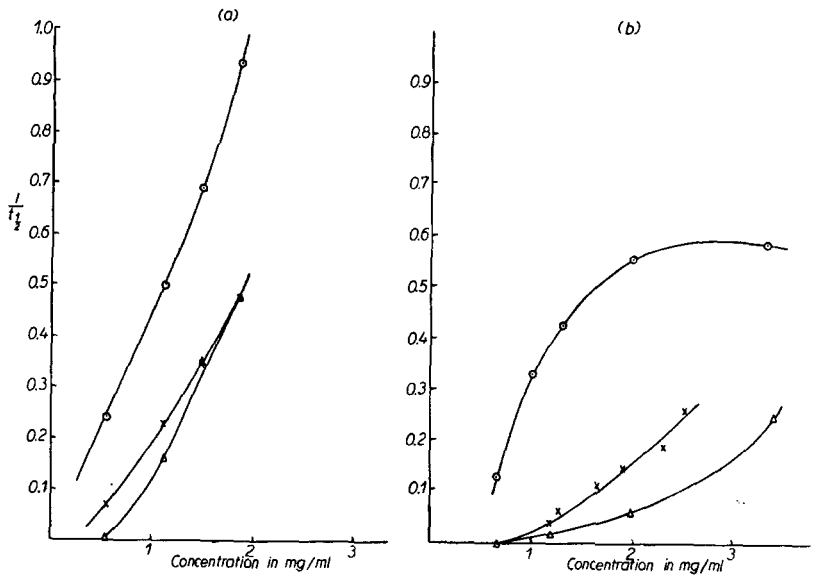


Fig. 4. Influence of protein concentration and neutral salts on the rate of "polymerisation" of actin at pH 7.
a. actin prepared from butanol-acetone treatment, pH 9.
b. actin prepared according to FEUER *et al.*[3]
Symbols for the "polymerisation" by salts same as in Fig. 3; ordinate: reciprocal of the half-time value (FEUER *et al.*[3]).

References p. 113.

whether the influence of tropomyosin on the course of polymerisation is due to specific interactions or to general electrostatic effects.

It may be appropriate here to discuss further the polymerisation behaviour of actin obtained from muscle residue treated at different pH's and dried with acetone. The influence of actin concentration, 0.1 *M* KCl, 0.001 *M* Mg^{++} or Ca^{++} on the rate of polymerisation at pH 7 is shown in Fig. 4. In this figure, (a) represents a preparation with previous treatment at pH 9 and (b) that by the procedures of FEUER *et al.*[3], with previous treatment at pH 10 (0.05 *M* Na_2CO_3, 0.05 *M* $NaHCO_3$). The general feature of the rate-concentration curves for preparations with pretreatments at pH 7 and 8 are identical with that at pH 9. In Fig. 4(b), it seems that (1) the occurrence of a threshold concentration of G-actin (0.05%) below which no polymerisation took place and (2) the comparatively slow rate of polymerisation with K^+, $K^+ + Mg^{++}$ or $K^+ + Ca^{++}$, are probably indications, comparing with Fig. 4(a), of the presence of more denatured actin in the preparation of FEUER *et al.*

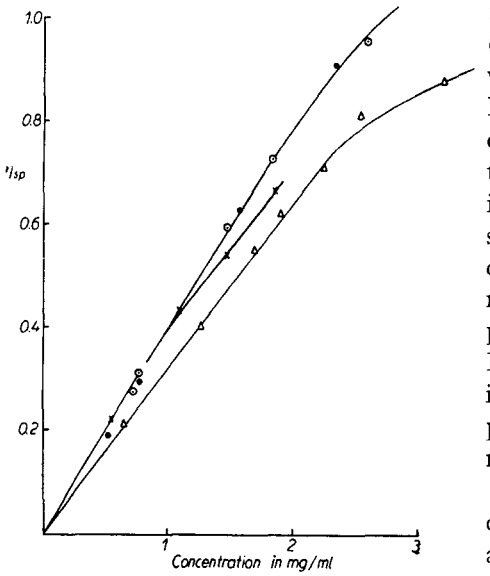


Fig. 5. "Specific Viscosity" of F-actin.
—△— prepared according to FEUER *et al.*[3]
—×— prepared by acetone treatment, pH 9.
—⊙— prepared by acetone treatment, pH 6.3.
—●— homogeneous actin by the present procedure.
"Specific viscosity" measured under conditions described under Table I.

The same conclusions can be reached if one compares the "specific viscosity" (Fig. 5) and actomyosin-forming ability (Table I) of actin prepared from residues pretreated at different pHs. When the pH of pretreatment exceeded 9, inactivation of the extracted actin occurs to a considerable extent.

The foregoing results on the effect of pH and of butanol and acetone treatment on the extraction of actin and tropomyosin form the basis of the following procedure for the preparation of pure actin.

TABLE I

ACTOMYOSIN-FORMING ABILITY OF ACTIN

Preparation	*pH of pretreatment*	η_r *A+M*	η_r *A+M+ATP**	*ATP*** *sensitivity*
FEUER *et al.*[3]	10	1.85	1.32	1.21
Acetone	8	1.94	1.32	1.38
Acetone	6	1.98	1.32	1.45
Butanol-acetone	6	1.96	1.32	1.42
Butanol-acetone (homogeneous)	7	2.02	1.34	1.40

* The conditions for the test are as follows: myosin 1.50 mg/ml, actin 0.50 mg/ml, Na ATP 0.0008 *M*, phosphate buffer of pH 7 0.01 *M*, KCl 0.5 *M*; 22.0° C; Viscosity measured with TSUDA[22] viscometer; capillary diameter 0.059 cm, length 29.4 cm, pressure head 67.2 cm H_2O.

** Defined (WEBER[5]) as $\frac{\log \eta_r - \log \eta_{r\ ATP}}{\log \eta_{r\ ATP}} \times 100$

Butanol-acetone procedure

The initial steps follow closely those of STRAUB's procedure. The distinct features lie in the use of (1) neutral pH (6.5–7) throughout the preparation to diminish the yield of inactive actin, (2) butanol to remove thoroughly the phospholipids and (3) 30% acetone instead of water as extractant to suppress the extraction of tropomyosin.

Hind leg and back muscles of the rabbit are coarsely minced into three volumes of an ice cold acid phosphate-KCl solution of GUBA AND STRAUB[1]* and extracted for 10 minutes under constant, gentle stirring. The suspension is diluted with water, 4 vol for every vol of the salt solution employed. The solution is then strained through a cloth and the residue washed first with 4 vol of a 0.4% $NaHCO_3$ solution (pH of final suspension 7.0) and then with 10–15 vol of distilled water. Each washing lasts for 20–30 minutes under constant stirring at room temperature. The residue is pressed in a cloth as dry as possible, and is macerated for one minute in the Waring blender with 5 vol of *n*-butanol which has been precooled so that the temperature never rises above 20° C during maceration. After standing for ½ hour, butanol is strained out through a cloth and the residue washed with 2–3 vol of acetone, and again strained out. The lumps of debris are disintegrated in the Waring blender with acetone for a few seconds and strained out, followed by another two washings with acetone. The powder is air-dried overnight.

The following operations are carried out in the cold room with all reagents at 0° C.

Every 10 g of the air-dried fibre are extracted with 200–300 ml of 30% (v/v) A.R. acetone containing 40 μg each of Na ATP and neutralised ascorbic acid per ml. After extraction for 30 minutes with constant slow stirring, the liquor is strained through a cloth onto a Buchner funnel containing a thin layer of paper pulp (washed with 30% acetone) which removes fine debris. Molar acetate buffer (0.5 ml, pH 4.65) is added to the clear filtrate and the precipitate spun down. A few drops of 5% $NaHCO_3$ are added to the precipitate with stirring which transforms into a transparent and strongly thixotropic gel. This is dialysed against several changes of 0.1 *M* KCl containing 40 μg each of ATP and ascorbic acid per ml to remove acetone. If instead the gel is dialysed against distilled water (also containing ATP and ascorbic acid) F-actin is quickly and reversibly depolymerised into the "globular" form.

The total yield of actin is comparable to that in the procedure of FEUER *et al.* if the final extraction is carried out with gentle mechanical stirring, or about 1/3 to 1/2 of that amount without.

The electrophoretic diagrams at pH 2 and 10 are given in Fig. 1g,h, the "specific viscosity" included in Fig. 5, and the actomyosin-forming ability in Table I. The product appears to be electrophoretically homogeneous with maximal activity.

SOME CHEMICAL PROPERTIES

Sulphydryl groups

In the initial experiments a method suggested by Mr M. W. Rees was explored for the colorimetric determination of SH groups by a quantitative nitroprusside (NP) reaction. This was eventually discarded because of irregularities traced to the sensitivity of the colour to slight variations in pH. It was discovered, however, that given a fairly stable NP colour, an SH reagent such as N-ethylmaleimide (NEM) (FRIEDMANN,

* 0.3 *M* KCl, 0.15 *M* potassium phosphate pH 6.5.

References p. 113.

MARRIAN AND SIMON-REUSS[23]) could be used to "titrate away" the colour, and under the conditions adopted, standard solutions of pure NEM and pure glutathione (GSH) titrate each other exactly. The use of NEM was suggested by the pioneer studies of FRIEDMANN *et al.* (1949) who showed that it reacts instantaneously with GSH to give a saturated adduct, and that it is antimitotic in concentrations of 10^{-6}.

Reagents. (a) Saturated guanidine HCl containing 0.1 *M*-acetate buffer pH 4.6: this reagent is purified by twice crystallizing the carbonate in presence of a little versenate from hot 50–60% (v/v) ethanol, converting to the chloride and recrystallizing from water. (b) Freshly prepared 0.1 *M* KCN. (c) LUGG's[24] (1933) glycine citrate buffer (7.5 g glycine, 11.8 g tertiary Na citrate (2 H_2O), 25 ml *N* NaOH diluted to 100 ml (pH 9.7). (d) Freshly prepared 10% (w/v) Na nitroprusside. (e) Standard glutathione (0.0033 *M*) and standard NEM (0.002 *M*). The latter is readily synthesized by the method of MARRIAN[25] and is purified by sublimation: it should be entirely water-soluble and neutral.

Method. The GSH standard or protein solution in a final volume of 0.5 ml is mixed with 3.35 ml of the guanidine solution and thoroughly stirred with a fine rod. Citrate buffer (1 ml) is gently run in to float on top of the guanidine, and to this is added in order 0.1 ml of cyanide which helps to stabilize the colour and 0.05 ml nitroprusside. The contents are mixed and NEM is added from a microburette until the colour fades through a rose-pink to a pure yellow. The first titration should be rapid and approximate, and in the second, NEM can be added after all the reagents but before mixing so as to give a faint NP reaction on which the observer can concentrate.

The accuracy of the method is checked from time to time with fresh GSH: for example, 0.00326 *M* GSH by weight was found to be 0.00326 *M* by titration, and for another solution the corresponding figures were: 0.00266 and 0.00263 respectively. The routine amount of SH (as cysteine) adopted for each titre is 0.06–0.12 mg and for a protein such as myosin containing approx. 1% CSH, about 10 mg. A pink colour is discernible with only 3 μg CSH and the end point is accurate to about 5%.

An interesting feature of the method is the possibility of determining the extent of reaction of a native protein with various types of SH reagent, including NEM itself. After treatment with the reagent, the protein is precipitated by the minimal amount of trichloroacetic acid (TCA), washed once with 1% TCA and dissolved in the guanidine. The number of SH groups reacting is then calculated by the difference in titre of the untreated native protein and the treated. The procedure is best described by an actual example: An aliquot sample of an actin solution (4 ml), precipitated with TCA, dissolved and titrated in guanidine required 0.46 ml of 0.00266 NEM. Similar samples, in presence of phosphate buffer pH 7.4, were allowed to react for 20 min at room temperature with 0.5, 0.75 and 1.0 ml NEM. These were precipitated with TCA, washed with TCA, dissolved in guanidine and titrated. The respective titres were 0.26, 0.28, 0.26 ml of NEM, showing that the extent of reaction is constant even with different amounts of excess reagent. The reactivity towards NEM is thus 44%.

SH content of actin and myosin. The cysteine content of very pure four-times crystallized ovalbumin by our new method gives a value of 1.3%, in good agreement with the accepted values of 1.24–1.29% obtained by other types of method[26].

In the literature, the total cysteine content of (acto-) myosin lies within the range 0.65–1.15%, the higher values being obtained in guanidine by porphyrindin titration (GREENSTEIN AND EDSALL[27]). FREDERICQ[28] reports a value of 0.66% for SZENT-GYÖRGYI's myosin A by the ferricyanide method. The present results also refer to myosin A twice precipitated, which contains some actin; rigorous purification was not attempted because of the dangers of oxidative loss.

The cysteine content of various preparations lies between 0.85–0.9% and the reactivity of the groups towards NEM being of the order of 40–50% (Table II).

BAILEY AND PERRY[29] first noted the strong NP test given by actin, and Table II shows that its cysteine content is nearly as high as that of myosin, though the reactivity towards NEM is rather less.

The role of SH groups in the transformation of G- to F-actin. On the effect of SH reagents upon the change of G- to F-actin and its reversal, the literature is rather confused. BAILEY AND PERRY[17] noted that the inactivation of actin by oxidants (H_2O_2 and iodosobenzoate), which were very effective in suppressing the ATPase activity of

References p. 113.

TABLE II

SH CONTENT OF MYOSIN AND ACTIN BY TITRATION WITH N-ETHYLMALEIMIDE IN GUANIDINE

(Results as g cysteine/100 g protein)

Protein	*Description*	*Total SH*	*Groups reacting with NEM at pH 7.4 as % of the total SH*
Ovalbumin	4 × recrystallized	1.30	—
Myosin A	2 × precipitated	0.9	—
Myosin A	2 weeks at 0° C	0.9	50
Myosin A	2 × precipitated	0.85	42
F-actin	extracted 30% acetone	0.85	40
G-actin	extracted water	0.8	44
G-actin	extracted 30% acetone	0.85	38
F-actin	above sample "polymerised"	0.85	36

myosin, was extremely small; and the nitroprusside reaction could be abolished with little effect on the actomyosin-forming ability. FEUER *et al.*[3], however, reported that although high concentrations of ferricyanide or quinones were necessary to inactivate, cystine or methylene blue (0.001 *M*) showed some effect. In a series of papers, KUSCHINSKY AND TURBA[29,30,31] have examined the action of certain drugs such as Salyrgan, and have interpreted their effectiveness as due to combination with SH groups which they consider essential for the transformation. There is no doubt about the effectiveness of compounds containing heavy metals (*e.g.* chloromercuribenzoate) and it is noteworthy that the arsenicals used contained heavy metals; one which did not Oxarsan (*m*-amino-*p*-oxyphenyl-arsen (III)) oxide had only a slight effect.

In Fig. 6 are shown the effects of *p*-chloromercuribenzoate and NEM on the transformation of G-actin to F-actin; the first prevents any rise in viscosity and the second has no effect. If F-actin, prepared according to the present method, is depolymerized by dialysis to a state intermediate between that of G- and F-actin, the increment of viscosity between the G- and F-forms can be taken as the difference in viscosity of the mercury-poisoned system and that in presence of KCl. The poisons can then be tested as to their inhibition of the transformation to F-actin (*i.e.* inhibitor added before KCl) or the reverse (KCl followed by inhibitor). Fig. 6 shows that again NEM has little effect, and iodosobenzoate appears to act in a curious way: it maintains the viscosity at the value of the starting material.

Anticipating the paper which follows, it is unlikely that F-actin in solution is a polymer of the type shown in electron microscope studies. It seems to be a special

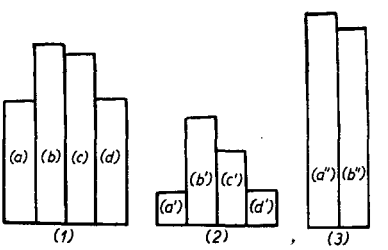


Fig. 6. Effect of SH reagents (~ 0.0025 *M*) on the viscosity of actin. Treated for 20 min at room temp.
Ordinates represent increment of viscosity, with the mercury-poisoned system representative of G-actin.
1. (a) original solution (butanol actin); (b) KCl alone; (c) KCl then NEM; (d) KCl then iodosobenzoate.
2. (a′) original solution (butanol actin); (b′) KCl alone; (c′) NEM then KCl; (d′) iodosobenzoate then KCl.
3. (a″) Straub actin, KCl only; (b″) KCl then NEM. Rates of polymerisation also identical.
Protein concentration: 1. ~ 1 mg/ml; 2. ~ 0.6 mg/ml; 3. ~ 1.5 mg/ml.

References p. 113.

thixotropic state of the dimeric form of actin, in which the monomer of 70,000 molecular weight is linked via the nucleotide prosthetic group and through magnesium to another molecule. There is much justification for the view, deriving from studies on myosin ATPase and other enzymes for which ATP acts as substrate, that binding occurs at centres which contain SH groups. If this generalization is extended to actin, then one may suppose that any reagent capable of reacting with its SH groups will prevent the transformation by displacing the essential prosthetic group, and the effect will not be a primary one. Even so, it remains to be shown, in the light of the relative ineffectiveness of SH poisons other than those containing heavy metals, whether inhibitors act in this way, or whether the effective poisons act by displacing magnesium from the dimer complex.

Tyrosine and tryptophan. FEUER *et al.*[3] have reported a minimal molecular weight of 70,000 based on a tryptophan content of 0.31%. For STRAUB actin, PERRY[32] found by the LUGG method[33] a content of 1.2%; for a pure actin and one preparation of STRAUB actin, respectively, we find: tryptophan 1.15, 1.20: tyrosine 4.7, 4.65 g/100 g protein (N content 16.2%). Although the M.Wt of the actin monomer is in fact 70,000, the value derived by FEUER *et al.* is thus fortuitously correct.

DISCUSSION

The purification procedures of MOMMAERTS[7] and A. G. SZENT-GYÖRGYI[8] depend either upon the fractional sedimentation in the ultracentrifuge or the fractional precipitation with cold ethanol and dialysis, both applied to actin in the solution phase. The procedure outlined in this paper offers an alternative route by eliminating or suppressing the impurities in the solid phase before extraction. Through the use of moderate pH and butanol for the treatment of muscle brei, and of 30% acetone for the extraction of actin, the protein can be obtained electrophoretically homogeneous with maximal activity once it is shed into solution.

Since in the present procedure the loss of tropomyosin through the treatment of muscle mince with alkaline buffer is avoided, and since the extraction of tropomyosin from the dried powder is suppressed by employing 30% acetone, it is possible to use the final residue directly for the extraction of tropomyosin by the procedures of BAILEY[18]. One can obtain, therefore, from the same muscle mince, three structural proteins: myosin, tropomyosin and actin, all electrophoretically homogeneous and all in high yield.

It is not the purpose of the present work to investigate how actin exists in muscle. However, some of the preliminary observations on the extraction of actin may be of relevance to this problem. HASSELBACH AND SCHNEIDER[19] have shown that after removal of myosin, the rate of extraction of actin from fresh muscle brei depends only on the state of subdivision of the latter; and that above pH 6 actin went into any aqueous extractant if the muscle brei was macerated fine enough in the blender. They suggested that the solubility behaviour of actin existing in muscle was the same as that in the extracted state. On maceration, the long insoluble threads of F-actin in muscle were fragmented, eventually becoming small enough to be extracted. With rabbit muscle, it was possible to confirm the findings of HASSELBACH AND SCHNEIDER, but there was some uncertainty about the results of the extraction with water itself. If the myosin-free brei was washed with water until free from salt and then macerated with water in the Waring blender for 4 minutes and spun, the supernatant was very

References p. 113.

viscous, but the viscosity fell drastically on the addition of neutral salts. There was no evidence that actin came out into the extract. The viscosity fall, in one case from η rel = 10.1 to 1.8, was found to be due to nucleotropomyosin which has been isolated and crystallised. Under comparable conditions, and when the brei was finely macerated in the blender, the extent of extraction of nucleotropomyosin by M KCl was about 40 times that by water (TSAO[21]). It may be that the solubilisation of actin follows a similar course and that the amount of actin extracted by water alone is insufficient to respond to the actomyosin-ATP test.

The parallel behaviours of actin and nucleotropomyosin/tropomyosin in their isolation are of interest. The extraction of both is markedly favoured by organic solvent treatment, alkaline pH, or high salt concentration or by any combination of these factors. It is difficult to imagine that the two highly soluble proteins exist in muscle mainly by virtue of the high degree of polymerisation. It is not improbable, considering the extraction behaviour, that *in situ* actin forms some kind of complex with the stroma and that in this complex formation lipo-nucleoprotein linkages are actually involved.

ACKNOWLEDGEMENTS

We are greatly indebted for a grant made available to one of us (K. BAILEY) from Imperial Chemical Industries, which made this work possible.

SUMMARY

1. The protein impurities in conventional preparations of actin have been identified: one is tropomyosin and the other may be a flavo-protein.

2. A procedure for the preparation of electrophoretically homogeneous actin with maximal activity is described. The method permits the preparation, from the same muscle mince, of three structural proteins, myosin, actin and tropomyosin, all in high yield.

3. A new method for the determination of –SH groups in proteins is described. This makes use of the disappearance of the nitroprusside colour by titration with N-ethylmaleimide.

4. The cysteine content of both myosin and actin is 0.8–0.9%.

RÉSUMÉ

1. Les impuretés protéiniques des préparations usuelles d'actine ont été identifiées: l'une est la tropomyosine et l'autre pourrait être une flavoprotéine.

2. Un procédé de préparation d'actine électrophorétiquement homogène est décrit. Cette méthode permet de préparer trois protéines structurales, la myosine, l'actine et la tropomyosine, à partir d'une même préparation de muscle et toutes trois avec un bon rendement.

3. Une nouvelle méthode de détermination des groupes –SH dans les protéines est décrite. Elle fait usage de la disparition de la couleur du nitroprusside lors du titrage par l'N-éthylmaléimide.

4. La teneur en cystéine de la myosine aussi bien que de l'actine est de 0.8–0.9%.

ZUSAMMENFASSUNG

1. Die Verunreinigungen an Protein in den herkömmlichen Aktinpräparaten wurden identifiziert. Die eine erwies sich als Tropomyosin und die andere könnte ein Flavoprotein sein.

2. Ein Verfahren zur Darstellung von elektrophoretisch homogenem Aktin mit Maximalaktivität wird beschrieben. Die Methode gestattet die Darstellung von drei organischen Proteinen, dem Myosin, dem Aktin und dem Tropomyosin aus dem gleichen Muskelbrei in hohen Ausbeuten.

3. Es wird eine neue Methode der Bestimmung der –SH Gruppen in Proteinen beschrieben. Es wird dabei Gebrauch gemacht von dem Verschwinden der Nitroprussidfarbe bei der Titration mit N-Äthylmaleinsäureimid.

4. Der Cysteingehalt sowohl von Myosin als auch von Aktin ist 0.8–0.9%.

References p. 113.

REFERENCES

1 F. B. STRAUB, *Studies Inst. Med. Chem. Univ. Szeged*, 2 (1942) 1.
2 F. B. STRAUB, *Studies Inst. Med. Chem. Univ. Szeged*, 3 (1943) 23.
3 G. FEUER, F. MOLNÁR, E. PETTKÓ AND F. B. STRAUB, *Hungarica Acta Physiol.*, 1 (1948) 150.
4 A. SZENT-GYÖRGYI, *Chemistry of Muscular Contraction*, New York, Academic Press (1947).
5 H. H. WEBER, *Advances in Protein Chemistry*, 7 (1952), New York, Academic Press.
6 S. S. SPICER AND J. GERGELY, *J. Biol. Chem.*, 188 (1951) 179.
7 W. F. H. M. MOMMAERTS, *J. Biol. Chem.*, 188 (1951) 559.
8 A. G. SZENT-GYÖRGYI, *J. Biol. Chem.*, 192 (1951) 361.
9 F. B. STRAUB AND G. FEUER, *Biochim. Biophys. Acta*, 4 (1950) 455.
10 K. LAKI, W. J. BOWEN AND A. CLARK, *J. Gen. Physiol.*, 33 (1950) 437.
11 T.-C. TSAO, *Biochim. Biophys. Acta*, in the press.
12 T.-C. TSAO, *Biochim. Biophys. Acta*, in the press.
13 M. DUBUISSON, *Biochim. Biophys. Acta*, 5 (1950) 426.
14 K. LAKI AND A. CLARK, *Arch. Biochem.*, 30 (1951) 187.
15 N. M. LJUBIMOVA AND G. M. POPOVA, *Doklady Akad. Nauk S.S.S.R.*, 66 (1949) 433.
16 F. G. HOPKINS, E. J. MORGAN AND C. LUTWAK-MANN, *Biochem. J.*, 32 (1938) 1829.
17 K. BAILEY AND S. V. PERRY, *Biochim. Biophys. Acta*, 1 (1947) 506.
18 K. BAILEY, *Biochem. J.*, 43 (1948) 271.
19 W. HASSELBACH AND G. SCHNEIDER, *Biochem. Z.*, 321 (1951) 462.
20 R. K. MORTON, *Nature*, 166 (1950) 1092.
21 T.-C. TSAO, Unpublished.
22 S. TSUDA, *Kolloid-Z.*, 45 (1928) 325.
23 E. FRIEDMANN, D. H. MARRIAN AND I. SIMON-REUSS, *Brit. J. Pharm. and Chemotherap.*, 3 (1948) 335.
24 J. W. H. LUGG, *Biochem. J.*, 27 (1933) 668.
25 D. H. MARRIAN, *J. Chem. Soc.*, (1949) 1515.
26 H. NEURATH, J. P. GREENSTEIN, F. W. PUTNAM AND J. O. ERICKSON, *Chem. Rev.*, 34 (1944) 157.
27 J. P. GREENSTEIN AND J. T. EDSALL, *J. Biol. Chem.*, 133 (1940) 397.
28 E. FREDERICQ, Thesis, Liege, 1943.
29 G. KUSCHINSKY AND F. TURBA, *Naturwissenschaften*, 37 (1950) 425.
30 F. TURBA, G. KUSCHINSKY AND H. THOMANN, *Naturwissenschaften*, 37 (1950) 453.
31 F. TURBA AND G. KUSCHINSKY, *Biochim. Biophys. Acta*, 8 (1952) 76.
32 S. V. PERRY, Dissertation, Cambridge (1947).
33 J. W. H. LUGG, *Biochem. J.*, 31 (1937) 1422.

Received November 13th, 1952